Morphology, Molecular Codes, and Circuitry Produce the Three-Dimensional Complexity of the Cerebellum

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Annu. Rev. Cell Dev. Biol. 2007. 23:549–77

First published online as a Review in Advance on July 3, 2007

The *Annual Review of Cell and Developmental Biology* is online at http://cellbio.annualreviews.org

This article's doi:
10.1146/annurev.cellbio.23.090506.123237

1081-0706/07/1110-0549$20.00

Key Words

patterning, coordinate system, *Engrailed*, *Sonic hedgehog*, Zebrin

Abstract

The most noticeable morphological feature of the cerebellum is its folded appearance, whereby fissures separate its anterior-posterior extent into lobules. Each lobule is molecularly coded along the medial-lateral axis by parasagittal stripes of gene expression in one cell type, the Purkinje cells (PCs). Additionally, within each lobule distinct combinations of afferents terminate and supply the cerebellum with synchronized sensory and motor information. Strikingly, afferent terminal fields are organized into parasagittal domains, and this pattern bears a close relationship to PC molecular coding. Thus, cerebellum three-dimensional complexity obeys a basic coordinate system that can be broken down into morphology and molecular coding. In this review, we summarize the sequential stages of cerebellum development that produce its laminar structure, foliation, and molecular organization. We also introduce genes that regulate morphology and molecular coding, and discuss the establishment of topographical circuits within the context of the two coordinate systems. Finally, we discuss how abnormal cerebellar organization may result in neurological disorders like autism.

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MOLECULAR CODES AND MORPHOLOGY DISTINGUISH THE THREE-DIMENSIONAL ARCHITECTURE OF THE CEREBELLUM

Overview of Cerebellum Development

The neuroepithelium that gives rise to the cerebellum undergoes several major structural and genetic transformations that occur at sequential stages during development to produce a complex foliated structure with molecular coding. Separated from the midbrain by a lineage boundary (Zervas et al. 2004), the cerebellum arises from dorsal rhombomere 1 (r1) of the developing hindbrain at embryonic (E) day 9 (E9) in mouse (Millet et al. 1996, Wingate & Hatten 1999, Zervas et al. 2004). Between E9 and E12 a 90-degree rotation converts the anterior-posterior (AP) axis of the dorsal neural tube into the medial-lateral (ML) axis of the cerebellar primordium, which takes on a bilateral, wing-like morphology (Sgaier et al. 2005) (**Figure 1**). This ML axis is maintained to adulthood. By E15.5 the cerebellum starts to transform into a simple sausage-shaped structure and begins to display ML organization in the form of parasagittal molecular domains (**Figure 1**). Further complexity is added to the cerebellum at E17, when four fissures initiate and separate the

AP: anterior-posterior

ML: medial-lateral

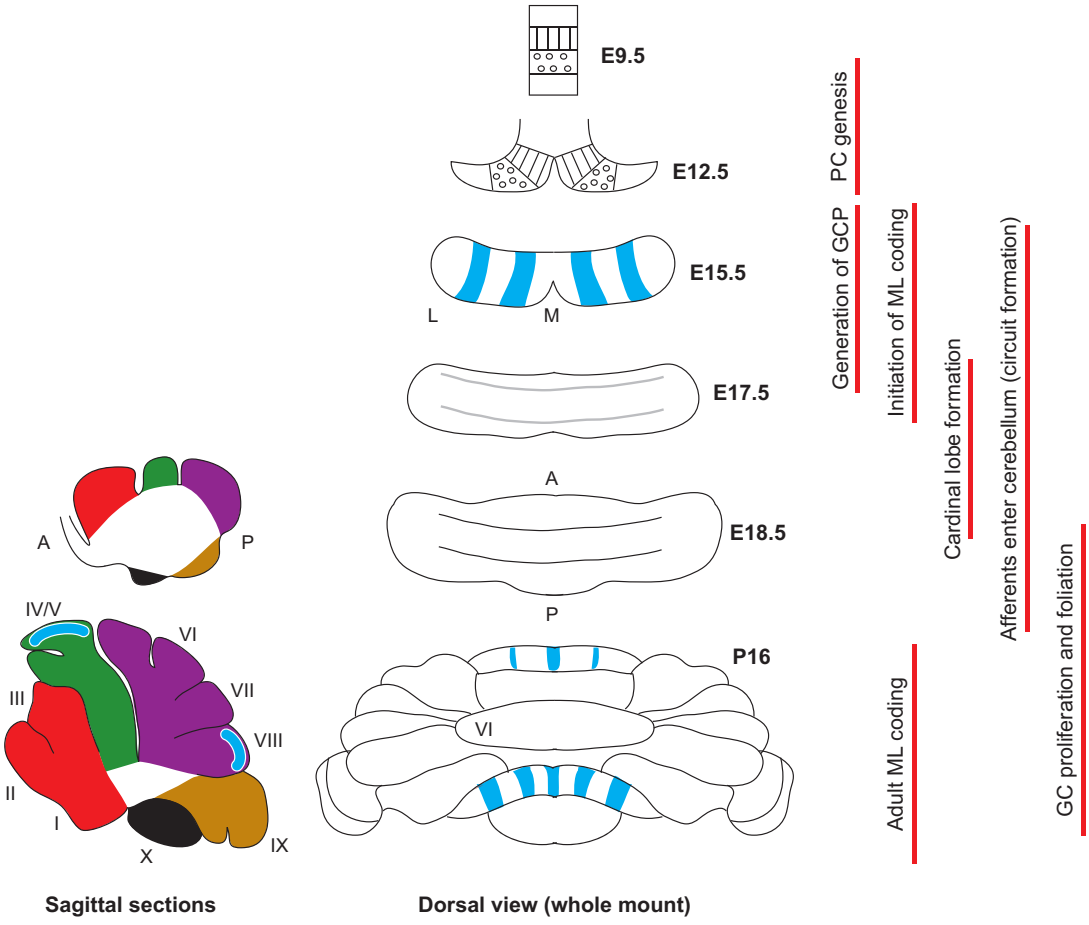

Figure 1

Two coordinate systems orchestrate cerebellum development. The figure shows dorsal views of the cerebellum illustrating changes in the anterior-posterior (AP) morphology and medial-lateral (ML) molecular coding from embryo to adult. Also shown are sagittal schematics illustrating the lobules in the AP axis. Other abbreviations used: A, anterior; P, posterior; M, medial; L, lateral; GC, granule cell; GCP, granule cell precursor; PC, Purkinje cell.

cerebellum into five folds along the AP axis (**Figure 1**). By E18.5 the cerebellum has acquired a three-dimensional (3-D) architecture containing AP folds intersected by ML molecular domains (**Figure 1**). Furthermore, by the animal's birth the circuitry of the cerebellum has established a rudimentary set of synaptic connections that begin to become topographically organized within the context of the AP folds and ML molecular domains (**Figure 1**). By postnatal (P) day 16 (P16) in mouse, the cerebellum has organized all its cell types in layers, and the external lobule morphology and molecular domains are correlated with the localization of specific cerebellar circuits (**Figure 1**).

Foliation Demarcates an Anterior-Posterior Coordinate System in the Adult Cerebellum

The landmark studies performed by Olof Larsell (1970), which involved a comparative analysis of cerebellar lobule morphology,

GC: granule cell

Circuit: a pathway in the brain composed of two or more interconnected neurons carrying signals related to a particular function(s)

established a nomenclature for naming lobules across a wide range of species. In mammals, the medial portion of the cerebellum (vermis) has a foliation pattern along the AP axis that is distinct from the lateral extensions (hemispheres) (**Figure 2*a***), with an intermediate zone (also referred to as the paravermal zone) separating them. More lateral are the paraflocculi and flocculi, which extend outward from the ventral portion of the posterior cerebellum; each also contains distinct foliation patterns. Thus, morphological landmarks can also be used to divide the cerebellum along the ML axis broadly into four regions. Additionally, each of these regions is molecularly distinct on the basis of gene expression patterns as well as functionally distinct, as evidenced by localized lesions that affect specific motor functions, imaging studies that use specific paradigms to selectively activate one of the four regions (Chen et al. 1996), and finally by the termination patterns of afferents that

Lobule: morphological fold in the cerebellum of all mammals; anatomically the mammalian cerebellum is divided into ten lobules

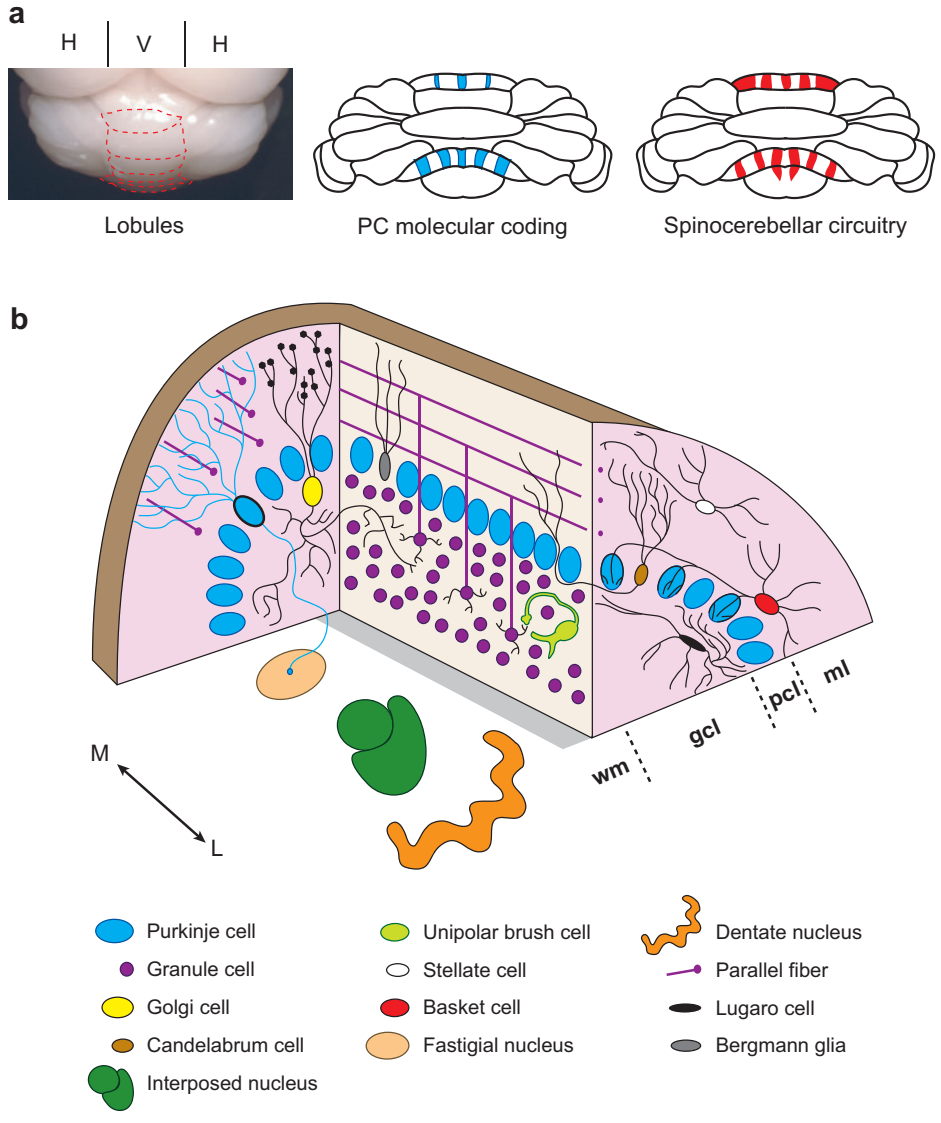

Figure 2

The adult cerebellum has intricate coordinate systems and circuitry. (*a*) The cerebellum can be divided into anterior-posterior (AP) lobules and a medial-lateral (ML) molecular code. The spinocerebellar projections terminate in specific AP and ML locations. (*b*) Cytology in the cerebellum. Note that the illustration shows a crenated dentate nucleus (*orange*), as seen in higher mammals. Abbreviations: ml, molecular layer; pcl, Purkinje cell layer; gcl, granule cell layer; wm, white matter; M, medial; L, lateral; V, vermis; H, hemisphere, PC, Purkinje cell.

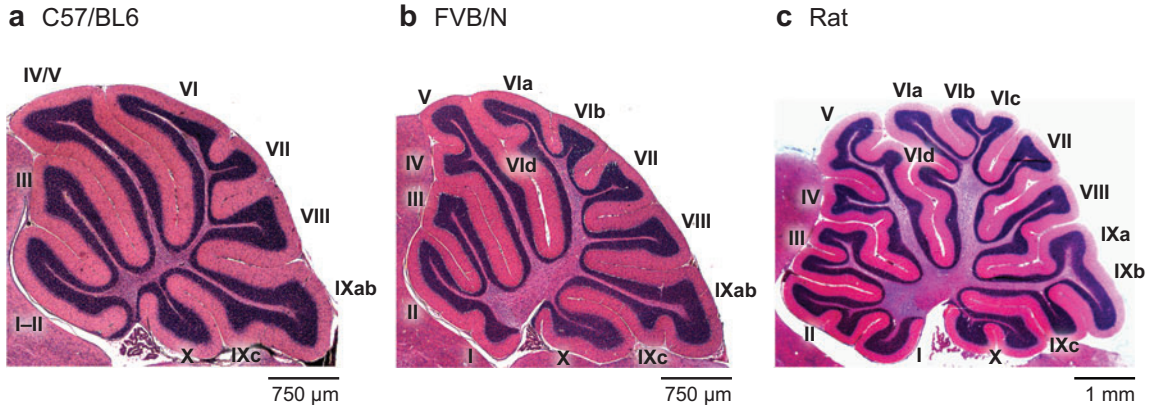

Figure 3

Foliation is more complex in FVB/N than in C57/BL6 mice. (*a*) C57/BL6. (*b*) FVB/N. The more complex foliation pattern in rat (*c*) is shown for comparison. The lobule numbers are indicated by Roman numerals.

carry specific information to distinct ML locations in the cerebellum (Chockkan & Hawkes 1994, Hallem et al. 1999, Schonewille et al. 2006).

Through the use of Larsell's criteria for the vermis, the cerebellum of all mammals can be divided into ten basic lobules; on the basis of the species, each of the primary lobules is further divided into secondary and tertiary sublobules (Larsell 1970). Despite the convenience of this nomenclature, several factors must be taken into consideration when interspecies differences are examined in detail. For example, in mouse (**Figure 3**), the identification of lobule I can be difficult because of the poor formation of the precentral fissure in most strains (Inouye & Oda 1980). In addition, because of a shallow or absent intraculminate fissure, the separation between lobules IV and V is not always clear, and morphologically only one fold appears to encompass both lobules (Inouye & Oda 1980). We have found that FVB/N mice have a much more complex foliation pattern than do C57/BL6 mice; these are two strains commonly used in mouse genetics experiments (**Figure 3**). In the FVB/N strain, but not the C57/BL6 strain, lobules I and II are separated by a shallow precentral fissure (compared with rat), lobules IV and V are easily distinguishable on either side of the intraculminate fissure, and sublobules VIa and VIb are separated by an obvious declival sulcus. In both strains, sublobules IXab and IXc are separated by an uvular sulcus. Despite these characteristic differences, the remarkable conservation of lobules across mammals suggests that the genetic mechanism(s) underlying the process of foliation became fixed early during evolution of the mammalian cerebellum.

Gene Expression Domains Demarcate a Medial-Lateral Coordinate System in the Adult Cerebellum

Although the laminar histology of the cerebellar cortex appears uniform throughout the ML axis, an elaborate array of fine subdivisions into modules is revealed by gene expression (Hawkes et al. 1985; reviewed in Hawkes & Gravel 1991, Hawkes 1997) (**Figure 4**) and by the topography of afferent projections (Voogd & Ruigrok 1997, Voogd & Glickstein 1998) (**Figure 5**). The Purkinje cell (PC) efferent system also projects axons into the deep cerebellar nuclei (DCN) in a ML organization. Parasagittal molecular domains in the cerebellar cortex were first described by Scott (1963), using the enzyme

Axon: the portion of a neuron that carries signals away from the cell body toward a synapse with a receiving neuron

Purkinje cells (PCs): one of the largest cell types in the brain, these neurons are the only output of the cerebellar cortex

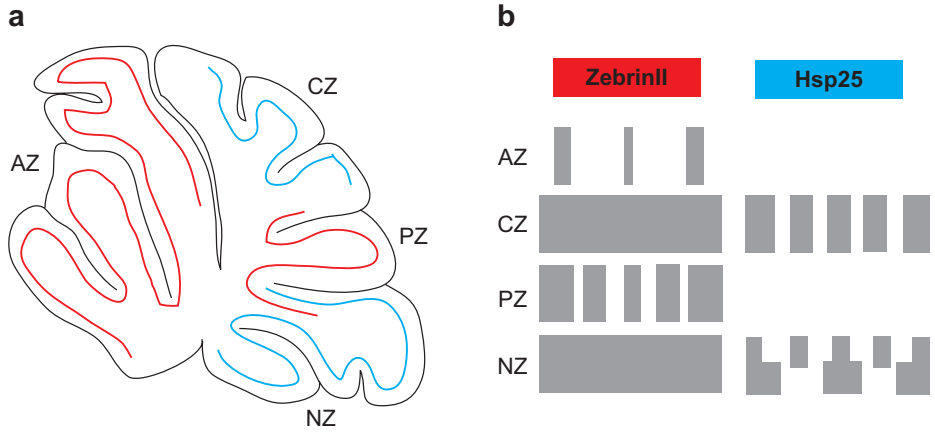

Figure 4

ZebrinII and Hsp25 expression patterns delineate four anterior-posterior (AP) transverse zones in the adult cerebellum. (*a*) ZebrinII [*red* in anterior zone (AZ), posterior zone (PZ)] and Heat shock protein 25 (Hsp25) [*blue* in central zone (CZ), nodular zone (NZ)] parasagittal domains occupy distinct AP zones. (*b*) Illustration of ZebrinII (AZ, PZ) and Hsp25 (CZ, NZ) Purkinje cell (PC) parasagittal molecular domains in the vermis of an adult mouse cerebellum. Note that ZebrinII is homogeneous in the CZ and NZ and that Hsp25 is absent from the AZ and PZ.

5′-nucleotidase. Since then studies have revealed a plethora of molecules expressed in parasagittal domains in the embryonic and adult cerebellum (Armstrong & Hawkes 2000, Larouche & Hawkes 2006). The most thoroughly documented of the adult stripe markers is ZebrinII, first described by Brochu et al. (1990) and later shown to be aldolase C (Ahn et al. 1994; reviewed in Hawkes et al. 1992). The ML molecular coding is not homogeneous along the AP axis. Instead, it appears to be divided into four major domains that are joined by transition areas (Ozol et al. 1999, Sgaier et al. 2007). ZebrinII is expressed in parasagittal stripes in lobules I-V and lobule VIII, whereas most of lobules VI-VII and IX-X uniformly express ZebrinII. In contrast, lobules that uniformly express ZebrinII express the small heat shock protein Hsp25 in a striking array of parasagittal stripes (Armstrong et al. 2000) (**Figure 4**). Thus, the expression of ZebrinII and Hsp25 reveals parasagittal stripes in four distinct AP transverse zones in the cerebellum (Hawkes & Eisenman 1997, Ozol et al. 1999, Armstrong et al. 2000). Through the use of these molecular markers, ML and AP patterns can be superimposed on the lobules (also an AP pattern), resulting in an intrinsic grid-like organization or coordinate system of the cerebellum.

The transitions between transverse zones do not correspond precisely to lobule boundaries (depth of fissures), but in general the anterior zone is composed of lobules I–V, the central zone is composed of lobules VI and VII, the posterior zone is composed of lobules VIII and dorsal IX, and the nodular zone includes ventral lobule IX and lobule X (Ozol et al. 1999) (**Figures 4** and **5**). However, a recent comparative study using the basal insectivore tenrec (*Echinops telfairi*), an animal with a lissiform cerebellum with only five lobules, nevertheless has an AP organization of ZebrinII of four transverse zones (Sillitoe et al. 2003b). For example, the AP transition between lobules VI/VII [central zone (CZ) homogeneous ZebrinII expression] and lobule VIII [posterior zone (PZ) ZebrinII stripes] in tenrec is easily recognizable on its smooth cerebellar surface, which lacks the fissure between lobules VII and VIII (Sillitoe et al. 2003b, 2005). The data from tenrec suggest

Parasagittal domain: distinct division along the ML axis with unique gene expression and molecular signature (molecular code)

ZebrinII: also known as aldolase C, this brain-specific respiratory isoenzyme is strongly expressed in Purkinje cell parasagittal domains

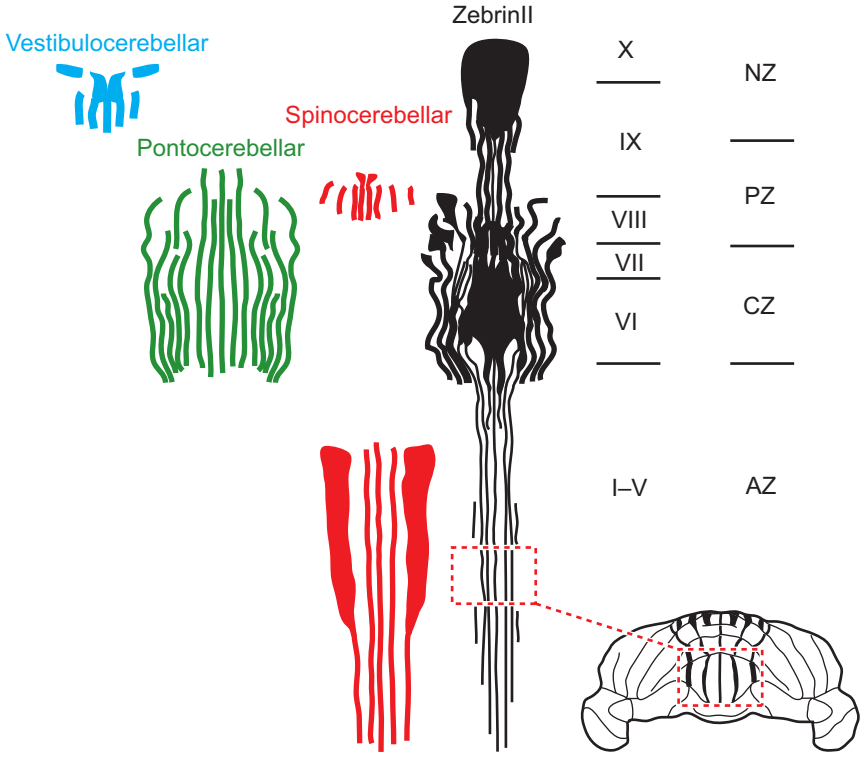

Figure 5

ZebrinII medial-lateral (ML) coding corresponds to mossy fiber terminal field organization. The figure shows an unfolded vermis illustrating the ZebrinII ML code and various mossy fiber parasagittal domains. At the bottom right, ZebrinII stripes in the anterior lobules are superimposed onto a drawing of the anterior cerebellum. The pontocerebellar projection is based on the rat vermis and hemispheres (Serapide et al. 2001), and the vestibulocerebellar (Sillitoe et al. 2003a) and spinocerebellar (Vogel et al. 1996) projections are based on the mouse vermis. The mouse ZebrinII schematic was redrawn from Eisenman & Hawkes (1993), with permission. Lobule numbers are indicated by Roman numerals.

that dividing the cerebellum into distinct lobules is not a prerequisite for the broader partitioning of the cerebellum into four AP transverse zones with elaborate ML coding of molecular domains.

Not all molecular maps in the cerebellum are exactly congruent with ZebrinII and Hsp25. One example is P-path, a monoclonal antibody that recognizes a cerebellar ganglioside, 9-*O*-acetyl-GD3. P-path was identified as the first marker expressed predominantly in ZebrinII-negative (–) PCs (Leclerc et al. 1992). Another example is the human natural killer cell antigen HNK1, a cell surface carbohydrate epitope that is most notably expressed in ZebrinII (–) PCs (Eisenman & Hawkes 1993). Although P-path and HNK1 predominantly mark the ZebrinII (–) PC subset, both are also expressed in a small subset of ZebrinII-positive (+) PCs (Leclerc et al. 1992, Eisenman & Hawkes 1993, Marzban et al. 2003). More recently, the expression patterns of phospholipase Cβ3 and Cβ4 have been shown to have a near perfect congruence with the ZebrinII (+) and (–) PCs, respectively (Sarna et al. 2006). In summary, PC parasagittal stripes may represent part of a ML molecular code composed of specific gene expression domains, each with a unique molecular tag. Together, the ML molecular code and

Mossy fiber: a specific type of projection providing input to the cerebellum from many regions of the brain and spinal cord

AP folds form a grid-like pattern in the cerebellum. We suggest that this organization may provide the spatial framework on which specialized circuits develop.

Climbing fiber: a specific type of projection providing a powerful input to the cerebellum from a single source in the brainstem, the inferior olive

CNS: central nervous system

ISO: isthmic organizer

THE CELLS THAT MAKE UP THE CEREBELLUM

Despite the complexity of the cerebellum at the level of morphology and molecular coding, the cerebellar cortex is histologically homogeneous and divided into three distinct cellular layers. These layers overly an inner core composed of white matter and three pairs of symmetrical clusters of deep cerebellar nuclei (DCN) (**Figure 2*b***). Bordering the white-matter core and lying immediately above it is the granule cell (GC) layer. The small GCs (the most numerous neuronal cell type in the brain) comprise the great majority of this layer. The somata of the Golgi cell and Lugaro cell interneurons as well as the unipolar brush cells (UBCs), recognized only recently as a distinct neuronal type, also reside in the granular layer (Mugnaini et al. 1997). The next layer is called the PC layer because it is primarily a monolayer of PC somata; it also contains the somata of Bergmann glia (Voogd & Glickstein 1998) and candelabrum cells (Laine & Axelrad 1994), which are both wedged between the much larger PC soma. The molecular layer is most superficial and is made up primarily of PC dendrites and GC axons (parallel fibers) but also contains stellate and basket cell interneurons in addition to palisades of Bergmann glia fibers (Voogd & Glickstein 1998). The cerebellum receives electrical impulses from most regions of the brain as well as the spinal cord via two major afferent systems. Mossy fibers and climbing fibers constitute the majority of the afferents entering the cerebellum and terminate within the GC layer and the molecular layer, respectively. Thus, in addition to cells that are intrinsic to the cerebellum, a major portion of the cerebellar architecture is made up of extrinsic cytological components.

EARLY SPECIFICATION OF THE CEREBELLAR ANLAGE

Although not fully appreciated, the molecular dynamics that participate in establishing the cerebellar anlage have been intensively studied via mouse molecular genetics, chicken embryology, and, more recently, zebrafish developmental genetics. By ~E9.5 in mouse, the major central nervous system (CNS) regions can be morphologically distinguished from anterior to posterior: the telencephalon and diencephalon (forebrain), mesencephalon (mes) (midbrain), and the metencephalon and myelencephalon (hindbrain). During development the mes is separated from the metencephalon (r1 and r2) by a morphological constriction called the mid/hindbrain junction, or isthmus. Patterning of the midbrain and r1 along the AP axis is dependent on signals from an organizing center located in the isthmus, the isthmic organizer (ISO) (Brand et al. 1996, Liu & Joyner 2001a, Wurst & Bally-Cuif 2001, Nakamura et al. 2005, Zervas et al. 2005) (**Figure 6**). Fibroblast growth factor 8 (FGF8) is the primary organizer molecule expressed and secreted by the ISO (Liu & Joyner 2001a, Wurst & Bally-Cuif 2001, Nakamura et al. 2005). In gain-of-function experiments in mouse and chick embryos, FGF8 protein or isthmus grafts induce the formation of ectopic tecta (the dorsal part of the midbrain) or ectopic cerebella, depending on the site in the brain and the isoform of FGF8 expressed (Crossley et al. 1996, Hidalgo-Sanchez et al. 1999, Liu et al. 1999, Martinez et al. 1999, Sato et al. 2001). Conversely, in loss-of-function experiments, inactivation of *Fgf8* leads to loss of the entire tectum and cerebellum (Meyers et al. 1998, Reifers et al. 1998, Chi et al. 2003). A secreted molecule, WNT1, is expressed immediately anterior to the FGF8 domain in the mes (**Figure 6**). Unlike FGF8, WNT1 does not have mes/r1 inductive activity but is essential for midbrain and cerebellum development, likely because *Fgf8* expression is dependent on Wnt1 (McMahon & Bradley 1990,

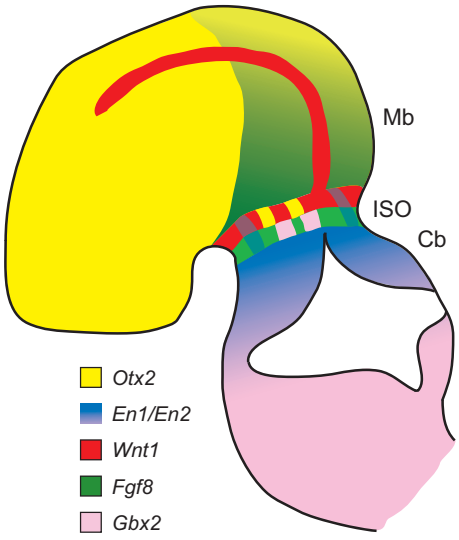

Figure 6

Complex genetic interactions are required for the establishment of the cerebellar anlage. Illustration of the mouse embryo at approximately embryonic day 9.5 showing the genes required for cerebellum morphogenesis. Abbreviations: Mb, midbrain; ISO, isthmic organizer; Cb, cerebellum.

Thomas & Capecchi 1990, Lee et al. 1997). Thus, among the earliest known molecules secreted in the isthmus, FGF8 has emerged as the key molecule for mediating the inductive activity of the ISO.

In addition to the action of FGF8 and WNT1, a complex transcriptional network operates during midbrain and cerebellum development. The homeobox genes *Otx2* and *Gbx2*, among the earliest genes expressed in the CNS, initially mark the anterior and posterior epiblast, respectively (Joyner et al. 2000, Liu & Joyner 2001a, Simeone 2000, Wurst & Bally-Cuif 2001, Simeone et al. 2002, Nakamura et al. 2005). Within the neural plate at E8.5, the border of *Otx2* and *Gbx2* expression corresponds to the future posterior border of the mes. Otx2 and Gbx2 act antagonistically to demarcate the position of the *Fgf8* and *Wnt1* expression domains, respectively; however, they are not required for the induction of either gene (Rhinn et al. 1998, 1999; Broccoli et al. 1999; Millet et al. 1999; Li & Joyner 2001). Conversely, it has recently been shown in zebrafish that *Fgf8* is required to maintain the posterior boundary of *Otx2* expression (Foucher et al. 2006), consistent with FGF8-soaked-bead studies showing that FGF8b can repress *Otx2* (Liu et al. 1999, Martinez et al. 1999). *Wnt1*, along with two transcription factors, En1 and Pax2, is expressed earlier than *Fgf8* in the mes/r1, whereas *En2* and *Pax5* are expressed later in the mes/r1 (Crossley & Martin 1995, Liu & Joyner 2001a). Whereas *En1/En2* are required for the maintenance but not for the initiation of *Fgf8* or *Wnt1* expression (Liu and Joyner 2001b), *Pax2* is necessary and sufficient for induction of *Fgf8* in the r1 (Ye et al. 2001). Moreover, the LIM homeodomain transcription factor Lmx1b (orthologous to chicken Lmx1) is also necessary for the initiation of *Fgf8* expression and for the maintenance of several genes, including *Wnt1*, *En1*, *En2*, *Pax2*, and *Gbx2* (Adams et al. 2000, Matsunaga et al. 2002, O'Hara et al. 2005, Guo et al. 2007). Together, the combination of precise spatial and temporal activation of transcription factors and secreted molecules is necessary and sufficient for setting up a cerebellar territory by E9 and forming the bilateral wing-like cerebellar primordium by E12.5.

ORIGINS AND MIGRATORY ROUTES OF CELLS IN THE DEVELOPING CEREBELLUM

The cerebellar primordium serves as a platform from which the various cerebellar cell types are generated. The cerebellum is unique in the brain because it has two specialized germinal zones from which neurons and glia arise. The GCs, some deep nuclear neurons, and unipolar brush cells are generated by a transient germinal epithelium called the rhombic lip (RL) (Machold & Fishell 2005, Wang et al. 2005, Englund et al. 2006). The RL is located at the interface between the dorsal neural tube and the widened portion of the fourth ventricle's roofplate in the posteriormost region of r1 (Wingate 2001) (**Figure 7**).

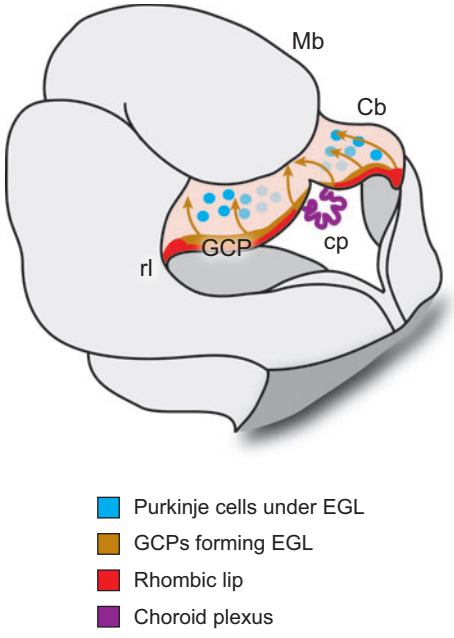

Figure 7

Schematic of an embryonic day 12.5 cerebellum showing the tangential migration of granule cell precursors (GCPs) away from their origin in the rhombic lip. The GCPs migrate over the surface of the cerebellum. Below the surface, Purkinje cells (PCs) are seen within the cerebellum. Abbreviations: Mb, midbrain; Cb, cerebellum; cp, choroid plexus; GCP, granule cell precursor; rl, rhombic lip.

GC precursors (GCPs) in the RL migrate over the entire surface of the cerebellum from all ML points along the RL (Wingate & Hatten 1999) to form a mitotically active region called the external granular layer (EGL). In mice the EGL forms by ~E15, and GCPs within the EGL remain mitotically active into the first two postnatal weeks. GCPs reach their peak of proliferation at ~P8 in mice (Fugita et al. 1966, Fugita 1967). At approximately the time of birth, some GCPs start to exit the cell cycle and differentiate into mature GCs as they undergo axon extension and tangential (medial to lateral) migration within the deep layer of the EGL. GCs then migrate radially along Bergmann glial fibers into the developing cerebellar cortex (past the developing PCs) and form the internal granular layer (IGL), the final position of adult GCs (Altman & Bayer 1997, Wang & Zoghbi 2001). The migration and maturation of GCs are complete by P20, a few days after the disappearance of the EGL (reviewed in Goldowitz & Hamre 1998).

The second germinal zone of the cerebellum is the ventricular zone (VZ), located along the lining of the dorsal aspect of the fourth ventricle. The VZ gives rise to PCs and most interneurons in the cerebellum (Altman & Bayer 1997). PCs in mouse become postmitotic between E11 and E13 (Miale & Sidman 1961). Postmitotic PCs apparently migrate radially along radial glial fibers (Edwards et al. 1990, Morales & Hatten 2006) and transiently form a multilayer below the EGL (reviewed in Armstrong & Hawkes 2000) (**Figure 7**).

One major cellular rearrangement that occurs in the developing cerebellum around the time of birth is the dispersal of PCs from a multilayered structure into a single monolayer that becomes the mature PC layer. The dispersal of PCs is dependent on expansion of the cerebellum through GC proliferation and is at least in part controlled by the Reelin signaling pathway. Reelin is a large extracellular matrix protein secreted by EGL cells (reviewed in Rice & Curran 2001). Reelin binds to at least two transmembrane receptors on PCs: the very-low-density lipoprotein receptor (VLDLr) and the Apolipoprotein E Receptor 2 (ApoER2) (Rice & Curran 2001). The cytoplasmic domains of these receptors bind to and induce tyrosine phosphorylation of the intracellular protein Disabled-1 (Dab-1). This triggers an intracellular, kinase-dependent cascade that is necessary for PCs to disperse into a monolayer, perhaps by reducing PC-PC homophilic binding (Rice & Curran 2001).

The DCN are unique in the cerebellum in that they appear to be derived from both the RL and the VZ (Hoshino et al. 2005, Machold & Fishell 2005, Wang et al. 2005). The difference in the lineage of cells may be related to their excitatory versus inhibitory function (Hoshino et al. 2005). DCN neurons derived

EGL: external granular layer

VZ: ventricular zone

from both germinal zones become postmitotic between E10 and E12 and thereafter migrate to their final position through the use of distinct routes, depending on the germinal zone from which they are derived. DCN neurons originating from the RL initially migrate over the cerebellar cortex, using a similar route as the GCPs (Machold & Fishell 2005, Wang et al. 2005), but eventually migrate radially into the cerebellar core. VZ-derived DCN neurons apparently migrate radially to their final location within the cerebellum (Altman & Bayer 1997, Hoshino et al. 2005).

The origin and migratory routes of UBC interneurons have not been fully resolved. Like stellate and basket cell interneurons of the cerebellar cortex, UBCs may arise from the VZ and migrate dorsally to reside predominantly in the posterior cerebellum (Ilijic et al. 2005). However, a more recent study has shown that UBCs are generated from the RL (Englund et al. 2006) between ~E13.5 and early neonatal periods in mice (Hevner et al. 2006). Whether UBCs arise from the VZ and/or the RL, their exact migratory routes within the cerebellum have yet to be defined. Although there is little evidence to support the hypothesis, UBCs may use existing white-matter tracts in the cerebellum as migration scaffolds (Hevner et al. 2006). By birth, the cerebellum has already acquired several levels of structural complexity, as seen by the dispersal of cells into ordered layers and the initial folding of the cortical layers of the cerebellum.

REGULATION OF THE POSITION OF FOLIA

Just before PCs begin to disperse into a monolayer (the late embryonic stages), the cerebellum begins one of its most remarkable morphogenetic maneuvers, foliation. The formation of folds (folia) may arise through a genetically determined series of cell movements and structural changes. The morphogenetic processes of folding the cerebellum can be divided into two general stages: The first stage is the formation of the cardinal lobes, which occurs embryonically, and the second stage is the formation of the lobules and sublobules, which occurs postnatally.

The surface of the cerebellar anlage is initially smooth, with no external morphological landmarks distinguishing particular regions along the AP axis. However, by late embryonic development (~E17 in mouse and ~E22 in rat), four shallow fissures begin to form in the vermis to produce the five cardinal lobes (**Figure 8**). During postnatal development the cardinal lobes undergo extensive outgrowth and subdivision into lobules in a stereotyped sequence common to all mammals, culminating in the formation of ten distinct lobules (Altman & Bayer 1997, Larsell 1952, 1970; Inouye & Oda 1980). Subsequently, the specific lobules, designated by Roman numerals I–X, are further divided into a species-specific number of sublobules. In mouse, foliation is complete by ~P14 (**Figure 3**).

The cellular and genetic cues that regulate the early stages of cardinal lobe formation are poorly understood. Altman & Bayer (1997) have proposed that anchor points in the PC layer are crucial to the folding of the smooth surface of the cerebellum. Basically, PCs positioned at the future base of each fissure are anchored via their axons to the DCN, and as a result of GC proliferation the surface of the cerebellum balloons out in between. The folding process from this point onward would be due largely to mechanical forces. One assumption of this model is that PCs are inherently heterogeneous in structure soon after their birth. This is certainly a plausible assumption in light of evidence that PC subsets along the ML axis differentially express a variety of molecular markers starting as early as E14 (Oberdick et al. 1993, Millen et al. 1995, Larouche & Hawkes 2006). Among these ML molecular markers of PC subsets are cell adhesion–type molecules such as the cadherins (Arndt et al. 1998, Luo et al. 2004). Furthermore, the morphogen Sonic hedgehog (Shh) divides the E17 cerebellum along the AP axis into four domains (Corrales et al. 2004). Although it is

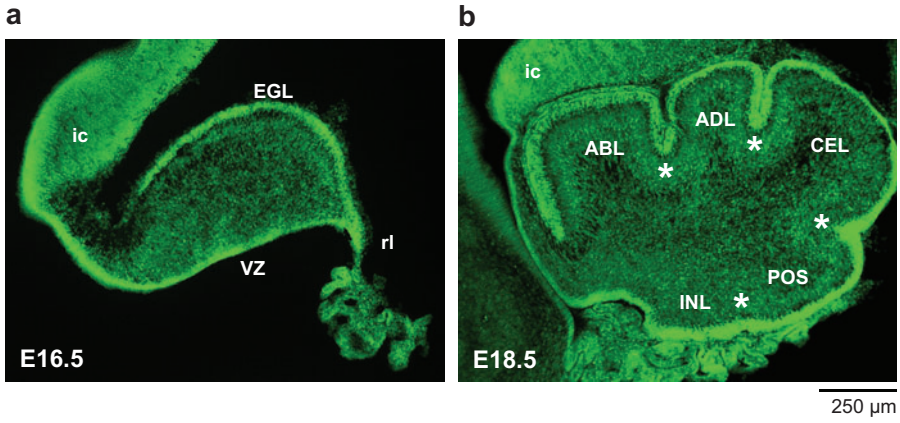

Figure 8

The five cardinal lobes are formed at approximately embryonic day (E) 17.5 in the mouse cerebellum. (*a*) Sagittal section cut through the E16.5 cerebellum. (*b*) Sagittal section cut through the E18.5 cerebellum. The asterisks indicate the four fissures separating the cerebellum into five cardinal lobes. From anterior to posterior they are the preculminate, primary, secondary, and posterolateral fissures (Altman & Bayer 1997). Abbreviations: ic, inferior colliculus, EGL, external granular layer; rl, rhombic lip; VZ, ventricular zone; ABL, anterobasal lobe; ADL, anterodorsal lobe; CEL, central lobe; POS, posterior lobe; INL, inferior lobe. The scale bar in panel *b* applies to both panels.

intriguing to pinpoint a single cell type as the master regulator of lobe position, one must consider that early-arriving mossy fibers, climbing fibers, glia, and even the GCs may contribute to anchoring the base of fissures. We also know that besides PCs, other cerebellar components are heterogeneous at multiple levels. For example, parvalbumin immunoreactive climbing fibers divide the cerebellum into global AP regions, as seen by selective staining in the posterior and inferior lobes of perinatal rats (Wassef et al. 1992). Because of the obvious complexity that already exists in the cerebellum at ~E17 (during initial stages of lobe formation), it is likely that several genetic cues govern the formation of specific anchor points.

It is important to use sophisticated molecular and genetic strategies to determine whether the base of fissures act as physical anchor points and, if so, which genes regulate the cell behaviors required to form and position each anchor point. Conditional activation and inactivation of specific genes will be particularly useful for exploring the initial trigger(s) that initiate fissure formation. In addition, if candidate genes expressed by anchor points are identified, genetic inducible fate mapping (GIFM) (Joyner & Zervas 2006) will be invaluable in following the movements of cells within each anchor point before, during, and after lobe formation.

The second stage of cerebellar folding, lobule formation, occurs during the postnatal period of massive GC proliferation in mouse. Differential rates of proliferation, with higher rates at the base of fissures, may contribute to this process (Mares & Lodin 1970). Work in our laboratory and others argues that the Shh pathway is critical for the degree to which foliation proceeds but not the position of the fissures and that Shh primarily regulates GC proliferation (Corrales et al. 2004, 2006; Lewis et al. 2004).

REGULATION OF GRANULE CELL PROLIFERATION BY Shh DETERMINES THE EXTENT OF FOLIATION

The massive GC proliferation and migration clearly are required for development of

GIFM: genetic inducible fate mapping

cerebellar folds because mutant mice with GC defects have a reduction or loss of folds. Furthermore, PCs are necessary for GC proliferation (Sidman et al. 1962, Caddy & Biscoe 1979, Wetts & Herrup 1982, Herrup 1983). PCs express the mitogen Shh from ~E17 onward, and this molecule with its associated signaling pathway is critical for GC proliferation (Corrales et al. 2004, 2006; Lewis et al. 2004). The first studies showed Shh to be capable of inducing proliferation of GCPs in culture, and the injection of Shh antibodies into the cerebellum reduces GC proliferation (Dahmane & Ruiz-i-Altaba 1999, Wallace 1999, Wechsler-Reya & Scott 1999).

Recently, the conditional inactivation of *Shh* in the mouse cerebellum showed the necessity of Shh for expansion of the GC precursor pool in vivo and also showed the requirement of Shh for cerebellar foliation (Lewis et al. 2004). Recently, our laboratory undertook a series of mouse genetics studies, using the Shh signaling pathway as an entry point into understanding the regulation of cerebellar foliation (Corrales et al. 2004, 2006). We selectively upregulated Shh signaling in the cerebellum, using a transgenic mouse line that overexpresses Shh and has a reduction in levels of the negative regulator Patched (Ptc). We also downregulated Shh signaling, using conditional mutagenesis of *Gli2* and *Smoothened* (*Smo*). The Gli2 transcription factor functions as the main activator of Shh-induced proliferation of GCPs (Corrales et al. 2006), whereas the Smo protein functions as an essential component of the receptor complex for Shh signaling (Murone et al. 1999). Increasing Shh levels via the use of a *Shh-P1* transgene produced a more complex foliation pattern, whereas progressively decreasing Shh levels resulted in a progressively simpler foliation pattern that reflected the normal stages of cerebellar foliation (Corrales et al. 2004, 2006). One interesting outcome of increasing Shh (*Shh-P1;Ptc*$^{+/-}$ mice) is the formation of an extra fold in the rostral aspect of lobule VI (Corrales et al. 2006). It is striking that this extra fold is both consistently observed in *Shh-P1;Ptc*$^{+/-}$ mice and found in a location similar to a fold in the normal rat cerebellum. Thus, Shh signaling from PCs appears to regulate a conserved genetic mechanism to achieve the correct number of folds in the cerebellum.

In support of the link between PC/GC interactions and cerebellar folding, genetic and experimental manipulations of the GCPs produce results similar to defects targeted to PCs. The selective partial elimination of GCPs in rat at late embryonic or early postnatal stages through the use of irradiation (Doughty et al. 1998) or hyperthyroidism (Lauder et al. 1974) also produces simpler foliation patterns. Strikingly, the patterns of foliation produced by these alterations mimic the patterns seen in our allelic series of Shh signaling–deficient mutant mice (Corrales et al. 2006). Conversely, hypothyroidism prolongs the presence of the EGL (and as a result prolongs the foliation process) and increases the final number of fissures (Lauder et al. 1974). Furthermore, in accordance with PC expression of Shh and the necessary interaction between PCs and GCs for foliation, mutant mice with PC defects (Sidman et al. 1962, Caddy & Biscoe 1979, Wetts & Herrup 1982, Herrup 1983) or specific ablation of PCs (Smeyne et al. 1995) also have simpler foliation patterns. For example, in *Staggerer* and *Lurcher* mutants, foliation is prematurely arrested, and the structure of the cerebellum is similar to *Gli2* mutants (Sidman et al. 1962, Caddy & Biscoe 1979, Wetts & Herrup 1982, Herrup 1983). In summary, the results obtained from in vivo genetic manipulations and spontaneous mouse mutants demonstrate that the pattern of foliation proceeds normally and arrests prematurely in register with the level of Shh signaling. This result suggests that Shh is a permissive factor, allowing foliation to proceed by stimulating GC proliferation. Consistent with this, Shh signaling appears to be evenly dispersed along the base and the crown of the developing lobules (Corrales et al. 2004). It is interesting to speculate what determines when GCs stop proliferating in different species and thus determines the

complexity of foliation. The evidence is thus accumulating in favor of a genetic basis for foliation dependent on Shh signaling (Inouye & Oda 1980; Neumann et al. 1990, 1993; Corrales et al. 2006). However, none of the mechanisms account for the conserved positions of fissures.

If the apparent precise genetic regulation of fold position and the tightly regulated developmental progress of each fold are considered, each fold may demarcate an AP coordinate in the 3-D structure of the cerebellum. Within each developing fold, a unique molecular program could then be initiated, depending on the specific coordinate in relation to other folds along the AP axis. The molecular program would serve as a second coordinate system, allowing the addition of further complexity to the cerebellum. One striking feature of the cerebellum, parasagittal molecular domains, can easily represent this potential second coordinate system.

DEVELOPMENT OF PARASAGITTAL ORGANIZATION IN THE CEREBELLUM

The organization of the cerebellum into adult parasagittal domains arises through a dynamic series of expression patterns in which homogeneous domains are replaced by parasagittal ones; alternatively, initial parasagittal domains are replaced by ubiquitous expression. During development, ZebrinII expression is first evident in the posterior lobe of the vermis at P5 and gradually spreads to all lobules by P12, so that almost all PCs express the gene at some level (Leclerc et al. 1988; Reviewed by Armstrong & Hawkes 2000). Parasagittal stripes become clear after P15 owing to the suppression of ZebrinII in specific PCs to eventually produce the mature parasagittal organization. Interestingly, Hsp25 expression in the cerebellum is first detected at approximately the time of birth and is localized to the anterior lobules within a series of parasagittal stripes (Armstrong et al. 2001). The pattern changes progressively over the first postnatal week such that the stripes are replaced by an entirely Hsp25-positive cerebellum. Finally, by P21 the adult pattern emerges to form distinct stripes of Hsp25 in the vermis of lobules VI–VII and IX–X (Armstrong et al. 2001).

The developmental expression of ZebrinII and Hsp25 is in contrast to other markers, such as Calbindin (Wassef et al. 1985), *L7/Pcp2* (Oberdick et al. 1993), and *En1/En2* (Millen et al. 1995), that are transiently expressed in subsets of PCs in the embryo. The embryonic ML parasagittal domains generally fall into two categories: those that overlap with the expression of *En1* and *En2* and those with complementary expression that overlap with the expression of *L7/Pcp2*. There are very few markers that are expressed in subsets of PCs throughout development and in the adult. One continuous marker of parasagittal stripes was generated by truncation of the enhancer of the *L7/Pcp2* gene and the creation of a transgenic mouse that expresses *lacZ* in parasagittal stripes throughout embryonic and postnatal cerebellar development (*L7βG3*) (Oberdick et al. 1993, Ozol et al. 1999). More recently, Neurogranin was found to label PC subsets from ~E17 to ~P20 (Larouche et al. 2006). The expression patterns of *L7βG3* (Ozol et al. 1999) and Neurogranin (Larouche et al. 2006) were both compared with that of ZebrinII. The overlap of ZebrinII expression with *L7βG3* and Neurogranin expression in the juvenile mouse cerebellum (~P15) suggests a transfer of ML patterning between the embryonic and adult cerebellum (reviewed by Larouche & Hawkes 2006). Although the basic pattern of ML stripes seems to be conserved from embryo to adult, it is difficult to know whether the same cells within a domain constitutively express a particular marker. GIFM now makes it possible to directly analyze the relation between embryonic and adult ML parasagittal domains.

An important question is, what regulates striped expression in the cerebellum? An intriguing proposal is that the time of PC birth

is directly linked to the ML positions of PCs. One study, using adenoviral vectors that apparently label PCs on the day they undergo terminal differentiation, showed that PCs subsequently migrate to ML positions reminiscent of *L7/Pcp2* or *En1/En2* domains in the embryo and ZebrinII stripes in the adult (Hashimoto & Mikoshiba 2003). The data from this study suggest that PCs born at a particular time have a tendency to migrate to and accumulate in similar ML positions. Although there is some evidence arguing against lineage restriction within cerebellum stripes (Hawkes et al. 1998, Lin & Cepko 1999), it is possible that PCs accumulate at specific ML locations soon after their birth and express particular genes within parasagittal stripes on the basis of their birthdates during embryogenesis and that the embryonic expression domains lead to the expression of adult genes that produce the organization of adult parasagittal domains. Finally, it seems highly likely that molecular coding is relevant to the overall function of the cerebellum.

By the superimposition of the internal molecular parasagittal organization on the external lobule architecture, the cerebellum can be thought of as a 3-D structure composed of two basic coordinates: ML parasagittal domains and AP lobules. Although experimental evidence suggests that the development of the two coordinate systems are not necessarily linked (Ozol et al. 1999, Sillitoe et al. 2003b), early developmental events clearly set in motion a process that produces the two coordinate systems. In terms of the genetic regulation of the coordinate systems, studies from our laboratory have shown a requirement for *En2* in the patterning of both foliation and ML coding (Joyner et al. 1991, Millen et al. 1994). Because the major afferents to the cerebellum from unique CNS locations terminate in specific ML locations within particular lobules, it is possible that cerebellar afferents use topographical cues reflected by the two coordinate systems to acquire their own precise spatial organization within the cerebellum. Thus, the ML molecular code and AP lobules may determine the general constraints of each functional circuit and preserve topography between the CNS and the cerebellum.

THE MAJOR CIRCUITS AND CONNECTIONS IN THE CEREBELLUM

The Major Afferent Projections to the Cerebellum

Three classes of afferents project to the cerebellum and provide a multitude of signals to all lobules. The first class, mossy fibers, originates from many locations in the brain and spinal cord, and the terminals of each axon are anatomically recognizable as rosettes located within the granular cell layer. The mossy fiber rosettes are one component of the glomerulus, which also includes GC dendritic boutons and Golgi cell axon terminals (Ito 1984). In the rodent brain, mossy fiber axons are sent to the cerebellum, primarily from the basilar pontine nuclei, vestibular nuclei, lateral reticular nuclei, and external cuneate nucleus. All levels of the rodent spinal cord also project mossy fibers to the cerebellum, primarily from Clarke's column and the dorsal-lateral part of lamina VII (spinal border cells) (Berretta et al. 1991). Depending on the source of mossy fibers, their termination within the cerebellum can be predominantly ipsilateral (e.g., cuneocerebellar) or contralateral (e.g., spinocerebellar), is restricted to particular lobules, and also forms ML domains. The fasciculated bundles of mossy fiber afferents enter the cerebellum through the superior (spinocerebellar), middle (pontocerebellar), or inferior cerebellar (spinocerebellar, cuneocerebellar, vestibulocerebellar, reticulocerebellar) peduncles (the fiber bundles connecting the cerebellum to the brainstem).

The second class of afferents, climbing fibers, originates solely from the various nuclei of the inferior olive, located within the medulla oblongata of the brainstem. Climbing fibers terminate only in the molecular layer of the mature cerebellar cortex, where

Inferior olive: a complex structure in the brainstem composed of several individual subnuclei that project climbing fibers only to the cerebellum

each climbing fiber interacts with the dendritic tree of one PC. However, a recent study shows that climbing fibers also contact NG2+ glia (glial progenitors in adult cerebellum) in the molecular layer, where each climbing fiber is able to contact multiple glial cells (Lin et al. 2005). Climbing fibers cross the midline in the brainstem, enter the cerebellum through the inferior cerebellar peduncle, and terminate contralaterally within the cerebellum. Afferents from each nucleus project to particular lobules and terminate in ML domains.

In addition to climbing fibers and mossy fibers, a third class of afferents projects to the cerebellum. This last class consists of a diffuse set of afferents, including noradrenergic afferents from the locus coeruleus (Abbott & Sotelo 2000), cholinergic afferents from the pedunculopontine nucleus (Jaarsma et al. 1997), and serotonergic afferents from the raphe nucleus (Strahlendorf & Hubbard 1983). The terminals of the third class of afferents are mapped within all layers of the cerebellar cortex and do not appear to be localized to particular lobules. It is unknown whether these afferents are restricted to specific ML parasagittal stripes in the cerebellum. It will be interesting to know whether this class of afferents has a similar ML organization within the cerebellum as mossy or climbing fibers.

In the adult cerebellum, mossy (Gravel & Hawkes 1990, Matsushita et al. 1991, Ji & Hawkes 1994, Voogd et al. 1996, Schonewille et al. 2006) and climbing fiber (Gravel et al. 1987, Wassef et al. 1992, Voogd et al. 2003) afferents terminate in stripes that are similar to the patterns set up by ZebrinII/Hsp25 PC stripes (**Figure 5**, **Figure 9**). Therefore, parasagittal domains in the cerebellum are highly complex and composed of multiple interacting cell types (**Figure 9**). The flow of information from mossy fiber to GC and from climbing fiber to PC can potentially occur within the limits of individual parasagittal stripes. Thus, the functional flow of information into the cerebellum is highly correlated to the relationship between the lobules and their underlying molecular maps.

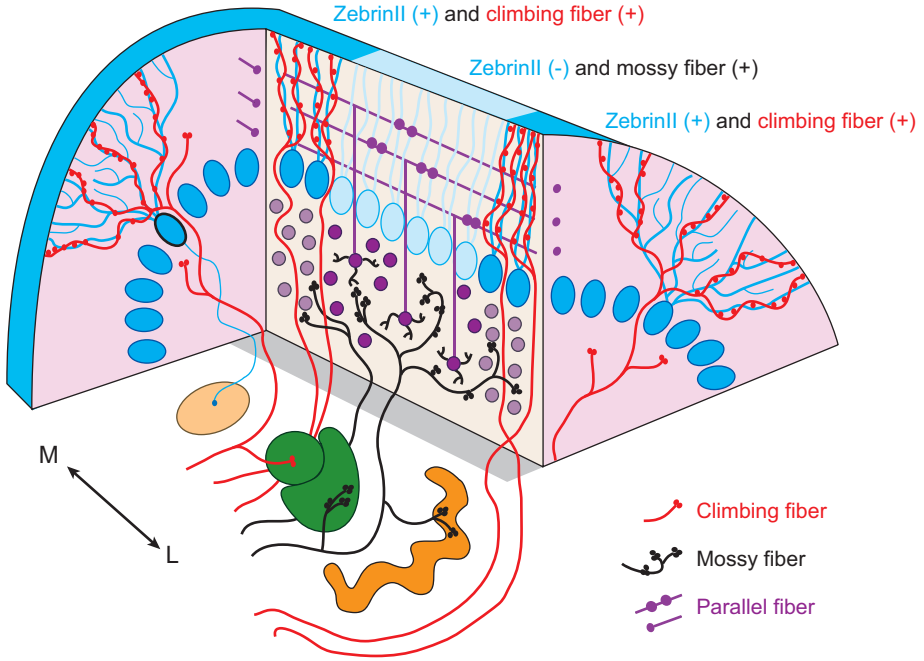

Figure 9

Mossy and climbing fiber terminal fields are organized into parasagittal domains that align with ZebrinII Purkinje cell (PC) parasagittal domains. Whereas mossy fibers (*black*) often extend into neighboring domains, climbing fibers (*red*) respect the limits of PC parasagittal domains. M, medial; L, lateral.

The Circuitry and General Neurochemistry within the Cerebellum

The GCs are the major excitatory cell type found in the cerebellum and use glutamate as their neurotransmitter (reviewed in Voogd et al. 1996); some DCN neurons and UBCs also use glutamate (Nunzi et al. 2001, Hoshino et al. 2005). All other neurons use the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Mossy fibers contact GCs directly within the glomerulus (Ito 1984). In turn, the GCs send their axons into the molecular layer, where they bifurcate as parallel fibers running along the ML axis and make synaptic contact with the spiny branches of multiple PC dendrites. The plane of the PC dendritic tree is perpendicular to the plane of GC parallel fibers (**Figures 2*b*** and **9**). However, there is also evidence for synaptic contacts between the ascending branch of the GC axon and the PC somatodendritic compartment (Gundappa-Sulur et al. 1999). One result of this organization is that a single mossy fiber may influence hundreds of PCs whereas only one PC is stimulated by input from a single climbing fiber (Altman & Bayer 1997).

Additionally, parallel fibers of GCs make excitatory contact with the dendrites of Golgi and stellate and basket cells. The Golgi cells make inhibitory contacts with the glomerulus and thus modulate the excitatory input of the mossy fibers. The basket and stellate cells inhibit the PCs directly via synapses on the cell somata and distal portions of the dendrites, respectively. The climbing fibers synapse directly with the proximal shafts of the PC dendrites. Thus, information from both afferent systems converges on the PCs, which integrate all incoming information. The PCs relay this information through inhibitory connections with the three pairs of DCN that reside in the medullary center as well as the medial and lateral vestibular nuclei, located in the brainstem. These projections are arranged topographically such that PCs in the vermis project to more medial nuclei and the vestibular nucleus in the brainstem, whereas projections from the hemispheres project to the more laterally positioned DCN (e.g., Hawkes & Leclerc 1986, Tabuchi et al. 1989, Paallysaho et al. 1990). Through the integration and modulation of afferent information, the cerebellum is able to play a major functional role in the regulation of fine motor control, sensory-motor learning, memory, and perhaps cognition (e.g., Kim & Thompson 1997, Allin et al. 2001).

Synapse: the junction between two neurons where chemical signals are translated into electrical signals, providing a means of communication between neurons

THE ARRIVAL OF PROJECTIONS IN THE CEREBELLUM AND THE DEVELOPMENT OF CIRCUIT MAPS

Afferents originating from specific locations in the CNS terminate within specific AP locations within the developing cerebellum, and these locations later correlate with specific lobules. Furthermore, each afferent subset terminates within specific ML parasagittal domains that bear a consistent relationship to the organization of PC parasagittal molecular domains. Thus, to establish their own organization, afferents likely use positional and molecular cues set up by the two major coordinate systems of the cerebellum.

In vitro 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI) retrograde tracing in fixed embryonic rat tissue has shown that the arrival of afferents to the cerebellum spans from the mid-embryonic to early postnatal periods (Ashwell & Zhang 1992). On the basis of this study, mossy fibers from the vestibular ganglion are the first mossy fibers to arrive in the rat cerebellum and are present in the anlage by E13 (Ashwell & Zhang 1992). The next mossy fiber subsets to arrive are from the vestibular nuclei and spinal cord at E15, and these are followed by climbing fibers from the inferior olive at E17 (Ashwell & Zhang 1992). Consistently, in vitro anterograde tracing experiments in fetal mice labeled spinocerebellar mossy fibers in the cerebellum as early as E13/E14 (Grishkat & Eisenman 1995)

and climbing fibers at E14/E15 (Paradies & Eisenman 1993). Finally, at P0 in rat, mossy fibers originating from the lateral reticular nucleus and the pontine nuclei reach the cerebellum (Ashwell & Zhang 1992). The arrival of mossy fibers from nuclei such as the external cuneate nuclei was never labeled in the Ashwell & Zhang (1992) study, raising the possibility that mossy fibers from these nuclei reach the cerebellum during early postnatal development.

Although the retrograde labeling studies by Ashwell & Zhang (1992) provide details regarding the sequential arrival of afferents to the cerebellum, these studies did not address whether the initial organization of afferents correlates with the molecular coding inherent to the early cerebellum. Elegant studies by Constatino Sotelo and colleagues used the chick as a model system to test whether the development of cerebellar afferent organization, and in particular that of the climbing fiber afferents, is dependent on cues provided by the cerebellum, or vice versa (Chedotal et al. 1997; reviewed in Sotelo 2004, Sotelo & Chedotal 2005).

Cerebellum mossy fibers contact GC dendrites in the GC layer in the adult. However, the scenario in the embryo is different: Mossy fibers form transient contacts with developing PCs (Mason & Gregory 1984, Manzini et al. 2006). The transient interaction between PCs and mossy fibers and the analysis of mutant mice with correlating disruptions in PC and spinocerebellar topography have suggested that mossy fiber–PC interactions are critical for the segregation of afferents into parasagittal stripes (Arsenio Nunes et al. 1988, Sotelo & Wassef 1991, Ji & Hawkes 1995). However, because only spinocerebellar organization has been analyzed in mutants with PC defects, it is difficult to conclude that PC domains affect all mossy fibers equally. Furthermore, the mossy fiber afferents do not partition into ML domains until after birth (Arsenio Nunes & Sotelo 1985; R.V. Sillitoe & A.L. Joyner, unpublished data).

Unlike mossy fibers, climbing fibers are organized into parasagittal stripes by late embryogenesis in the rat and mouse (Sotelo et al. 1984, Chedotal & Sotelo 1992, Paradies & Eisenman 1993, Paradies et al. 1996). Furthermore, climbing fiber parasagittal stripes have a rudimentary parasagittal organization by E15/16 in mice (Paradies & Eisenman 1993), a stage soon after the initial expression of several PC parasagittal markers (e.g., *En1/2* and *L7/Pcp2*). By E17 in mice, PC molecular coding and olivocerebellar topography show a strong correspondence (Paradies et al. 1996). Thus, PCs may influence the ML topography of climbing fibers, and in turn the afferents may influence ML molecular coding.

The work of Sotelo and collaborators provided evidence for bidirectional signaling in the olivocerebellar map. Using antibodies that stain embryonic PC subsets (Calbindin, GMP-cyclic dependent protein kinase, PC-specific glycoprotein, and PEP-19) and specific subnuclei in the inferior olive and their afferents [Calbindin, Parvalbumin, and Calcitonin gene-related peptide (CGRP)], Sotelo and colleagues postulated that gene expression domains match between the cerebellum and inferior olive. On the basis of matching positional cues shared between the afferents and their targets, these researchers hypothesize that the identities of inferior olivary subsets match specific PC subsets within the cerebellum, allowing the formation of a precise topographical projection map (Sotelo & Chedotal 2005).

There is also evidence in support of intrinsic ML spatial cues in the cerebellum, such as the initiation of *L7/Pcp2* ML gene expression with a normal pattern (albeit delayed) in organ cultures derived from E14 cerebella (Oberdick et al. 1993). In addition, Zebrin-positive and Zebrin-negative PCs develop in the absence of afferent inputs (Wassef et al. 1990), and in *En2* mutants the embryonic and adult parasagittal domains are altered (Millen et al. 1995, Kuemerle et al. 1997). The cell-autonomous regulation of PC parasagittal domains provides critical evidence in support of

the idea of intrinsic control during the formation of the cerebellar topographical map.

Using a chick in vitro explant system, Nishida and coworkers (2002) provided compelling evidence for a possible molecular pathway regulating afferent patterning. In both chick and mouse embryonic cerebella, members of the *Eph/Ephrin* gene family are expressed in ML parasagittal domains (Karam et al. 2000, 2002). Overexpression of *Ephrin-A2* in the chick cerebellum via the use of retroviral vectors disrupts the topography of the olivocerebellar projection. Inferior olivary axons expressing high Eph receptor activity are prevented from entering into domains ectopically expressing Ephrin-A2 ligand (Nishida et al. 2002). These experiments show that the Eph/Ephrin signaling pathway may provide positional information during afferent/efferent matching, and demonstrate that repulsive signals can play a key role during cerebellar map formation. In addition to *En2* regulation of the patterning of foliation and ML molecular codes, there is also evidence that *En2* regulates circuitry because $En2^{-/-}$ mice have mild defects in the parasagittal organization of the spinocerebellar mossy fibers that project to lobule VIII (Vogel et al. 1996). In addition, ectopic expression of *En2* disrupts the parasagittal spinocerebellar mossy fiber pattern in lobule VIII (Baader et al. 1999). It may thus be significant that the En transcriptional repressors are expressed in complementary domains to EphA4 in the embryo (Y. Chan & A.L. Joyner, unpublished results). Although *En1* and *En2* are candidate regulators of afferent ML patterning, we are still lacking an in-depth understanding of how early prepatterns in the cerebellum translate into adult patterns and how cellular interactions during development shape circuit architecture.

It is not clear what determines the specificity of climbing and mossy fiber projections to particular lobules in the AP axis. Nevertheless, Sotelo's group used chick brain explant cultures (reviewed in Sotelo 2004) to show that the embryonic cerebellum has cues that direct climbing fibers from different regions of the inferior olive to different AP positions immediately on entering the cerebellar primordium (Chedotal et al. 1997). Strikingly, if the AP axis of the cerebellar primordium is reversed, then the projection map is reversed (Chedotal et al. 1997). Furthermore, if the anterior cerebellum is removed, the map appears to be compressed, whereas if the posterior cerebellar primordium is removed, the climbing fibers that normally enter this region do not project into the remaining anterior cerebellar primordium (Chedotal et al. 1997). Thus, it is possible that the initial coarse circuitry map of the afferents along the AP axis is determined by spatial cues provided by the cerebellum. Furthermore, Sotelo has proposed that the En transcription factors may play a role in setting up these cues (reviewed in Sotelo 2004).

DEVELOPMENTAL DISORDERS AND POSSIBLE CIRCUIT FORMATION ABNORMALITIES

The functional roles of lobules, as well as parasagittal molecular domains and topographical circuitry organization, are still speculative. It is clear that a massive amount of sensory information is channeled to the cerebellum via multiple afferent pathways. Each pathway likely carries several distinct sets of sensory information, and thus the cerebellum may process information in parallel. Besides the obvious difference in function based on the source of each afferent projection, it is not clear whether each lobule-specific stripe processes information in a particular manner. It is interesting to speculate that specific insults to the coordinate systems during development may result in altered connections at multiple levels of the cerebellar circuit. The changes in synaptic interactions would either impede the correct integration of signals delivered to each PC parasagittal domain or induce compensatory circuit modifications and thereafter diminish the efficacy of the cerebellar response.

Obvious cytological or morphological defects in the cerebellum often result in motor abnormities such as ataxia. However, there may be diseases that affect the cerebellum but do not show obvious pathology, similar to *En2* mutant mice in which morphological defects are subtle. Nevertheless, ML coding may be affected in such diseases and lead to severe circuitry defects. Strikingly, studies have implicated the cerebellum as one structure affected in autism spectrum disorder (ASD) (Dum & Strick 2006, Kuemerle et al. 2007), and recently it was shown that mutations in the human *ENGRAILED2* gene are associated with ASD susceptibility (Gharani et al. 2004). The unique gene expression domains of PCs and the specific mossy and climbing fiber termination patterns found in each lobule offer a potential entry point into understanding how developmental neurological disorders affect the human cerebellum. The alteration of precise cerebellar circuits during development may potentially have a physiological impact in ASD. With the appropriate sophisticated genetic tools now available, it will be important to deconstruct the circuitry of the cerebellum and its related areas in animal models to understand fully the implications for specific genetic brain disorders. We propose that the two coordinate systems of the cerebellum, lobules in the AP axis and parasagittal molecular domains in the ML axis, underlie the functional framework of the cerebellum. A clear understanding of how gene networks set up the coordinates and the degree to which they shape the development of circuits, and perhaps more importantly the modularity of circuits, will shed critical light on their impact on complex neurological diseases of the cerebellum.

SUMMARY POINTS

1. The cerebellum is organized into a two-coordinate system composed of anatomical and molecular components. The anterior-posterior (AP) locations of the lobules constitute the first coordinate system. The Purkinje cell medial-lateral (ML) molecular code constitutes the second coordinate system. During development, the topography of the cerebellar global circuitry is likely guided by the two coordinate systems.
2. The early patterning of the cerebellar anlage is established through complex bidirectional signaling cascades that include molecules that are secreted by the isthmic organizer, such as fibroblast growth factor 8, and downstream transcription factors like Engrailed.
3. The embryonic cerebellum is organized into a ML molecular code that shares a common topography with the ML code in the adult cerebellum.
4. The recent development in mouse of sophisticated genetic tools such as genetic inducible fate mapping (GIFM) has provided a means for analyzing the origins of different cerebellar cell types and their migratory routes and physiology in more detail.
5. The Sonic hedgehog (Shh) signaling pathway is critical for granule cell proliferation and as a consequence plays a role in establishing the correct number of folia in the postnatal cerebellum.
6. The position of folia is genetically determined, and creating the base of fissures may involve coordinated cell behaviors.
7. Afferents from different locations in the central nervous system reach the cerebellum sequentially during embryonic and perinatal development, project to particular lobules along the AP axis, and segregate into parasagittal domains.

FUTURE ISSUES

1. Genetic pathways that determine the position of fissures, as well as those that act cooperatively with the Shh signaling pathway, need to be identified to fully appreciate the temporal and spatial details of cerebellum lobule morphogenesis.
2. The genetic pathways and cell behaviors that establish parasagittal molecular domains in the embryo constitute a large void in our understanding. Importantly, the function of parasagittal molecular domains in the embryonic and adult cerebellum also needs to be deciphered.
3. There is a need for improved methods for detecting axons and their associated terminals with high resolution to analyze carefully the development of circuits in the cerebellum. The availability of genetically encoded neuronal tracers like GFP has sparked an initiative to further develop and refine the existing tracers for more sophisticated uses in mouse.
4. The molecules that regulate the guidance of axon projections to the cerebellum and those that pattern afferents along the AP and ML axis need to be identified.
5. Perhaps the most exciting and important discoveries now within our grasp involve identifying the cerebellar genes and molecules that are affected in developmental neurological disorders and defining the cellular behaviors that lead to adverse symptoms.

DISCLOSURE STATEMENT

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

ACKNOWLEDGMENTS

We are grateful to all members of the Joyner lab for many stimulating discussions and to Charles Levine for critical reading of an earlier version of the manuscript. RVS received support from the Alberta Heritage Foundation for Medical Research (AHFMR). ALJ is supported by grants from the NIH (HD35768 and HD50767) and Autism Speaks.

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