Development of Cerebellar Nuclei

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Abstract

The cerebellum is a foliated structure consisting of the laminated cerebellar cortex and a paired set of bilateral cerebellar nuclei (CN) located in the deep white matter adjacent to the roof of the fourth ventricle. The CN are comprised of multiple

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neuron types including large glutamatergic projection neurons, GABAergic projection neurons, and small GABAergic interneurons. Hodologically, CN receive afferent projections from Purkinje cells and give rise to major cerebellar output tracts. The developmental origins of CN have long been debated, although the consensus of evidence now indicates that different GABAergic (inhibitory) and glutamatergic (excitatory) neuronal populations are derived by sequential neurogenesis from distinct progenitor compartments. Each type of neuronal population is born at different times and follows a unique migratory route. The molecular mechanisms regulating CN neurogenesis, cellular migration, and axonal guidance of the efferent pathways are now being elucidated. This chapter highlights recent advances in embryonic cerebellar development, focusing on the development of CN and their connections and on molecular mechanisms underlying their development. Mouse mutant phenotypes involving the CN, as well as human malformations affecting CN morphology, illustrate the importance of CN in cerebellar function and pathology.

Keywords

Nuclear transitory zone · Tbr1 · Autism · Tbr2

Introduction

The cerebellum has been implicated in motor control (Ito 1984), cognition (Leiner et al. 1986; Schmahmann 1996), and visceral functions (Haines et al. 1990). These distinct functions are exerted through the connections from the cerebellar cortical output via the cerebellar nuclei (CN) onto a large number of different thalamic and brainstem nuclei (Voogd 1995). The CN receive extensive input from Purkinje cells, which are organized into parallel, parasagittally arrayed modules correlated with distinct molecular expression profiles (Armstrong and Hawkes 2000). In turn, the Purkinje cell modules receive different afferent projections from precerebellar nuclei (mainly the inferior olives and the pons), likewise organized into parasagittal axon terminal fields (Sillitoe and Joyner 2007). The CN basically represent the output of these modular pathways (Voogd 1995), and this may be reflected in segmental organization of some CN nuclei, especially the dentate nucleus. The CN integrate diverse neurotransmitter signals. These include glutamatergic, serotonergic, noradrenergic, and cholinergic excitatory input from mossy and climbing fiber collaterals and GABAergic inhibitory input from Purkinje cells (Voogd 1995; Jaarsma et al. 1997). Classical anatomical studies have identified four major subdivisions of the CN in rodents: the medial (fastigial in humans), anterior and posterior interposed (emboliform and globose, respectively), and lateral (dentate) nuclei. Anterograde tracing and degeneration studies demonstrated a basic arrangement of cortico-nuclear connections in which medial nucleus receives input from Purkinje cells from the vermis, interpositus nucleus receives input from the intermediate hemisphere, and lateral nucleus from the lateral hemispheres (Armstrong and Schild 1978a, b).

Although CN are central to cerebellar circuitry and function, surprisingly they received very little attention both regarding their embryonic development and functional importance. Older and current textbooks provide a very simplified description of CN in terms of neuronal subtypes and electrophysiological examinations, but recent studies have dramatically changed that view. The CN are comprised of several types of neurons: large glutamatergic neurons providing excitatory drive to various regions of the forebrain, brainstem, and spinal cord, medium-size GABAergic nucleo-olivary projection neurons providing inhibition to the inferior olive (Mugnaini 1985; Ruigrok 1997), and GABAergic and glycinergic interneurons providing local inhibition (Uusisaari et al. 2007; Uusisaari and Knopfel 2008). In addition, glycinergic neurons might also provide direct inhibitory feedback to the cerebellar cortex (Uusisaari and Knopfel 2010) or feed-forward inhibition to the ipsilateral brainstem nuclei (Bagnall et al. 2009).

This chapter will highlight the contributions of molecular biology and mouse genetics to enhance our knowledge of CN development. Recent studies have drastically altered our views of neuronal subtype specification, differentiation, migration, and integration into specific circuits. For example, it has been shown that neurogenesis in the cerebellum, as in some other structures such as the forebrain, is compartmentalized according to neurotransmitter fate. Specifically, glutamatergic and GABAergic neurons are produced from distinct germinal regions, known as the rhombic lip (RL) and the ventricular zone (VZ). The VZ is a neuroepithelial zone that lines the dorsolateral aspect of the fourth ventricle and is marked by the early expression of bHLH transcription factor (TF) *Ptf1a* (Hoshino et al. 2005; Pascual et al. 2007). In contrast, the cerebellar RL is a unique proliferative region that forms at the interface between the roof plate (dorsal midline) of the fourth ventricle and the adjacent neuroepithelium (Wingate 2001) and is marked by the expression of the bHLH TF *Math1* (*Atoh1*) (Ben-Arie et al. 1997).

Overview of Cerebellar Development

Recent studies have focused on understanding the molecular and cellular mechanisms underlying cerebellar neurogenesis, cellular migration, axonal guidance, dendritogenesis, and integration into functional circuits in various model systems. Given the high degree of conservation of cerebellar anatomy between mice and humans, knowledge of the developmental mechanisms of these processes in mouse will ultimately reveal new insights into the developmental basis of malformations in humans.

Patterning and Morphogenesis

The adult cerebellum is a bilateral foliated structure morphologically divided into a central vermis flanked by the intermediate and lateral hemispheres. Early during embryonic development, around embryonic (E) 9 in mouse, the cerebellum is

induced from the dorsal region of rhombomere (r) 1 of the anterior hindbrain (Sotelo 2004). Fate mapping, molecular expression studies, and mutant mouse analysis have shown that the isthmic organizer, located at the junction between the midbrain and hindbrain, produces molecules that have been shown to be important setting up the early regional pattern of the cerebellar anlage (Sotelo 2004; Sgaier et al. 2005). The combination of precise spatial and temporal activation of secreted molecules, such as Fgf8 and Wnt1, and transcription factors, such as Otx2, Gbx2, Pax genes, and Lmx1b, has been shown to be necessary and sufficient for setting up the cerebellar territory (Sillitoe and Joyner 2007). In addition, recent studies revealed the developing cerebellum is also patterned by molecules produced from the adjacent roof plate, which produces BMP and Wnts factors (Chizhikov et al. 2006, 2010). Further morphogenesis events during cerebellar development involve a 90° rotation of the cerebellar anlage converting the rostro-caudal axis into a medial-lateral axis leading to the formation of the bilateral wing-like cerebellar primordium by E12.5 in mouse (Sgaier et al. 2005). This morphogenetic event is followed by growth of the dorsal bilateral primordium to form the central vermis and lateral hemispheres (Louvi et al. 2003). At this early stage, the cerebellar anlage comprises two spatially and molecularly distinct germinative regions that give rise to all the cells of the cerebellum: VZ of the cerebellar plate and the upper (or cerebellar) RL.

Cerebellar Neuron Subtypes Are Produced Sequentially

Genetic fate mapping and targeted mutation in mice are modern methods that have contributed tremendously to the analysis of cell-fate determination, lineage specificity of progenitor compartments, and neuronal migration and final positioning in the adult cerebellum. The genesis of CN neurons is an integral part of overall cerebellar neurogenesis, in which a multitude of neuron types are produced. The majority are either glutamatergic excitatory neurons or GABAergic inhibitory neurons, in addition to some glycinergic neurons (Uusisaari et al. 2007). GABAergic neurons of the cerebellar cortex include stellate and basket cells in the molecular layer, Purkinje cells with their somata in the Purkinje cell layer, and Golgi cells in the granule cell layer. Cortical glutamatergic interneuron types include granule neurons (the most abundant neurons in the brain) and unipolar brush cells (UBCs), both located in the granule cell layer (Carletti and Rossi 2008).

Classical birthdating studies and recent genetic fate mapping have shown that all the cells that make up the adult cerebellum are generated sequentially from either the VZ, which gives rise to all inhibitory neurons, both GABAergic and possibly glycinergic cells, or from the upper RL, which gives rise to all cerebellar excitatory glutamatergic neurons. Consensus exists that the first neurons to be produced in the embryonic cerebellum in mice are the glutamatergic and GABAergic CN projection neurons (E10.5–E12.5), followed shortly by GABAergic Purkinje cell projection neurons (E11.5–E13.5) (Miale and Sidman 1961; Machold and Fishell 2005; Wang et al. 2005; Fink et al. 2006; Leto et al. 2006). GABAergic interneurons, including a subpopulation of CN interneurons, basket, stellate, and Golgi cells, arise from a

common VZ progenitor Pax2+ subpopulation that proliferate within the white matter as they migrate and differentiate from E13.5 to P15. After the generation of CN neurons from E10 to E12.5, the cerebellar RL gives rise sequentially at E13.5 to granule cell precursors forming the EGL, which will occupy the anterior cerebellum and later around E15.5 by granule cell precursors which will populate the posterior cerebellum (Machold and Fishell 2005; Wang et al. 2005). Granule cells, which are the most abundant neuron type in the brain, exit the cell cycle in the EGL in the first 2–3 postnatal weeks and migrate inwardly to position themselves in the IGL (Miale and Sidman 1961). Concurrently with granule cell precursors, UBCs are produced from E14.5 to early postnatal days (Englund et al. 2006). UBCs differentiate into three chemically defined UBC subtypes and migrate through cerebellar white matter to the IGL (Sekerkova et al. 2004; Englund et al. 2006). The cerebellar RL remains as an active progenitor compartment till early postnatal life, while the VZ, depleted of progenitors, involutes at late embryonic stages (Chizhikov et al. 2010) (Fig. 1).

Development of the Cerebellar Nuclei in Mammals

In mammals, four bilateral CN are distinguished: the medial (fastigial), posterior interposed (globose), anterior interposed (emboliform), and lateral (dentate) nuclei (Korneliussen 1968; Chan-Palay 1977). The CN each contain three main categories of neurons that differ from each other in size, molecular signature, neurotransmitter phenotype, and connectivity (de Zeeuw and Berrebi 1995). These are (1) large nucleofugal glutamatergic neurons that project contralaterally to diverse brain regions outside the cerebellum, (2) small- to medium-sized GABAergic nucleo-olivary projection neurons providing feedback to the inferior olive, and (3) small local GABAergic interneurons (Chan-Palay 1977; Fredette and Mugnaini 1991; de Zeeuw and Berrebi 1995). Other neuron types have been identified recently, notably large glycinergic projection neurons located primarily in the medial/fastigial nucleus projecting to the ipsilateral brainstem, and small local glycinergic neurons dispersed throughout the CN are also part of the neuronal circuitry of the CN (Helms et al. 2001; Uusisaari et al. 2007; Bagnall et al. 2009; Chung et al. 2009). Until the current millennium, it was thought that neurons of the CN and Purkinje cells arise from the VZ, while the rest of the cerebellar neurons arise from the EGL (Carletti and Rossi 2008). Recent studies challenged this view and showed that the cerebellar primordium is compartmentalized into a *Ptf1a*+VZ that gives rise to GABAergic cerebellar neurons and a *Math1*+ RL that gives rise to glutamatergic neurons.

Cerebellar Morphogenesis Leading to the Formation of CN

As stated previously, the first cerebellar neurons to be generated are the projections glutamatergic and GABAergic CN located deep in the white matter, followed shortly by the production of Purkinje cells (Miale and Sidman 1961; Pierce 1975; Altman and Bayer 1978; Leto et al. 2006). Based on early birthdating experiments in rats,

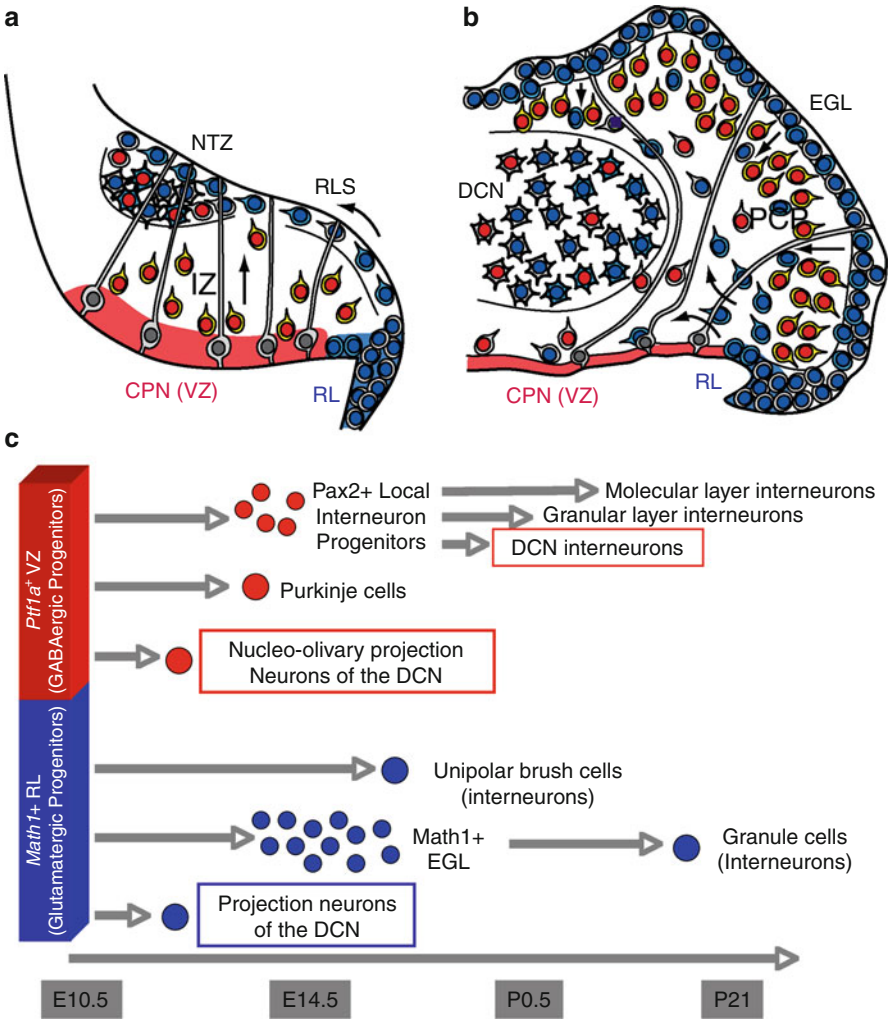

Fig. 1 Neurogenesis in cerebellar development. (**a**, **b**) Diagrams showing schematic views of the developing cerebellum in sagittal sections through the vermis, oriented with rostral to the left and dorsal to the top and (**c**) schematic illustrating cerebellar neurogenesis. (**a**) Early stage of cerebellar development (mouse E13.5) showing that cells derived from the upper rhombic lip (*RL*) (*blue nuclei*) migrate nonradially through the rostral rhombic lip migratory stream (*RLS*) to the nuclear transitory zone (*NTZ*). At the same time, Purkinje cells (*red nuclei*) migrate radially from the ventricular zone (*VZ*) of the cerebellar plate neuroepithelium (*CPN*) along radial glial cells (*gray*) through the intermediate zone (*IZ*), toward the RLS and NTZ. Other GABAergic neurons are not depicted in this model. (**b**) At later stages of embryonic cerebellar development (mouse E17.5), the Purkinje cell plate (*PCP*) has formed, and the external granular layer has replaced the RLS. Cells from the EGL migrate radially inward through the PCP, while unipolar brush cells (*UBCs*) migrate directly from the RL into the IZ. The deep cerebellar nuclei (*DCN*) contain glutamatergic neurons derived from the NTZ (*blue*) that have migrated radially inward toward the VZ settling underneath the white matter and GABAergic neurons (*red*) derived from the VZ. (**c**) Within the cerebellar

Altman and Bayer (1978, 1985a) describe the migratory path of neurons of the CN, in which they report the formation of a superficial migratory band extending from the cerebellar RL (or lateral cerebellar primordium) toward a more medial nuclear transitory zone (NTZ) locations. This subpial stream into the superficially located NTZ appears to correspond to rostral rhombic lip migratory stream (RLS) (Altman and Bayer 1985a, b, c). The subsequent radial translocation of neurons from the NTZ (subpial) to the CN (adjacent to the fourth ventricular surface) was thought to be guided by penetrating afferent fibers of the inferior cerebellar peduncle (Altman and Bayer 1985b). These birthdating studies also suggested that the CN neuronal precursors begin to send axons before exiting the NTZ, by E15 in rat. The CN neuronal precursors begin their descent from NTZ to CN shortly after E16 in rat (E14 in mouse). Altman and Bayer (1985b) called this movement "translocation" to distinguish it from the initial mode of transverse subpial migration toward the NTZ. However, it remains unclear whether the CN neurons migrate radially toward deeper positions or are displaced due to other morphogenetic movements that shape the cerebellum, such as Purkinje cell migrations. Likewise, it remains unclear whether CN neurons make contact with radially migrating Purkinje cells or whether Purkinje cells may actually make a detour around the descending CN neurons when migrating radially toward the forming cerebellar cortex.

Formation of Deep Cerebellar Neurons: Differentiation, Cellular Migration, and Transcription Factor Expression

Together with events such as progenitor proliferation, differentiation, and survival, processes of cell migration bring major morphogenetic transformations during cerebellar development. Different migratory routes and distinct cellular substrates have been distinguished during cerebellar development in studies of multiple model organisms. These studies have revealed that cellular migration relies on either cell-cell interaction (neuron-neuron or neuron-radial glial interaction) or by production of extracellular factors, which guides their migration. Whereas the migratory pathways of glutamatergic CN neuron precursors from the cerebellar RL to NTZ have been

Fig. 1 (continued) primordium, distinct progenitors give rise to glutamatergic and GABAergic neurons. Math1+URL (*blue*) gives rise sequentially to glutamatergic projection neurons of the DCN, EGL progenitors that will produce granule cell interneurons of the internal granular layer (*IGL*), and UBCs that populate the IGL. Ptf1a+VZ (*red*) gives rise sequentially to GABAergic nucleo-olivary projection neurons of the DCN, Purkinje cells (projection neurons), and GABAergic interneurons arising from a Pax2+ progenitor pool that generates GABAergic interneurons of the DCN, granular layer, and molecular layer. The DCN is therefore a heterogeneous population of neurons (*rectangles*) derived from different germinal zones, comprising glutamatergic projection neurons, GABAergic nucleo-olivary projection neurons, and GABAergic interneurons. Developmental timeline is indicated at the bottom

somewhat studied, very little is known about the molecular determinants underlying the specific routes, modes, and dynamics of this migration process. Likewise, the migration and cues for migration of VZ-derived GABAergic CN neurons have not been extensively studied.

Differentiation and Migration of CN GABAergic Neurons

Classical birthdating studies using radiolabeled thymidine indicated that the first GABAergic neurons to be born are the Purkinje cells, generated between E11 and E13 in mice (Miale and Sidman 1961). More recently, BrdU birthdating showed that GABAergic nucleo-olivary projection neurons are born slightly before and for the most part overlap with Purkinje cells production, from E13 to E15 in rats (E11–E13 in mice) (Leto et al. 2006). Nothing is known about the position and molecular profile of the GABAergic nucleo-olivary projection neurons, and whether they initially migrate into the NTZ, but they are thought to originate from Ptf1a+VZ. Since both glutamatergic and GABAergic projection neurons are born early, it is conceivable that they migrate to a common zone (NTZ) before they descend into deeper positions and may be marked by Lhx2/9 (glutamatergic neurons), IRX3, and Meis2 (GABAergic neurons) (Morales and Hatten 2006). VZ-derived Purkinje cells became postmitotic between E11 and E13 in mouse and settle under the NTZ, as revealed by the expression of Lhx1/5 and the GTPase-activating protein RGC8 (Morales and Hatten 2006). As Purkinje cells migrate radially along Bergmann glia cells to settle beneath the newly forming EGL (around E14.5–E17.5), they start expressing calbindin, a marker associated with Purkinje cells in adult cerebellum. As the generation of GABAergic projection neurons (nucleo-olivary and Purkinje cells) reaches completion at E13.5 in mouse, GABAergic interneurons are produced next, during late embryonic and early postnatal development. The GABAergic neurons are produced according to an inside-out sequence, with CN interneurons produced first, followed by granule layer interneurons, and finally molecular layer interneurons (Carletti and Rossi 2008). Retroviral tracing, used to map lineages of VZ-derived progenitors in the adult cerebellum, indicated that Golgi cells, basket neurons, and stellate cells originate from a common VZ-derived pool of mitotic progenitors dispersed in the white matter as they migrate during late embryonic and early postnatal development (Zhang and Goldman 1996). Another approach to clonal analysis, using genetic recombination to randomly label single cells using a modified *LaacZ/LacZ* reporter, suggested that cortical interneurons, Purkinje cells, and CN neurons (presumably those comprising the GABAergic projection neurons and interneurons) share a clonal origin, indicating a common VZ-derived progenitor pool that gives rise to all GABAergic cell types (Mathis et al. 1997; Mathis and Nicolas 2003). It is important to note that the histochemical phenotype of CN neurons was not characterized in these lineage-tracing studies. Later studies analyzing the expression of transcription factor Pax2 allowed Maricich and Herrup (1999) to distinguish a population of VZ progenitors at E13.5 that subsequently spreads and divides through the cerebellar white matter (Maricich and Herrup 1999). These progenitors expressed GABAergic phenotypes and give rise to both the CN and the cerebellar cortex, suggesting that GABAergic

interneurons are generated from a common progenitor pool, different from those that produce cerebellar GABAergic projection neurons. Using Pax2-EGFP transgenic mice, a recent report further confirms these findings (Weisheit et al. 2006).

To investigate the mechanism underlying the generation of different cerebellar GABAergic interneurons, recent heterochronic and heterotopic transplantation studies have shown that GABAergic interneuron precursors are not intrinsically determined to give rise to specific interneuron subtypes but instead maintain full developmental potential until late stages and adopt mature identities in response to local unidentified yet instructive cues (Leto et al. 2006, 2009; Carletti and Rossi 2008). Whereas Ptf1a and Pax2 emerge as key regulators in GABAergic lineages, further molecular interactions that regulate GABAergic subtype specification and the mechanisms of their migration are largely unknown. It is unclear whether different combinations of TFs are expressed in different VZ progenitor pools, which are in turn related to subtype specificity (Morales and Hatten 2006). The specific inductive signals that specify progenitor subpopulations have not been identified, although some evidence exists that roof plate secretes BMP and Wnt signals that might regulate progenitor phenotype (Chizhikov et al. 2006). The specific inductive signals that trigger the emergence of the first wave of migration from the VZ of the GABAergic nucleo-olivary projection neurons are not known. In addition, cues and mechanisms that lead to the generation and migration of small GABAergic interneurons of the CN are also not known.

Differentiation and Migration of CN Glutamatergic Neurons

Until recently, the large glutamatergic projection neurons of the CN were thought to be generated in the VZ from a common progenitor pool that also gave rise to Purkinje cells (Carletti and Rossi 2008). Korneliussen (1967) raised the possibility that some CN may originate from the RL (Korneliussen 1967), but this idea did not gain experimental support until the most recent decade. Studies using clonal lineage analysis showed that CN neurons may be produced from separate progenitors than those generating Purkinje cells, but their origin was nevertheless attributed to the VZ (Mathis and Nicolas 2003). In the last 10 years, genetic lineage-tracing analysis and organotypic slice assays suggested that most CN projection neurons, including Tbr1+ glutamatergic neurons, are produced from Math1+ progenitors in the RL (Machold and Fishell 2005; Wang et al. 2005; Fink et al. 2006). The cerebellar RL is a remarkable proliferative region that produces sequentially specific hindbrain nuclei, CN glutamatergic projection neurons, UBCs, and granule cell precursors in a temporally controlled manner by an ongoing production of *Math1* in the cerebellar RL (Wingate 2005).

To define the RL and the neurons that are derived from it, Wang et al. (2005) used a *LacZ* reporter gene targeted to the *Math1* locus (Ben-Arie et al. 1997), tagging the cells with β-galactosidase (β-gal) protein and tracking their migration for several days, while β-gal expression persisted in the daughter cells despite *Math-1* downregulation. β-gal expression was detected as early as E11.5 in the developing rostral RL, and positive cells entered the RLS. In the absence of Math1

due to genetic mutation, the first wave of neuronal precursors that make up the RLS did not form. This first wave gave rise to neurons that migrated to extracerebellar nuclei (PMT, LL, and parabrachial nuclei), indicating that these nuclei are derived from the upper RL, migrate through the RLS, and are Math-1 dependent. At E11.5 the RLS covers the entire surface of the developing cerebellum, and from E12.5 to E14.5 β-gal+cells accumulate in the rostral cerebellum, including the NTZ, thought to represent a transient differentiation zone for cells destined to become CN neurons (Altman and Bayer 1985b). From E14.5 to E16.5, β-gal+cellular aggregate descended from the NTZ into the deep cerebellum, as expected for CN. During this time the caudal portion of the RLS remained superficial and formed the EGL. While a precise border between the NTZ and EGL is not clear on E13.5 in mice, the regions of these two populations can be distinguished. In *Math1* null mutant mice, the NTZ and the EGL were both deficient, although cells in the cerebellar RL continued to express β-gal. Moreover, on E16.5, *Math1* null embryos lacked CN neurons, consistent with earlier absence of the NTZ. The large β-gal+cells in medial CN of control E16.5 mice expressed Lhx2/9 and Tbr1 transcriptional factors (Wang et al. 2005; Fink et al. 2006). The interposed and lateral nuclei were also labeled by Lhx2/9. In *Math1* null mice, Lhx2/9 and Tbr1 expression was absent. These findings indicated that a subset of CN neurons are derived from the upper RL and are *Math1* dependent. Similar conclusions were obtained in a study using an in vivo inducible fate mapping strategy to label cohorts of progenitor cells leaving the RL at different developmental times (Machold and Fishell 2005). In addition, this work revealed a temporal sequence in cerebellar RL neurogenesis, in which Math1-expressing precursors that migrated from the RL prior to E12.5 became CN neurons, while those that exited later became different cohorts of granule cells.

Analyzing a panel of TFs including Pax6, Tbr2, and Tbr1, Fink and others (2006) showed that this TF cascade delineated stages of neuronal maturation along the RLS pathway. Pax6 was expressed in the cerebellar RL and RLS, Tbr2 in the RLS and NTZ, and Tbr1 in the NTZ. The origin of the subpial stream from cerebellar RL was inferred from Pax6 expression (Engelkamp et al. 1999; Fink et al. 2006). In addition, Fink and others (2006) used organotypic slice cocultures to provide direct evidence that the cerebellar RL is necessary and sufficient to supply cells migrating through the RLS to the NTZ. Combinatorial expression of transcription factors plays a critical role in neuronal subtype specification during CNS development. Thus, further analysis of molecular markers together with real-time imaging of migratory behavior of CN neurons using transgenic mice will profoundly advance and improve our understanding of the cellular and molecular mechanisms underlying cerebellar morphogenesis.

Overall, the cerebellar VZ and RL appear to be specialized compartments for the production of GABAergic and glutamatergic cerebellar neurons, respectively (Fig. 1b). This hypothesis resembles neurogenesis in the forebrain, where glutamatergic neurons are produced in the cortical VZ and subventricular zone (SVZ), while GABAergic neurons are produced in basal forebrain regions (Hevner et al. 2006).

Anatomical and Molecular Classifications of CN Neurons

Whereas the cerebellar cortex is highly compartmentalized into genetically determined and reproducible topographic units (transverse zones and parasagittal stripes) that can be identified by molecular expression patterns (Sillitoe and Joyner 2007), it seems likely that the CN may be organized in a related manner. However, very little is known about the presence of topographical molecular maps within the four subdivisions of the CN. Morphological studies have identified a series of segmental partitions within the major CN, but molecular criteria have not been applied to these subdivisions (Voogd 1995). Morphological subdivisions of the CN have been described for the rat by Voogd (1995) and may be applied in the mouse. From medial to lateral (on each side of the midline) these are (1) the caudomedial, middle, and dorsolateral protuberance subdivisions of the medial nucleus, (2) the anterior interposed nucleus, (3) the posterior interposed nucleus and dorsolateral hump, and (4) the lateral cerebellar nucleus.

Using a panel of molecular markers assessed individually and in combination, a recent study suggested that the CN can be subdivided into at least 12 expression domains in mouse and proposed that such neurochemical heterogeneity could represent counterparts of zones and stripes in the cerebellar cortex (Chung et al. 2009). Using *GlyT2*-EGFP reporter mice, glycinergic neurons were identified as a fraction of both large and small neurons within the CN, mainly in the medial nucleus. Tbr1, previously shown to be expressed in a subset of medial CN (Fink et al. 2006), was also identified in this study as a marker of neurons in the caudal domain of the medial CN. HNK1, a cell surface-associated carbohydrate epitope, was expressed in a subset of Purkinje cells, Golgi cells, and large neurons of the CN but excluded from caudal regions of the medial and dorsolateral medial nuclei. PLCβ4, a key component in the transduction of metabotropic glutamate receptor 1 signaling, was expressed mainly in the interpositus and lateral nuclei. In *Thy1*-YFP transgenic mice, transgene expression was also localized in large somata in CN, mainly the interposed and lateral nuclei. Using these and other markers, each medial nucleus was subdivided into six expression domains; the anterior and posterior interposed nuclei were subdivided into four domains; and the lateral nuclei were subdivided into two domains (Chung et al. 2009).

Previous studies have also used molecular markers to distinguish between the different types of CN neurons: NeuN labels virtually all nuclear neurons in rats (Weyer and Schilling 2003; Leto et al. 2006). In contrast, SMI-32 and calretinin identify two largely nonoverlapping neuronal subtypes: SMI32 is expressed in large polygonal neurons corresponding to projection neurons (Jankovski et al. 1996; Hoshino et al. 2005; Fink et al. 2006), whereas calretinin is expressed in smaller ovoid-shaped cells representing GABAergic neurons of the CN (Bastianelli 2003; Leto et al. 2006). Brn2 also labels the majority of CN neurons (Fink et al. 2006). The application of CN subtype-specific molecular markers to morphological mapping will aid in the precise histological description of the CN and in the analysis of CN phenotypes in mutant animals.

Efferent Projections: Molecular Determinants for Axonal Guidance

The cerebellum receives excitatory input from multiple precerebellar nuclei located in the brainstem and sends processed information to diverse brain structures via the CN. Both neuronal migration and guidance of axonal projection are required for the proper formation of the complex circuitry of the cerebellar system. Although some molecules that regulate the migration of precerebellar neurons and the guidance of their projections to the cerebellum are known (Sotelo 2004), the signaling pathways and molecules necessary for the guidance of CN axonal projections to their final targets are largely unknown.

The principal efferent fibers of the cerebellum arise from the CN and are organized into two main systems, contained in three separate bundles. The main efferent systems are the superior cerebellar peduncles (SCP) and the fastigial efferent projections (Sotelo 2004). The SCP is the largest cerebellar efferent bundle and is formed by fibers arising from cells in the lateral (dentate) and interposed (emboliform and globose) nuclei. This composite group of fibers passes ventrorostrally into the upper pons and then ventromedially into the midbrain tegmentum. All SCP fibers decussate in the caudal midbrain, and most of these fibers ascend to enter the contralateral red nucleus and thalamus, which relay motor information to the cortex (Malcolm and Carpenter 1978). Axoplasmic transport studies have shown that the CN receive also inputs from the contralateral inferior olivary nucleus, and these nuclei have reciprocal connections with specific parts of the inferior olivary complex (Martin et al. 1976). Medial/fastigial nucleus efferent projections emerge from the cerebellum via the uncinate fasciculus (Russell bundle) and the juxtarestiform body (a subdivision of the inferior cerebellar peduncle). Fibers in the uncinate fasciculus, both crossed and uncrossed, arch around the SCP, while fibers entering the juxtarestiform body are uncrossed and pass medial to the SCP toward the vestibular nuclei. Both of these efferent bundles send fibers to parts of all vestibular nuclei. Some fibers projecting in the uncinate fasciculus bypass the vestibular nuclei and enter regions of the reticular formation. This oversimplified classification of the cerebellar efferent system suggests that, functionally, each half of the cerebellum is organized into three longitudinal zones, each of which has specialized connections through subdivisions of the CN (Malcolm and Carpenter 1978).

Recent studies using molecular markers have identified major axonal tracts of the CN. Fink et al. (2006) showed that immunoreactivity for calretinin and VGLUT2 is present in the NTZ and hook bundle as early as E14.5 and E17.5, respectively. Unilateral DiI tracing from the medial, interposed, and lateral cerebellar nuclei similarly revealed the decussating hook bundle axons emerging from the medial DCN and the robust axonal projections through the superior cerebellar peduncle to the red nuclei and the pons (Fink et al. 2006). In an effort to identify molecular determinants important for the formation of these major efferent tracts in vivo, several knockout mouse mutants have been studied.

Recent studies have shown that axon guidance signaling systems that control axonal trajectories in many regions of the nervous system, such as Robo/Slit and

Unc5/netrin, may also regulate the navigation of efferent axonal projections from the CN (Tamada et al. 2008; Kim and Ackerman 2011). Netrin-1 and its receptor DCC, and members of the Slit/Robo guidance system, have been found to regulate the ventral guidance and midline crossing of precerebellar neurons and their axons (Bloch-Gallego et al. 1999; Causeret et al. 2002). Unc5c is a repulsive receptor for netrin, and Unc5c (also known as Unc5h3) is expressed in the medial cerebellar neurons within the NTZ (Kim and Ackerman 2011). *Unc5c* mutant mice have abnormal ipsilateral projections of the medial deep neurons with a concomitant reduction of the cerebellar commissure. Direct labeling demonstrated that in the Unc5c mutant mice, the hook bundle projects toward the ipsilateral mesencephalic floor plate, indicating that Unc5c may play a role in repelling the hook bundle from the floor plate (Kim and Ackerman 2011). Interestingly, in addition to axonal guidance defects, disruption of Unc5c signaling in mice also leads to rostral cerebellar malformations in which granule and Purkinje cells spread ectopically into the caudal mesencephalon (Ackerman et al. 1997).

Similarly, ventral guidance and/or crossing of migrating axons of lateral/intermediate CN neurons has recently been shown to be under Rig-1 (Robo3) and Robo1/2 control (Tamada et al. 2008). In *Rig-1*-deficient mice embryos, efferent axons extended longitudinally on the ipsilateral side, failing to cross the midline, indicating that Rig1 (Robo3) is required for the approach of efferent axons to the midline. In *Robo1/2* double knockout mice, many efferent axons reached the midline but failed to fully cross, indicating that Robo1 and/or Robo2 are required for exit of axons from the midline. Interestingly, *Robo1* and *Robo2* mRNA are detected in the somata of NTZ neurons as early as E13 in rat embryos (E11.5 equivalent in mice), and Robo1 and Robo2 proteins were detected in axonal processes at the same time, a stage when axons initiate growth circumferentially toward the floor plate (Tamada et al. 2008). These studies have begun to elucidate the molecular mechanism that guides the efferent axonal trajectories from the deep CN to other regions of the brain, but much additional work remains to be done.

Development of Human Cerebellar Nuclei

Neuroblasts that form the human CN migrate from germinal zones to the intermediate zone in two broad streams during the embryonic stages from 3 to 8 gestational weeks (gw). Cells in the median stream segregate before 16 gw into fastigial (FN), emboliform (EN), globose (GN), and dentate (DN) nuclei, while those in the lateral stream contribute almost exclusively to the formation of DN (Yamaguchi et al. 1989). These CN migratory streams have been thought to arise from the cerebellar VZ, but in light of the recent findings from experimental work, it is likely that CN neurons arise also from the cerebellar RL in humans, as in other rodents.

The DN in humans is much larger and more complex than in rodents and has been the focus of most anatomical investigations. The human DN develops more slowly than the FN and the intermediate nuclei (including EN and GN, which are not clearly discriminated during fetal ages). The DN is first delineated at about 11–12.5 gw

(Gudovic et al. 1987; Hayaran et al. 1992b) and coalesces within the deep white matter at around 16 gw as a thick band of cells (Mihajlovic and Zecevic 1986). The DN surface is smooth at 20–22 gw, but the DN cells reorganize to ultimately form a highly convoluted, near monolayer configuration. This process of DN cellular reorganization is completed by approximately 35 gw (Mihajlovic and Zecevic 1986).

Different subdivisions of the human DN can be recognized on the basis of morphology and cell size, and these subdivisions undergo differential growth and development. The rostral DN is smaller with microgyric folding, while the caudal part is larger with macrogyric configuration. The DN also displays distinct dorsomedial and ventrolateral subdivisions that undergo differential growth during the period from 20 to 24.5 gw (Gudovic et al. 1987). The dorsomedial, magnocellular region begins to grow at 13 gw and to fold at 20 gw, whereas the ventrolateral, parvocellular region grows rapidly at 20 gw and begins folding around 23.5 gw (Gudovic et al. 1987).

Several types of immature and mature neurons have been described in the developing human DN. Bipolar cellular morphology is recognized around 14–15 gw. By 19–20 gw, three cell types are identifiable: bipolar, hemispheric, and pyriform. Neurons of the dorsomedial region mature earlier than those of the ventrolateral (Hayaran et al. 1992a), but the greater part of subsequent DN development is due to the growth of its ventrolateral region (Matano 2001). Developing DN neurons at 20 gw express MAP2; synaptophysin; nerve growth factor receptor (NGFR); neurofilament proteins of light (NF-L), medium (NF-M), and heavy (NF-H) molecular weights; and alpha-internexin (Yachnis et al. 1993). Differentiation of the dentate neurons is especially intensive during the mid-gestational period (20–25 gw), because the cell bodies increase size and develop profusely branching dendrites with spines. A second, slower phase of maturation consisting of addition of secondary and tertiary branches continues into the postnatal period. Interestingly, at all prenatal ages, dentate neurons appear morphologically more mature than the Purkinje cells in the overlying cortex (Mihajlovic and Zecevic 1986).

Regarding the axonal growth of CN in humans, virtually nothing is known about the timing or guidance cues.

Cerebellar Nuclei Abnormalities

Although CN are key to cerebellum function, in that they integrate information from Purkinje cells and provide the main output from cerebellum to other parts of the brain, so far there is no mouse mutant in which the CN are primarily affected by gene mutation. Interestingly, it seems that CN are numerically preserved in many mouse mutants with severe cerebellar cortex phenotypes, and only a few mouse mutants show cell deficiencies in the deep nuclei. In principle, abnormalities of CN development affecting either glutamatergic or GABAergic neuronal components may result from defects of CN cell-type specification, fusion of the winged-liked cerebellar primordium at the midline, cellular differentiation, migration, axon growth and guidance, and afferent input defects. Interestingly, abnormalities of CN

development are more recognizable in some human brain malformations, and pathological abnormalities of CN morphology have been described more extensively in humans than in rodents.

Cerebellar Nuclei Defects: Mouse Mutant Phenotypes

In the cerebellum, as in other brain regions, neuronal specification requires the actions of genes encoding specific bHLH transcription factors. Math1 is required for the specification and differentiation of glutamatergic lineages from the cerebellar RL, including CN as well as granule neurons and UBCs (Ben-Arie et al. 1997; Englund et al. 2006; Fink et al. 2006). In *Math1* null mice, the development of NTZ at E13.5 is impaired, and its derivatives, the glutamatergic projection neurons of the CN, are consequently deficient (Wang et al. 2005; Fink et al. 2006). *Math1* null mice also display loss of the EGL and UBCs. Neurod1 is another bHLH transcription factor important in granule neuron differentiation (Miyata et al. 1999), but its role in CN development (if any) has not been defined. A different bHLH transcription factor, Ptf1a, is required for the specification of GABAergic neurons from the VZ, including small GABAergic interneurons of the CN, as well as Purkinje cells (Hoshino et al. 2005; Pascual et al. 2007). *Ptf1a* null mice (*cerebelless* mutants) display deficits in the GABAergic DCN formation, in addition to complete lack of Purkinje cells and other GABAergic interneurons, further indicating its essential role in cerebellar GABAergic neuronal specification. Interestingly, *Ptf1a* mutation in humans is responsible for cerebellar agenesis (Sellick et al. 2004), confirming the important role this TF plays during cerebellar development.

Recently, progress has been made in studying the development of the cerebellar RL, the source of glutamatergic CN neurons. A LIM-homeodomain TF, *Lmx1a*, was identified as an important upstream regulator of cell-fate decisions during cerebellar neurogenesis (Chizhikov et al. 2006, 2010; Millen and Gleeson 2008). In embryonic mice, Lmx1a is expressed in the cerebellar roof plate (dorsal midline) at E10.5 where it functions together with BMP molecules to pattern the adjacent cerebellar anlage (Chizhikov et al. 2006). Lmx1a is also expressed in the RL progenitors starting at E12.5 in a partially overlapping pattern of expression with Math1, revealing molecular heterogeneity in the RL (Chizhikov et al. 2010). Lmx1a is also detected in the NTZ at E13.5, and within CN neurons, and *Lmx1a* null mice (*dreher* mutants) display loss of markers associated with medial nuclei of the cerebellum, indicating its role in the development of some components of the glutamatergic lineage. The role of Lmx1a in the development of GABAergic CN neurons has not been determined yet.

Previous studies have linked Reelin, a secreted signaling protein, to abnormal cerebellar development (Goffinet 1983). Reelin protein expression is first detected in the developing mouse cerebellum at E13, along the dorsal cerebellar surface corresponding to the RLS and NTZ (Fink et al. 2006). At later stage, Reelin-positive cells are scattered along the inner border of EGL and in deep areas coinciding with the CN and surrounding white matter. Expression of Reelin in the

deep areas disappears during the first postnatal week but is maintained in the inner half of the EGL during postnatal development and in some granule neurons of adult mice (Sotelo 2004). Reelin-deficient mice (*reeler*) and rats (*SRK*) display severe cerebellar hypoplasia and disorganization (Goffinet 1983; Goffinet et al. 1984) (Fig. 2a–h). In *reeler* mice (Goffinet 1983), the first defect to appear (E14.5) is deficient formation of the Purkinje cell plate. By E17.5, when foliation begins, *reeler* mutants exhibit not only Purkinje cells migration defects but also reduced tangential migration of the EGL and absent foliation. The NTZ develops virtually normally in *reeler* mice, but the CN are malformed due to migration defects. The lateral CN are disorganized (Goffinet 1983), and the medial CN are displaced laterally (Goffinet et al. 1984). Mice with deficiencies of downstream Reelin signaling pathway components include cell surface receptor molecules VDLR/ApoER2 and intracellular signaling molecules Disabled-1 (Dab1), and tyrosine kinases Src and Fyn exhibit essentially identical cerebellar malformations as in *reeler* (Rice et al. 2001), although a recent study of *Dab1*-deficent mice (*scrambler* mice) reported normal CN morphology (Chung et al. 2007). This suggests that CN development is not directly affected by the disruption of the *Reelin* signaling pathway but may be deformed due to cerebellar cortex malformation.

As mentioned above, Pax6, Tbr2, and Tbr1 are expressed sequentially in the RL and NTZ, suggesting that these transcription factors might be important for the development of glutamatergic CN neurons (Fink et al. 2006). Development of the CN has not yet been analyzed in *Pax6* null mutant or *Tbr2* conditional mutant mice but was found to be abnormal in *Tbr1* mutants. In particular, early postnatal *Tbr1* mutants were found to show defective morphogenesis of the medial CN, which displayed an irregular shape suggestive of migration defects (Fink et al. 2006) (Fig. 2i–p). More specifically, the dorsolateral protuberance of the medial CN (Voogd 1995) was not formed in *Tbr1* mutants. The interposed and lateral nuclei also appeared irregular but were less severely affected than the medial CN. Despite the abnormal histology of the CN, there was no apparent neuronal loss or apoptosis nor were nucleofugal axons abnormal in *Tbr1* mutants (Fink et al. 2006).

Another signaling pathway that regulates neuron migration in many brain regions and may be important in CN development is the chemokine receptor 4 (CXCR4)-chemokine ligand 12 (CXCL12) system. CXCR4 receptor expression is first detected in immature neuronal precursors that emerge from the RL as early as E12.5 (Tissir et al. 2004). CXCR4 expression persists in cells of the EGL and NTZ as they migrate through the subpial RLS. At the same time, the cognate ligand CXCL12 is expressed in the meninges overlying this migratory pathway, indicating that CXCR4- and CXCL12-expressing cells are positioned for potential interactions to regulate EGL formation and neuroblast migration in the RLS. Supporting this idea, *Cxcr4* and *Cxcl12* mutant mice (Ma et al. 1998; Zou et al. 1998) show dislocation of proliferating granule cell progenitors toward deeper positions, away from the meninges. Further studies have shown that CXCL12 acts as an attractant for the proliferating cells (Reiss et al. 2002; Zhu et al. 2002) and maintains these cells in the peripheral EGL. Downregulation of CXCR4, and therefore its interaction with the meningeal-produced CXCL12 ligand, leads to inward radial migration of granule cell precursors away from the meningeal surface and furthermore causes these cells

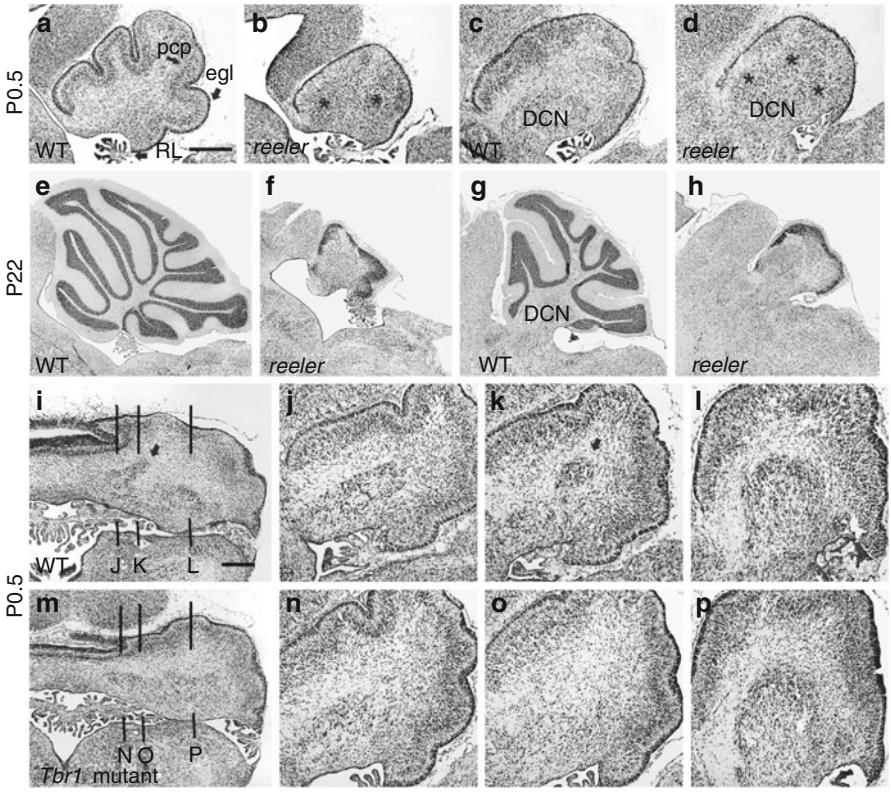

Fig. 2 Cerebellar histology illustrating malformation of the DCN in Reelin-deficient (*reeler)* and Tbr1 mutant mice. (**a**–**h**) Cresyl violet-stained sagittal sections through the WT (**a**, **c**, **e**, **g**) and *reeler* cerebellum (**b**, **d**, **f**, **g**) at vermal positions (**a**, **b**, **e**, **f**) and hemispheres (**c**, **d**, **g**, **h**) at P0.5 (**a**–**d**) and P22 (**e**–**h**). Sections are oriented dorsal top and anterior to the left. In P0.5 controls, Purkinje cells had migrated to the Purkinje cell plate (*PCP*), and folia developed by migration of proliferating precursors in the external granular layer (*EGL*), which originated from the rhombic lip (*RL*). In P0.5 reeler mice, the cerebellum was hypoplastic with folial formation defects, and Purkinje cells formed large, centrally located ectopic clusters (*asterisks* in **b**, **d**). The hypoplasia and defective foliation were even more obvious by P22 in the *reeler* cerebellum (**f**, **h**). The deep cerebellar nuclei (*DCN*) in reeler are located near their normal position but are somewhat distorted by the Purkinje cells ectopia (Goffinet 1983; Goffinet et al. 1984). (**i**–**p**) Cresyl violet stained P0.5 coronal (*dorsal to the top*) (**i**, **m**) and sagittal (**j**–**l**, **n**–**p**) sections through the WT (**i**–**l**) and *Tbr1* mutant (**m**–**p**) cerebellum. Lines in (**i**, **m**) indicate levels of sections in (**j**–**l**, **n**–**p**), respectively. The normal DCN had a smooth, ribbon-like contour, with a prominent dorsolateral protuberance in the medial nucleus (*arrows* in **a**, **k**). The *Tbr1* mutant DCN had irregular contours and appeared disorganized, and the dorsolateral protuberance did not form recognizably (compare **k**, **o**). *Scale bars* for *reeler* (in **a**): (**a**–**d**), 400 mm; (**e**–**h**), 1,000 mm and for *Tbr1* mutants (in **i**): (**i**, **m**), 400 mm; (**j**–**l**, **n**–**p**), 200 mm. (Modified with permission from Fink et al. 2006 and Fatemi 2008 Reelin Glycoprotein)

to exit the cell cycle. The specific role of CXCR4/CXCL12 signaling in development of the NTZ and CN has not been investigated, but it is tempting to speculate, based on the expression patterns and mechanism of action, that these molecules may facilitate the migration of neurons in the subpial stream toward the NTZ and/or

detachment of NTZ neurons from the surface to descend toward deeper position beneath the white matter.

CN defects may also arise secondarily from perturbations of cerebellar cortex development or degeneration. This is thought to be the mechanism of CN deficiency in *Purkinje cell degeneration* (*pcd*) (Triarhou et al. 1987) and *weaver* (Maricich et al. 1997) mouse mutants. The *pcd* mouse (*Nna1*-deficient mouse) is characterized behaviorally by moderate ataxia that develops between 3 and 4 weeks of age, correlating with the degeneration of Purkinje cells. The Purkinje cell loss causes secondary degeneration of cerebellar granule cells (synaptic drivers of Purkinje cells) and CN neurons (recipients of Purkinje cell synapses). These correlations point to a scenario in which synaptic loss contributes to secondary deficits in granule cells and CN neurons (Chang and Ghetti 1993; Wang and Morgan 2007).

The mouse neurological mutant *weaver* (*Girk2* potassium channel-deficient mice) displays an atrophic cerebellar cortex with deficits in Purkinje cells, granule cells, and also CN neurons. In these mutants, the CN appear to be displaced laterally and show numerical loss of neurons, especially in the medial regions, corresponding to the localization of Purkinje cell and granule neuron deficits. While this may suggest a secondary degeneration as in *pcd* mutants, the *weaver* phenotype also includes a failure of the cell movements that lead to midline fusion of the bilateral cerebellar anlage. This failure of morphogenesis leaves some Purkinje cells and CN neurons in an abnormal position where they may be unable to make appropriate connections (Maricich et al. 1997; Marti et al. 2001).

Cerebellar Nuclei Defects: Insights from Human Malformations

CN abnormalities can be seen in a wide spectrum of neurodevelopmental diseases. They can occur in the context of isolated cerebellar anomalies or as part of more extensive malformation syndromes. Cerebellar malformations are increasingly diagnosed in the fetal period, usually by ultrasound examination (Parisi and Dobyns 2003). Unfortunately, no single classification of human cerebellar malformations has been widely accepted, and CN malformations remain difficult to categorize and describe (Patel and Barkovich 2002). Involvement of the CN can be a specific feature in some diseases, and once DN are deemed abnormal on imaging, appropriate differential diagnosis can act as a guide to clinicians regarding suitable further investigations (McErlean et al. 2010). Here, just a few examples of CN malformations are illustrated.

Joubert Syndrome

Joubert syndrome (JS), a primarily autosomal recessive condition, comprises a broad spectrum of mid-hindbrain anomalies. These include varying degrees of vermis aplasia, enlarged fourth ventricle, hypoplastic cerebellum, fragmentation of the dentate nucleus, and cerebellar heterotopia. Other typical features of JS include dysplastic inferior olives, non-decussation of the pyramidal tract and the superior cerebellar peduncle, fragmented spinal nucleus of the trigeminal cranial nerve,

dysplastic dorsal medulla including the nuclei/fasciculi gracilis and cuneatus and the solitary nuclei/tract, and abnormalities of the arcuate nucleus (Friede and Boltshauser 1978; Yachnis and Rorke 1999). Thus, the principal defects involving the CN include fragmentation of the dentate nuclei (Fig. 3b) and non-decussation of the superior cerebellar peduncle, that is, nucleofugal axons.

Clinically, JS manifests with hypotonia, abnormal respirations and eye movements, ataxia, and developmental delay (Joubert et al. 1969). A specific neuroradiological feature seen by magnetic resonance imaging (MRI) is the "molar tooth sign," named for the characteristic appearance of the midbrain and its junction with the cerebellum (Maria et al. 1999). JS has been linked to mutations in ten identified genes (*NPHP1, AHI1, CEP290, RPGRIP1L, MKS3/TMEM67, CC2D2A, ARL13B, INPP5E, TMEM216*, and *OFD1*) that encode components of primary cilia (PC), but

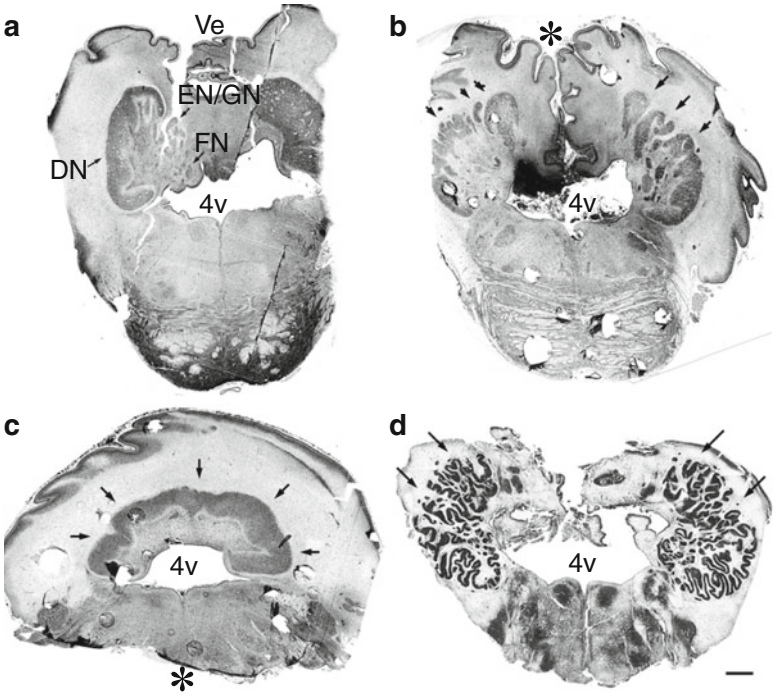

Fig. 3 Malformations of fetal human CN, as revealed by microtubule-associated protein 2 (*MAP-2*) immunoreactivity. (**a**) Normal cerebellum (21 gw). The fastigial (*FN*), emboliform/globose (*EN/GN*), and dentate (*DN*) nuclei are visible above the fourth ventricle (*4v*). The DN is largest, and at this fetal age forms a thick band with only slight convolutions. Note the midline vermis (*Ve*) separating the cerebellar hemispheres. (**b**) Joubert syndrome (21 gw). The vermis is absent (*asterisk*), and the cerebellar hemispheres are completely separated. The DN is fragmented (*arrows*). (**c**) Rhombencephalosynapsis (21 gw). The CN are fused across the midline (*arrows*) and appear morphologically to consist of only DN. The vermis is absent, but in contrast to Joubert syndrome, the cerebellar hemispheres are fused across the midline. The *asterisk* indicates sectioning artifact, removing the basis pontis. (**d**) Thanatophoric dysplasia (30 gw). The CN are enlarged and hyperconvoluted (*arrows*). Scale bar (*lower right*): 1 mm for (**a**–**c**); 2.5 mm for (**d**)

mutations in these genes account for only <50% of cases (Parisi et al. 2007). Little is known about how the PC function in brain development, although they seem to be specialized organelles with receptors to detect morphogens such as Wnt and Shh proteins (Doherty 2009; Parisi 2009). In the cerebellum, PC have been identified in both Purkinje cell and granule cell progenitors, and the primary defect in JS may be compromised granule cell proliferation, resulting in cerebellar hypoplasia (Millen and Gleeson 2008).

Rhombencephalosynapsis

Rhombencephalosynapsis (RES) is a rare malformation of the hindbrain, in which absence of the vermis leads to fusion of the cerebellar hemispheres and dentate nuclei at the midline (Fig. 3c). RES has been attributed to a failure of neuronal proliferation (Utsunomiya et al. 1998) and/or defective dorsal patterning (including the roof plate) at the midbrain-hindbrain boundary (Yachnis 2002; Pasquier et al. 2009). RES has been reported to occur in pure neurological conditions and in association with syndromes such as Gomez-Lopez-Hernandez and VACTERL-H (Pasquier et al. 2009). However, the underlying genetic mechanisms are unknown. MRI findings include a narrow, diamond-shaped ("keyhole") fourth ventricle on axial sections (Truwit et al. 1991). The clinical course is variable and ranges from mild truncal ataxia and normal cognitive abilities to severe learning disability, developmental delay, mental retardation, and cerebellar impairments (Utsunomiya et al. 1998; Toelle et al. 2002; Chemli et al. 2007; Kruer et al. 2009).

Thanatophoric Dysplasia

Thanatophoric dysplasia (TD) is a lethal form of short-limb skeletal dysplasia associated with macrocephaly and variable cloverleaf skull. Two subtypes of TD are recognized (TD1 and TD2), distinguished primarily by the radiologic features of the bones (Langer et al. 1987). Both subtypes are caused by activating ("gain-of-function") mutations in different functional domains of the fibroblast growth factor receptor 3 (FGFR3) gene, which fulfills multiple roles during brain development (Hevner 2005; Iwata and Hevner 2009). Neuropathological features include megalencephaly, hippocampal dysplasia and rudimentary dentate gyrus, polymicrogyria, enlarged temporal lobe, abnormal fiber bundles, neuronal heterotopia, cerebellar cortex abnormalities, dysplasia of the inferior olives, and dysplastic CN with enlarged, hyperconvoluted dentate nuclei (Fig. 3d) (Ho et al. 1984; Hevner 2005; Miller et al. 2009). The enlargement of dentate nuclei suggests that neurogenesis of these neurons is increased by FGFR3 signaling.

Pontocerebellar Hypoplasia

Pontocerebellar hypoplasia (PCH) represents a heterogeneous group of (mainly) autosomal recessive disorders with prenatal onset. Although there are several distinct types of PCH, the unifying features consist of cerebellar hypoplasia (most prominent in the hemispheres with relative sparing of the vermis), along with variable atrophy of the cerebellum and ventral pons, microcephaly, variable neocortical atrophy, and severe mental and motor impairments (Ramaekers et al. 1997; Gardner et al. 2001;

Parisi and Dobyns 2003). Histopathology of PCH reveals multiple cerebellar abnormalities including hypoplastic cerebellar folia, reduced volume of the cortex with scattered Purkinje cells loss, segmental loss of dentate nuclei neurons with preserved islands and reactive changes, loss of ventral pontine nuclei, and segmental cellular loss in the inferior olives (Patel et al. 2006; Barth et al. 2007).

Six subtypes of PCH have been defined according to the clinical and pathologic features, and several causative genes have been identified to date. Mutations in *PMM2* cause a congenital disorder of glycosylation with morphological features of PCH (Jaeken and Casaer 1997). Mutations in *VRK1* (Renbaum et al. 2009) and *RARS2* (Namavar et al. 2011) have been linked to PCH1; *TSEN2, TSNEN34, TSEN54*, and *RARS2* have been linked to PCH2 and PCH4, which appear to form a pathological spectrum (Graham et al. 2010; Namavar et al. 2011); and *RARS2* mutations have been linked to PCH6 (Rankin et al. 2010). The hindbrain structures affected in PCH are derivatives of the RL (Limperopoulos and du Plessis 2006). Moreover, the cerebellar cortex, CN, and olivary nuclei, all of which are affected in PCH, are functionally linked through the climbing fiber system (Barth et al. 2007). Pathologically, PCH appears to involve a combination of primary developmental anomaly and degeneration due to lack of appropriate connections.

Autism

Autism is a relatively common neurodevelopmental disorder characterized by impaired social interaction and communication and by repetitive and restricted behavior (La Malfa et al. 2004). Whereas its complete biological causes remain to be established, there is a strong genetic component. Neuropathological findings in autism have been found in the cerebellum, limbic system, brainstem, and neocortex. Notably, reduction of the Purkinje cell density in the cerebellar cortex and loss of neurons in DN are the most common findings in the cerebellum (Palmen et al. 2004). These findings suggest that in autism, interactions between the Purkinje cells and CN are modified on the structural, molecular, and functional level (Wegiel et al. 2010).

Conclusions and Future Directions

Recent studies have made tremendous progress in defining CN neuron types and their origins from distinct progenitor compartments, but our understanding of the embryologic and genetic mechanisms that control CN development and functional organization remains rudimentary. For example, little is known about gene expression in the developing CN, and there are few molecular markers of CN cell types and subdivisions. Progress on this front should be rapid, since large-scale projects have now mapped gene expression in the developing mouse brain. Expression of most genes can be examined in the developing brain by accessing GenePaint (genepaint.org), the Brain Gene Expression Map (stjudebgem.org), Eurexpress (eurexpress.org), or the Allen Institute for Brain Science (developingmouse.brain-map.org). These resources will facilitate the discovery of genes likely to be important in CN development that can be targeted for genetic mutation in mice.

Phases in the migration of CN neurons, from cerebellar RL to NTZ to CN, have been described only superficially, and relatively little is known about the underlying cellular and molecular mechanisms or the roles of radial glial and meninges. The recently introduced methodology of time-lapse video imaging in slice cultures could be highly informative about CN neuronal migrations.

The development of CN afferent and efferent axon pathways, and their relations to CN subdivisions, must be better described. Basic descriptions of stages in axon growth, trajectories, and turning points, as well as knowledge of molecular cues, are almost entirely lacking. Particular attention should be paid to subdivisions of the CN and their distinct afferent and efferent connections. How are Purkinje cell stripes related to CN subdivisions? Do different CN subdivisions project to different extracerebellar targets? Do different CN subdivisions subserve distinct neurological circuits or functions?

More focused attention on CN abnormalities and pathology will be valuable in studies of humans and other species and will be critical to progress against several human neurological and psychiatric disorders, including rare (such as RES) as well as common disorders (such as autism). Given the many questions that persist about CN developmental mechanisms, functional organization, and roles in disease pathogenesis, future studies will be both rewarding and relevant to human diseases.

References

Ackerman SL, Kozak LP, Przyborski SA, Rund LA, Boyer BB, Knowles BB (1997) The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein. Nature 386 (6627):838–842

Altman J, Bayer SA (1978) Prenatal development of the cerebellar system in the rat. I. Cytogenesis and histogenesis of the deep nuclei and the cortex of the cerebellum. J Comp Neurol 179 (1):23–48

Altman J, Bayer SA (1985a) Embryonic development of the rat cerebellum. I. Delineation of the cerebellar primordium and early cell movements. J Comp Neurol 231(1):1–26

Altman J, Bayer SA (1985b) Embryonic development of the rat cerebellum. II. Translocation and regional distribution of the deep neurons. J Comp Neurol 231(1):27–41

Altman J, Bayer SA (1985c) Embryonic development of the rat cerebellum. III. Regional differences in the time of origin, migration, and settling of Purkinje cells. J Comp Neurol 231 (1):42–65

Armstrong CL, Hawkes R (2000) Pattern formation in the cerebellar cortex. Biochem Cell Biol 78 (5):551–562

Armstrong DM, Schild RF (1978a) An investigation of the cerebellar cortico-nuclear projections in the rat using an autoradiographic tracing method. I. Projections from the vermis. Brain Res 141 (1):1–19

Armstrong DM, Schild RF (1978b) An investigation of the cerebellar corticonuclear projections in the rat using an autoradiographic tracing method. II. Projections from the hemisphere. Brain Res 141(2):235–249

Bagnall MW, Zingg B, Sakatos A, Moghadam SH, Zeilhofer HU, du Lac S (2009) Glycinergic projection neurons of the cerebellum. J Neurosci 29(32):10104–10110

Barth PG, Aronica E, de Vries L, Nikkels PG, Scheper W, Hoozemans JJ, Poll-The BT, Troost D (2007) Pontocerebellar hypoplasia type 2: a neuropathological update. Acta Neuropathol 114 (4):373–386

Bastianelli E (2003) Distribution of calcium-binding proteins in the cerebellum. Cerebellum 2 (4):242–262
Ben-Arie N, Bellen HJ, Armstrong DL, McCall AE, Gordadze PR, Guo Q, Matzuk MM, Zoghbi HY (1997) Math1 is essential for genesis of cerebellar granule neurons. Nature 390 (6656):169–172
Bloch-Gallego E, Ezan F, Tessier-Lavigne M, Sotelo C (1999) Floor plate and netrin-1 are involved in the migration and survival of inferior olivary neurons. J Neurosci 19(11):4407–4420
Carletti B, Rossi F (2008) Neurogenesis in the cerebellum. Neuroscientist 14(1):91–100
Causeret F, Danne F, Ezan F, Sotelo C, Bloch-Gallego E (2002) Slit antagonizes netrin-1 attractive effects during the migration of inferior olivary neurons. Dev Biol 246(2):429–440
Chang AC, Ghetti B (1993) Embryonic cerebellar graft development during acute phase of gliosis in the cerebellum of pcd mutant mice. Chin J Physiol 36(3):141–149
Chan-Palay V (1977) Cerebellar dentate nucleus. Organization, cytology and transmitters. JNEN 36 (6):978
Chemli J, Abroug M, Tlili K, Harbi A (2007) Rhombencephalosynapsis diagnosed in childhood: clinical and MRI findings. Eur J Paediatr Neurol 11(1):35–38
Chizhikov VV, Lindgren AG, Currle DS, Rose MF, Monuki ES, Millen KJ (2006) The roof plate regulates cerebellar cell-type specification and proliferation. Development 133(15):2793–2804
Chizhikov VV, Lindgren AG, Mishima Y, Roberts RW, Aldinger KA, Miesegaes GR, Currle DS, Monuki ES, Millen KJ (2010) Lmx1a regulates fates and location of cells originating from the cerebellar rhombic lip and telencephalic cortical hem. Proc Natl Acad Sci USA 107 (23):10725–10730
Chung S, Zhang Y, Van Der Hoorn F, Hawkes R (2007) The anatomy of the cerebellar nuclei in the normal and scrambler mouse as revealed by the expression of the microtubule-associated protein kinesin light chain 3. Brain Res 1140:120–131
Chung SH, Marzban H, Hawkes R (2009) Compartmentation of the cerebellar nuclei of the mouse. Neuroscience 161(1):123–138
de Zeeuw CI, Berrebi AS (1995) Postsynaptic targets of Purkinje cell terminals in the cerebellar and vestibular nuclei of the rat. Eur J Neurosci 7:2322–2333
Doherty D (2009) Joubert syndrome: insights into brain development, cilium biology, and complex disease. Semin Pediatr Neurol 16(3):143–154
Engelkamp D, Rashbass P, Seawright A, van Heyningen V (1999) Role of Pax6 in development of the cerebellar system. Development 126(16):3585–3596
Englund C, Kowalczyk T, Daza RA, Dagan A, Lau C, Rose MF, Hevner RF (2006) Unipolar brush cells of the cerebellum are produced in the rhombic lip and migrate through developing white matter. J Neurosci 26(36):9184–9195
Fatemi (2008) Reelin glycoprotein. Rober H Hevner Reelin and the cerebellum. Springer, New York, 141–158
Fink AJ, Englund C, Daza RA, Pham D, Lau C, Nivison M, Kowalczyk T, Hevner RF (2006) Development of the deep cerebellar nuclei: transcription factors and cell migration from the rhombic lip. J Neurosci 26(11):3066–3076
Fredette BJ, Mugnaini E (1991) The GABAergic cerebello-olivary projection in the rat. Anat Embryol (Berl) 184(3):225–243
Friede RL, Boltshauser E (1978) Uncommon syndromes of cerebellar vermis aplasia. I: Joubert syndrome. Dev Med Child Neurol 20(6):758–763
Gardner RJ, Coleman LT, Mitchell LA, Smith LJ, Harvey AS, Scheffer IE, Storey E, Nowotny MJ, Sloane RA, Lubitz L (2001) Near-total absence of the cerebellum. Neuropediatrics 32(2):62–68
Goffinet AM (1983) The embryonic development of the cerebellum in normal and reeler mutant mice. Anat Embryol (Berl) 168(1):73–86
Goffinet AM, So KF, Yamamoto M, Edwards M, Caviness VS Jr (1984) Architectonic and hodological organization of the cerebellum in reeler mutant mice. Brain Res 318(2):263–276
Graham JM Jr, Spencer AH, Grinberg I, Niesen CE, Platt LD, Maya M, Namavar Y, Baas F, Dobyns WB (2010) Molecular and neuroimaging findings in pontocerebellar hypoplasia type 2 (PCH2): is prenatal diagnosis possible? Am J Med Genet A 152A(9):2268–2276

Gudovic R, Marinkovic R, Aleksic S (1987) The development of the dentate nucleus in man. Anat Anz 163(3):233–238
Haines DE, May PJ, Dietrichs E (1990) Neuronal connections between the cerebellar nuclei and hypothalamus in *Macaca fascicularis*: cerebello-visceral circuits. J Comp Neurol 299(1):106–122
Hayaran A, Wadhwa S, Bijlani V (1992a) Cytoarchitectural development of the human dentate nucleus: a Golgi study. Dev Neurosci 14(3):181–194
Hayaran A, Wadhwa S, Gopinath G, Bijlani V (1992b) Developing dentate nucleus in man: a qualitative and quantitative study. Exp Brain Res 89(3):640–648
Helms AW, Gowan K, Abney A, Savage T, Johnson JE (2001) Overexpression of MATH1 disrupts the coordination of neural differentiation in cerebellum development. Mol Cell Neurosci 17 (4):671–682
Hevner RF (2005) The cerebral cortex malformation in thanatophoric dysplasia: neuropathology and pathogenesis. Acta Neuropathol 110(3):208–221
Hevner RF, Hodge RD, Daza RA, Englund C (2006) Transcription factors in glutamatergic neurogenesis: conserved programs in neocortex, cerebellum, and adult hippocampus. Neurosci Res 55(3):223–233
Ho KL, Chang CH, Yang SS, Chason JL (1984) Neuropathologic findings in thanatophoric dysplasia. Acta Neuropathol 63(3):218–228
Hoshino M, Nakamura S, Mori K, Kawauchi T, Terao M, Nishimura YV, Fukuda A, Fuse T, Matsuo N, Sone M et al (2005) Ptf1a, a bHLH transcriptional gene, defines GABAergic neuronal fates in cerebellum. Neuron 47(2):201–213
Ito M (1984) The cerebellum and neural control. Raven, New York
Iwata T, Hevner RF (2009) Fibroblast growth factor signaling in development of the cerebral cortex. Develop Growth Differ 51(3):299–323
Jaarsma D, Ruigrok TJ, Caffe R, Cozzari C, Levey AI, Mugnaini E, Voogd J (1997) Cholinergic innervation and receptors in the cerebellum. Prog Brain Res 114:67–96
Jaeken J, Casaer P (1997) Carbohydrate-deficient glycoconjugate (CDG) syndromes: a new chapter of neuropaediatrics. Eur J Paediatr Neurol 1(2–3):61–66
Jankovski A, Rossi F, Sotelo C (1996) Neuronal precursors in the postnatal mouse cerebellum are fully committed cells: evidence from heterochronic transplantations. Eur J Neurosci 8 (11):2308–2319
Joubert M, Eisenring JJ, Robb JP, Andermann F (1969) Familial agenesis of the cerebellar vermis. A syndrome of episodic hyperpnea, abnormal eye movements, ataxia, and retardation. Neurology 19(9):813–825
Kim D, Ackerman SL (2011) The UNC5C netrin receptor regulates dorsal guidance of mouse hindbrain axons. J Neurosci 31(6):2167–2179
Korneliussen HK (1967) Cerebellar corticogenesis in Cetaca, with special reference to regional variations. J Hirnforsch 9(2):151–185
Korneliussen HK (1968) On the morphology and subdivision of the cerebellar nuclei of the rat. J Hirnforsch 10(2):109–122
Kruer MC, Blasco PA, Anderson JC, Bardo DM, Pinter JD (2009) Truncal ataxia, hypotonia, and motor delay with isolated rhombencephalosynapsis. Pediatr Neurol 41(3):229–231
La Malfa G, Lassi S, Bertelli M, Salvini R, Placidi GF (2004) Autism and intellectual disability: a study of prevalence on a sample of the Italian population. J Intellect Disabil Res 48 (Pt 3):262–267
Langer LO Jr, Yang SS, Hall JG, Sommer A, Kottamasu SR, Golabi M, Krassikoff N (1987) Thanatophoric dysplasia and cloverleaf skull. Am J Med Genet Suppl 3:167–179
Leiner HC, Leiner AL, Dow RS (1986) Does the cerebellum contribute to mental skills? Behav Neurosci 100(4):443–454
Leto K, Carletti B, Williams IM, Magrassi L, Rossi F (2006) Different types of cerebellar GABAergic interneurons originate from a common pool of multipotent progenitor cells. J Neurosci 26(45):11682–11694

Leto K, Bartolini A, Yanagawa Y, Obata K, Magrassi L, Schilling K, Rossi F (2009) Laminar fate and phenotype specification of cerebellar GABAergic interneurons. J Neurosci 29 (21):7079–7091

Limperopoulos C, du Plessis AJ (2006) Disorders of cerebellar growth and development. Curr Opin Pediatr 18(6):621–627

Louvi A, Alexandre P, Metin C, Wurst W, Wassef M (2003) The isthmic neuroepithelium is essential for cerebellar midline fusion. Development 130(22):5319–5330

Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T, Bronson RT, Springer TA (1998) Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. Proc Natl Acad Sci USA 95(16):9448–9453

Machold R, Fishell G (2005) Math1 is expressed in temporally discrete pools of cerebellar rhombic-lip neural progenitors. Neuron 48(1):17–24

Malcolm B, Carpenter AB (1978) Core text of neuroanatomy, 2nd edn. Williams and Wilkins, Baltimore

Maria BL, Quisling RG, Rosainz LC, Yachnis AT, Gitten J, Dede D, Fennell E (1999) Molar tooth sign in Joubert syndrome: clinical, radiologic, and pathologic significance. J Child Neurol 14 (6):368–376

Maricich SM, Herrup K (1999) Pax-2 expression defines a subset of GABAergic interneurons and their precursors in the developing murine cerebellum. J Neurobiol 41(2):281–294

Maricich SM, Soha J, Trenkner E, Herrup K (1997) Failed cell migration and death of purkinje cells and deep nuclear neurons in the weaver cerebellum. J Neurosci 17(10):3675–3683

Marti J, Wills KV, Ghetti B, Bayer SA (2001) Evidence that the loss of Purkinje cells and deep cerebellar nuclei neurons in homozygous weaver is not related to neurogenetic patterns. Int J Dev Neurosci 19(6):599–610

Martin GF, Henkel CK, King JS (1976) Cerebello-olivary fibers: their origin, course and distribution in the North American opossum. Exp Brain Res 24:219–236

Matano S (2001) Brief communication: proportions of the ventral half of the cerebellar dentate nucleus in humans and great apes. Am J Phys Anthropol 114(2):163–165

Mathis L, Nicolas JF (2003) Progressive restriction of cell fates in relation to neuroepithelial cell mingling in the mouse cerebellum. Dev Biol 258(1):20–31

Mathis L, Bonnerot C, Puelles L, Nicolas JF (1997) Retrospective clonal analysis of the cerebellum using genetic laacZ/lacZ mouse mosaics. Development 124(20):4089–4104

McErlean A, Abdalla K, Donoghue V, Ryan S (2010) The dentate nucleus in children: normal development and patterns of disease. Pediatr Radiol 40(3):326–339

Miale IL, Sidman RL (1961) An autoradiographic analysis of histogenesis in the mouse cerebellum. Exp Neurol 4:277–296

Mihajlovic P, Zecevic N (1986) Development of the human dentate nucleus. Hum Neurobiol 5 (3):189–197

Millen KJ, Gleeson JG (2008) Cerebellar development and disease. Curr Opin Neurobiol 18 (1):12–19

Miller E, Blaser S, Shannon P, Widjaja E (2009) Brain and bone abnormalities of thanatophoric dwarfism. AJR Am J Roentgenol 192(1):48–51

Miyata T, Maeda T, Lee JE (1999) NeuroD is required for differentiation of the granule cells in the cerebellum and hippocampus. Genes Dev 13(13):1647–1652

Morales D, Hatten ME (2006) Molecular markers of neuronal progenitors in the embryonic cerebellar anlage. J Neurosci 26(47):12226–12236

Mugnaini EOWH (1985) GABAergic neurons and terminals in the rat CNS as revealed by GAD immuno-histochemistry. In: GABA and neuropeptides in the CNS: the handbook of chemical neuroanatomy part I. Elsevier, Amsterdam, pp 541–543

Namavar Y, Barth PG, Kasher PR, van Ruissen F, Brockmann K, Bernert G, Writzl K, Ventura K, Cheng EY, Ferriero DM et al (2011) Clinical, neuroradiological and genetic findings in pontocerebellar hypoplasia. Brain 134(Pt 1):143–156

Palmen SJ, van Engeland H, Hof PR, Schmitz C (2004) Neuropathological findings in autism. Brain 127(Pt 12):2572–2583

Parisi MA (2009) Clinical and molecular features of Joubert syndrome and related disorders. Am J Med Genet C Semin Med Genet 151C(4):326–340

Parisi MA, Dobyns WB (2003) Human malformations of the midbrain and hindbrain: review and proposed classification scheme. Mol Genet Metab 80(1–2):36–53

Parisi MA, Doherty D, Chance PF, Glass IA (2007) Joubert syndrome (and related disorders) (OMIM 213300). Eur J Hum Genet 15(5):511–521

Pascual M, Abasolo I, Mingorance-Le Meur A, Martinez A, Del Rio JA, Wright CV, Real FX, Soriano E (2007) Cerebellar GABAergic progenitors adopt an external granule cell-like phenotype in the absence of Ptf1a transcription factor expression. Proc Natl Acad Sci USA 104 (12):5193–5198

Pasquier L, Marcorelles P, Loget P, Pelluard F, Carles D, Perez MJ, Bendavid C, de La Rochebrochard C, Ferry M, David V et al (2009) Rhombencephalosynapsis and related anomalies: a neuropathological study of 40 fetal cases. Acta Neuropathol 117(2):185–200

Patel S, Barkovich AJ (2002) Analysis and classification of cerebellar malformations. AJNR Am J Neuroradiol 23(7):1074–1087

Patel MS, Becker LE, Toi A, Armstrong DL, Chitayat D (2006) Severe, fetal-onset form of olivopontocerebellar hypoplasia in three sibs: PCH type 5? Am J Med Genet A 140(6):594–603

Pierce ET (1975) Histogenesis of the deep cerebellar nuclei in the mouse: an autoradiographic study. Brain Res 95:503–518

Ramaekers VT, Heimann G, Reul J, Thron A, Jaeken J (1997) Genetic disorders and cerebellar structural abnormalities in childhood. Brain 120(Pt 10):1739–1751

Rankin J, Brown R, Dobyns WB, Harington J, Patel J, Quinn M, Brown G (2010) Pontocerebellar hypoplasia type 6: a British case with PEHO-like features. Am J Med Genet A 152A (8):2079–2084

Reiss K, Mentlein R, Sievers J, Hartmann D (2002) Stromal cell-derived factor 1 is secreted by meningeal cells and acts as chemotactic factor on neuronal stem cells of the cerebellar external granular layer. Neuroscience 115(1):295–305

Renbaum P, Kellerman E, Jaron R, Geiger D, Segel R, Lee M, King MC, Levy-Lahad E (2009) Spinal muscular atrophy with pontocerebellar hypoplasia is caused by a mutation in the VRK1 gene. Am J Hum Genet 85(2):281–289

Rice DS, Nusinowitz S, Azimi AM, Martinez A, Soriano E, Curran T (2001) The reelin pathway modulates the structure and function of retinal synaptic circuitry. Neuron 31(6):929–941

Ruigrok TJ (1997) Cerebellar nuclei: the olivary connection. Prog Brain Res 114:167–192

Schmahmann JD (1996) From movement to thought: anatomic substrates of the cerebellar contribution to cognitive processing. Hum Brain Mapp 4(3):174–198

Sekerkova G, Ilijic E, Mugnaini E (2004) Time of origin of unipolar brush cells in the rat cerebellum as observed by prenatal bromodeoxyuridine labeling. Neuroscience 127(4):845–858

Sellick GS, Barker KT, Stolte-Dijkstra I, Fleischmann C, Coleman RJ, Garrett C, Gloyn AL, Edghill EL, Hattersley AT, Wellauer PK et al (2004) Mutations in PTF1A cause pancreatic and cerebellar agenesis. Nat Genet 36(12):1301–1305

Sgaier SK, Millet S, Villanueva MP, Berenshteyn F, Song C, Joyner AL (2005) Morphogenetic and cellular movements that shape the mouse cerebellum; insights from genetic fate mapping. Neuron 45(1):27–40

Sillitoe RV, Joyner AL (2007) Morphology, molecular codes, and circuitry produce the three-dimensional complexity of the cerebellum. Annu Rev Cell Dev Biol 23:549–577

Sotelo C (2004) Cellular and genetic regulation of the development of the cerebellar system. Prog Neurobiol 72(5):295–339

Tamada A, Kumada T, Zhu Y, Matsumoto T, Hatanaka Y, Muguruma K, Chen Z, Tanabe Y, Torigoe M, Yamauchi K et al (2008) Crucial roles of Robo proteins in midline crossing of cerebellofugal axons and lack of their up-regulation after midline crossing. Neural Dev 3:29

Tissir F, Wang CE, Goffinet AM (2004) Expression of the chemokine receptor Cxcr4 mRNA during mouse brain development. Brain Res Dev Brain Res 149(1):63–71

Toelle SP, Yalcinkaya C, Kocer N, Deonna T, Overweg-Plandsoen WC, Bast T, Kalmanchey R, Barsi P, Schneider JF, Capone Mori A et al (2002) Rhombencephalosynapsis: clinical findings and neuroimaging in 9 children. Neuropediatrics 33(4):209–214

Triarhou LC, Norton J, Ghetti B (1987) Anterograde transsynaptic degeneration in the deep cerebellar nuclei of Purkinje cell degeneration (pcd) mutant mice. Exp Brain Res 66(3):577–588

Truwit CL, Barkovich AJ, Shanahan R, Maroldo TV (1991) MR imaging of rhombencephalosynapsis: report of three cases and review of the literature. AJNR Am J Neuroradiol 12 (5):957–965

Utsunomiya H, Takano K, Ogasawara T, Hashimoto T, Fukushima T, Okazaki M (1998) Rhombencephalosynapsis: cerebellar embryogenesis. AJNR Am J Neuroradiol 19(3):547–549

Uusisaari M, Knopfel T (2008) GABAergic synaptic communication in the GABAergic and non-GABAergic cells in the deep cerebellar nuclei. Neuroscience 156(3):537–549

Uusisaari M, Knopfel T (2010) GlyT2+ neurons in the lateral cerebellar nucleus. Cerebellum 9 (1):42–55

Uusisaari M, Obata K, Knopfel T (2007) Morphological and electrophysiological properties of GABAergic and non-GABAergic cells in the deep cerebellar nuclei. J Neurophysiol 97 (1):901–911

Voogd J (1995) Cerebellum. In: Paxinos G (ed) The rat nervous system, 2nd edn. Academic, San Diego, pp 309–350

Wang T, Morgan JI (2007) The Purkinje cell degeneration (pcd) mouse: an unexpected molecular link between neuronal degeneration and regeneration. Brain Res 1140:26–40

Wang VY, Rose MF, Zoghbi HY (2005) Math1 expression redefines the rhombic lip derivatives and reveals novel lineages within the brainstem and cerebellum. Neuron 48(1):31–43

Wegiel J, Kuchna I, Nowicki K, Imaki H, Marchi E, Ma SY, Chauhan A, Chauhan V, Bobrowicz TW, de Leon M et al (2010) The neuropathology of autism: defects of neurogenesis and neuronal migration, and dysplastic changes. Acta Neuropathol 119(6):755–770

Weisheit G, Gliem M, Endl E, Pfeffer PL, Busslinger M, Schilling K (2006) Postnatal development of the murine cerebellar cortex: formation and early dispersal of basket, stellate and Golgi neurons. Eur J Neurosci 24(2):466–478

Weyer A, Schilling K (2003) Developmental and cell type-specific expression of the neuronal marker NeuN in the murine cerebellum. J Neurosci Res 73(3):400–409

Wingate RJ (2001) The rhombic lip and early cerebellar development. Curr Opin Neurobiol 11 (1):82–88

Wingate R (2005) Math-map(ic)s. Neuron 48(1):1–4

Yachnis AT (2002) Rhombencephalosynapsis with massive hydrocephalus: case report and pathogenetic considerations. Acta Neuropathol 103(3):301–304

Yachnis AT, Rorke LB (1999) Cerebellar and brainstem development: an overview in relation to Joubert syndrome. J Child Neurol 14(9):570–573

Yachnis AT, Rorke LB, Lee VM, Trojanowski JQ (1993) Expression of neuronal and glial polypeptides during histogenesis of the human cerebellar cortex including observations on the dentate nucleus. J Comp Neurol 334(3):356–369

Yamaguchi K, Goto N, Yamamoto TY (1989) Development of human cerebellar nuclei. Morphometric study. Acta Anat (Basel) 136(1):61–68

Zhang L, Goldman JE (1996) Generation of cerebellar interneurons from dividing progenitors in white matter. Neuron 16(1):47–54

Zhu Y, Yu T, Zhang XC, Nagasawa T, Wu JY, Rao Y (2002) Role of the chemokine SDF-1 as the meningeal attractant for embryonic cerebellar neurons. Nat Neurosci 5(8):719–720

Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR (1998) Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature 393(6685):595–599