Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Molecular Genetics and Metabolism 80 (2003) 54–65
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Minireview

Development and malformations of the cerebellum in mice

Victor Chizhikov and Kathleen J. Millen*

Department of Human Genetics, University of Chicago, 920 E. 58th Street, CLSC 319, Chicago, IL 60637, USA

Received 1 July 2003; received in revised form 4 August 2003; accepted 4 August 2003

Abstract

The cerebellum is the primary motor coordination center of the CNS and is also involved in cognitive processing and sensory discrimination. Multiple cerebellar malformations have been described in humans, however, their developmental and genetic etiologies currently remain largely unknown. In contrast, there is extensive literature describing cerebellar malformations in the mouse. During the past decade, analysis of both spontaneous and gene-targeted neurological mutant mice has provided significant insight into the molecular and cellular mechanisms that regulate cerebellar development. Cerebellar development occurs in several distinct but interconnected steps. These include the establishment of the cerebellar territory along anterior–posterior and dorsal–ventral axes of the embryo, initial specification of the cerebellar cell types, their subsequent proliferation, differentiation and migration, and, finally, the interconnection of the cerebellar circuitry. Our understanding of the basis of these developmental processes is certain to provide insight into the nature of human cerebellar malformations.
© 2003 Elsevier Inc. All rights reserved.

Keywords: Cerebellum; Mid/hindbrain; Mouse; Mutants; Spontaneous neurological mouse mutants; Gene targeting; Human cerebellar malformations

Introduction

The cerebellum has long been recognized as the primary center of motor coordination in the central nervous system (CNS) [1]. Recent studies in humans have also implicated the cerebellum in cognitive processing and sensory discrimination in medical conditions as diverse as pervasive developmental disorders, autism, and cerebellar vascular injuries [2–6]. Numerous cerebellar and related hindbrain malformations have been described in humans including cerebellar vermian hypoplasia, pontocerebellar hypoplasia, Dandy–Walker malformation and molar tooth malformations. Most are associated with significant risk for mental retardation and other developmental disabilities such as ataxia, cerebral palsy and epilepsy. Although commonly described in the clinical literature, only very recently has there been significant effort directed towards classifying and precisely defining human congenital cerebellar malformations [7–9]. The developmental and genetic etiologies of these human cerebellar malformations remain largely unknown. This is in contrast to the rich literature describing the molecular mechanisms of cerebellar malformations in the mouse. Given the high degree of conservation of cerebellar anatomy and function between both the mouse and human, an understanding of mouse cerebellar development is likely to shed new insight upon the developmental basis of human cerebellar malformations.

The cerebellum is a relatively simple CNS structure with well-defined anatomy and physiology (Fig. 1). The cerebellum is morphologically divided into a central vermis, which is flanked by lateral hemispheres. Both the vermis and hemispheres are subdivided by a series of parallel fissures defining a conserved pattern of folia. The function of the cerebellum depends on the actions of three principal neuronal subclasses; (1) granule cells, (2) Purkinje cells, and (3) deep cerebellar neurons. Each neuronal type has a stereotypic and distinct morphology and is located in a discrete lamina within the cerebellum. In the adult cerebellum precise connections between the principal neurons are arranged in a stereotyped circuitry that is repeated throughout the cerebellum (Fig. 1C). Information is sent to the cerebellum via the precerebellar system, a group of nuclei including the pontine

* Corresponding author. Fax: 1-773-834-8470.
E-mail address: kmillen@genetics.bsd.uchicago.edu (K.J. Millen).

1096-7192/$ - see front matter © 2003 Elsevier Inc. All rights reserved.
doi:10.1016/j.ymgme.2003.08.019

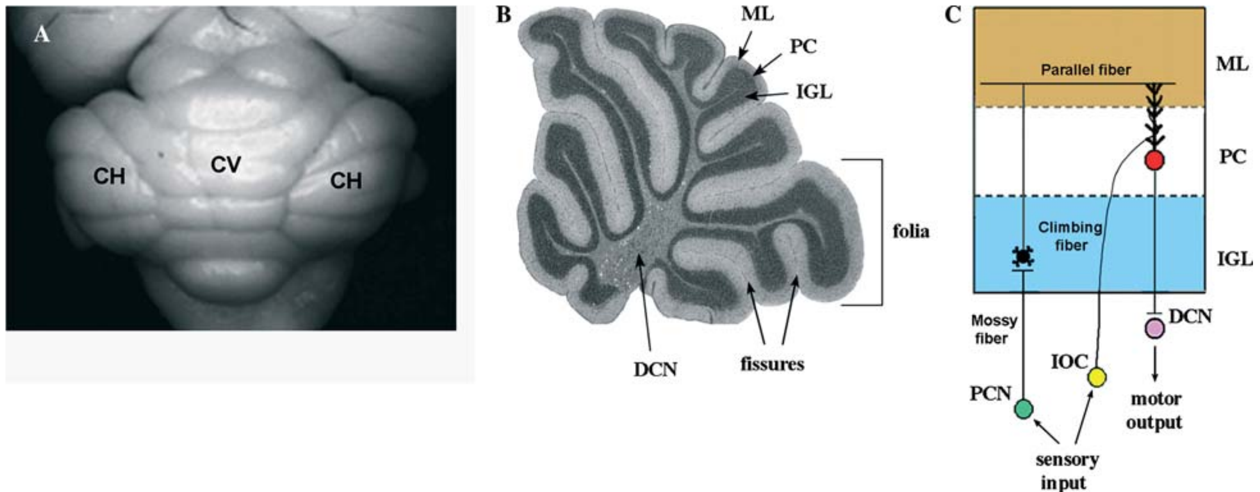

Fig. 1. Cerebellar structure and neuronal connections in the cerebellum of the adult wild-type mouse. (A) Dorsal view of the adult mouse cerebellum. The cerebellar vermis (CV) and the cerebellar hemispheres (CH) are indicated. (B) Para-sagittal section of the vermis. The molecular layer (ML), Purkinje cell layer (PC), internal granule cell layer (IGL), deep cerebellar nuclei (DCN), and parallel fissures, separating folia, are indicated by arrows. One folia is labeled by bracket. (C) Schematic diagram of the principle neuronal connections in the cerebellum. Purkinje cells are red, granule neurons are black, precerebellar nuclei (PCN) are green, inferior olivary complex (IOC) is yellow, and cells of deep cerebellar nuclei (DCN) are pink. Molecular layer, Purkinje cell layer, and internal granule cell layer are shown in brown, white, and blue, respectively. They are marked as in panel B and are not drawn to scale.

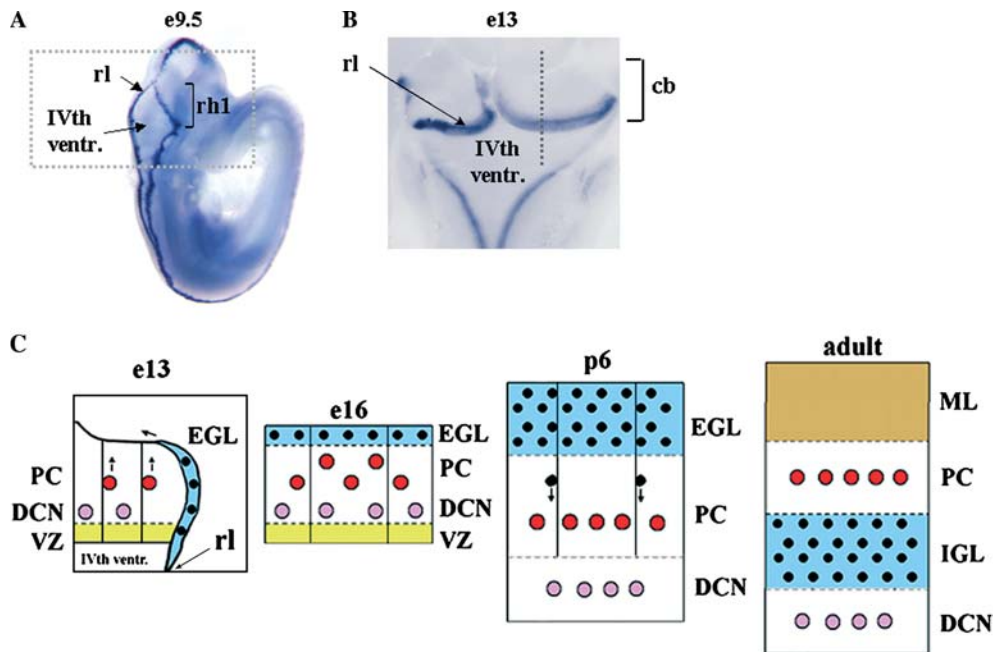

Fig. 3. Overview of cerebellar development. (A) Oblique dorsal view of a e9.5 mouse embryo stained for the expression of the *Gdf7* gene, which marks the roof plate of the CNS. At this stage expression of *Gdf7* dramatically outlines the edge of the ventriclar zone of the embryo adjacent to the fourth ventricle (IVth ventr.), which will give rise to the rhombic lip (rl). The cerebellum will arise from dorsal rhombomere 1 (rh1). (B) A magnified dorsal view of the boxed region in (A) four days later in development, at e13. This embryo is stained for expression of the *Math1* gene, which is expressed in the rhombic lip. The cerebellar anlage (cb) has formed at this stage. The dashed line indicates a parasagittal section, which is the plane of section represented in first schematic illustration in (C). (C) Schematic diagram of the developing cerebellum at e13, e16, and p6, demonstrating the extensive neuronal migrations that are required to achieve the final laminar structure of the adult cerebellum. In all diagrams ventricular zone (VZ) is yellow, Purkinje cell layer (PC) is white, external (EGL) and internal (IGL) granule cell layers are blue, and molecular layer (ML) is brown. Purkinje cells are red, granule cells are black, and cells of deep cerebellar nuclei (DCN) are pink. The directions of the cellular migrations are shown by the arrows. Glial fibers are drawn as black lines traversing the entire width of the developing cerebellum from the ventricular zone to the pial surface.

nuclei, and spinocerebellar pathways that send projections (mossy fibers) to granule neurons. Information to Purkinje cells from axons of granule cells (parallel fibers) is modulated by input from the climbing fibers of the inferior olivary complex, which also synapse with the dendrites of Purkinje cells. Purkinje cells, in turn, send axons to deep cerebellar nuclei, which provide the primary output from the cerebellar cortex [10,11].

Development of the cerebellum involves integration of both intrinsic and cell extrinsic events controlled by multiple genetic cascades. Cerebellar development occurs in several distinct but interconnected stages. They include establishing of the cerebellar territory along anterior–posterior (AP) and dorsal–ventral (DV) axes of the neural tube, initial specification of the cerebellar cell types, their subsequent proliferation, differentiation and migration, and finally, formation of the cerebellar circuitry. Much of our current understanding of cellular and molecular mechanisms directing formation of the cerebellum has come from analysis of mutant mice with cerebellar malformations. Approximately 60 spontaneous mouse mutant strains with cerebellar malformations have been identified over the last 100 years (http://www.informatics.jax.org/). Disruptions in cerebellar development and/or function often result in a typical uncoordinated phenotype in mice. Names of these mutant strains such as *weaver, lurcher, reeler*, and *swaying* clearly reflect this obvious uncoordinated phenotype. With the introduction of gene targeting technologies in the late 1980s, the roles of numerous additional genes in cerebellar development have been described enhancing our knowledge of the cerebellar development. Here we first briefly outline normal cerebellar development, and then review what is known about the molecular mechanisms that control development of the cerebellum in mouse and how these findings can help us to understand the nature of human cerebellar disorders.

Overview of cerebellar development

The vertebrate CNS derives from the neural plate, an epithelial sheet that arises from the dorsal ectoderm of the gastrula-stage embryo. Subsequently, the neural plate closes to form the neural tube, which becomes patterned along its AP and DV axes. Shortly after neural tube closure, a series of vesicles can be clearly distinguished morphologically at the anterior end of the neural tube of the mouse embryo, indicating its patterning along AP axis (Fig. 2). The most anterior end of the neural tube gives rise to the forebrain consisting of the telencephalon and the diencephalon, while more posterior regions form the midbrain, often referred to as the mesencephalon, the hindbrain, and the spinal cord. The hindbrain is divided into 7 segments, the rhombomeres [12]. The most anterior rhombomere is sometimes

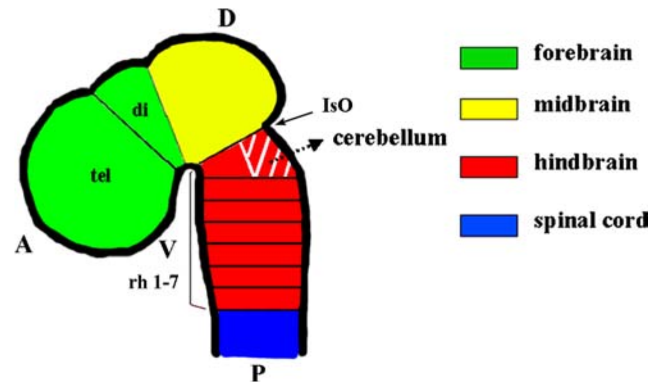

Fig. 2. Schematic diagram of the mouse CNS at embryonic day 9.5 (e9.5). The neural tube is divided into a series of segments. Forebrain, midbrain, hindbrain, and spinal cord territories are illustrated in green, yellow, red, and blue, respectively. The forebrain is further subdivided into the telencephalon (tel) and the diencephalon (di). The hindbrain is subdivided into its constituent rhombomeres (rh1-7). The junction of the mid-hindbrain is the location of the isthmic organizer (marked by the arrow). The presumptive cerebellar territory within the neural tube is illustrated as a hatched region in dorsal rhombomere 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

referred to as the metencephalon, and the remaining posterior rhombomeres of the hindbrain are often termed the rhombencephalon.

Although early chick–quail chimera experiments initially suggested that the cerebellum is derived from both presumptive midbrain and the hindbrain [13,14], subsequent gene expression and fate mapping studies showed that the cerebellum arises entirely from the anterior-most rhombomere of the hindbrain, rhombomere 1 (Fig. 2) [15,16]. It has been well documented that a signaling center called the isthmic organizer (IsO) plays an important role in establishment of the anterior limit of the cerebellar territory. The IsO develops at the boundary separating the midbrain and the hindbrain. IsO activity was first identified in chick. In particular, surgical movement of the isthmic tissue to more anterior regions (the caudal diencephalon) or more posterior regions (rhombomeres 2–5 of the hindbrain) of the neural tube of 10-somite stage chick embryos caused induction of ectopic midbrain and cerebellar structures in the host territories, which contacted the graft [17–19]. Less is understood regarding formation of the posterior boundary of the presumptive cerebellar territory. Currently, there is no evidence that activity of a specific signaling center restricts posterior extension of rhombomere 1 and the cerebellum. However, gene expression studies provide the candidate genes that may be involved in this process. Fate mapping studies have also located presumptive cerebellar territory along DV axis of the neural tube. Chick–quail chimera experiments showed that the cerebellar anlage originates from the alar plate of the rhombomere 1 [14,20].

Once the cerebellar territory is established, cerebellar development continues through the remaining embryonic period and is associated with extensive morphological changes of the presumptive cerebellar territory. The final structure of the mature cerebellum is not achieved until approximately post-natal day 15 (p15) in mice [21]. The cerebellar anlage arises in the midgestational period, between embryonic day 9.5 (e9.5) and e11.5, as the mouse neural tube closes and bends to establish pontine flexure. This results in formation of the mouth-like structure at the dorsal edges of the roof of the fourth ventricle adjacent to the cerebellar anlage (Figs. 3A and B). The rhombic lip is formed as a specialized region of the ventricular zone directly adjacent to the roof of the fourth ventricle and later gives rise to granule cells, which are the most numerous class of the cells in the cerebellar cortex [22–24]. The ventricular zone adjacent to the rhombic lip produces precursors of cerebellar deep nuclei at e10-11, and later becomes a source of Purkinje cells [14,25]. Precursors of Purkinje cells migrate along a radial glial fiber system to populate the intermediate layer along DV axis of the cerebellar anlage (Fig. 3C) [26]. Granule neuron progenitors migrate anteriorly over the surface of the developing cerebellar anlage to form the external granule layer (EGL). Within the EGL, granule neuron progenitors proliferate extensively and then differentiate and migrate into the cortex of the cerebellum along the Bergmann glia to establish the internal granule layer (IGL) under a monolayer of Purkinje cells (Fig. 3C). The extensive post-natal proliferation of the EGL and subsequent migration of differentiating granule neural cells results in massive growth of the cerebellum [26,27]. Foliation of the cerebellum is closely associated with both granule cell proliferation and ingrowth of cerebellar afferents, which also occurs during post-natal development [23,28].

Molecular mechanisms that control cerebellar development

Otx2 and Gbx2 determine the position of IsO

Gene targeting experiments have revealed that two genes: *Otx2* and *Gbx2*, mouse homologues of the *Drosophila* genes *orthodenticle* and *unplugged*, respectively, play primary roles in the positioning of the IsO, and thus, in determining of the anterior limit of the cerebellar territory [29–31]. In the mouse neural plate and neural tube, these genes are expressed in complementary patterns. *Otx2* is expressed in the anterior CNS, with a posterior limit of expression at the mid/hindbrain junction. *Gbx2* is expressed in presumptive hindbrain with an anterior border abutting the posterior border of expression of *Otx2* (Fig. 4) [32,33]. The regionally defined expression patterns of *Otx2* and *Gbx2* occur shortly after the onset of gastrulation and represent one of the first known molecular indications of AP patterning in the mouse CNS.

Loss of *Gbx2* causes expansion of the midbrain at the expense of the cerebellum. All IsO markers, including the secreted signaling factors *Wnt1* and *Fgf8*, are still detected in *Gbx2−/−* mice, but their expression domains are shifted posteriorly [33,34]. Chimeric analysis and gene targeting experiments have shown that reduction of *Otx* gene dosage (*Otx1−/−; Otx2+/−*) results in an expanded cerebellum at the expense of the midbrain. IsO markers *Wnt1* and *Fgf8* are moved to more anterior positions of the neural tube in these *Otx* mutant embryos, the opposite phenotype to that seen in *Gbx2−/−* mice [35–38].

Misexpression experiments have produced data complementary to the loss-of-function studies. Ectopic expression of a *Wnt1* driven *Gbx2* transgene in midbrain caused repression of *Otx2* in this region and shifted expression domains of *Wnt1* and *Fgf8* to more anterior positions. This resulted in an expansion of the anterior hindbrain and a reduced midbrain at embryonic day 9.5–10 [34]. Ectopic expression of a *En1* driven *Otx2* transgene in hindbrain caused repression of *Gbx2* in this domain, and *Wnt1* and *Fgf8* expression domains were shifted to more posterior regions of the neural tube. This led to enlargement of the midbrain and partial deletion of the cerebellum [39]. Taken together, the data indicate that *Gbx2* and *Otx2* act to mutually repress each other's expression resulting in the formation of a sharp boundary between their expression domains. This boundary sets the position of the IsO, which ultimately defines the posterior limit of the midbrain and the anterior limit of the cerebellum.

Fgf8 mediates the patterning activity of IsO

Currently, only one signaling molecule, fibroblast growth factor 8 (Fgf8), has been found to be able to mimic patterning activity of the IsO by inducing midbrain and cerebellar structures when ectopically provided to chick or mouse diencephalon or hindbrain [40–44]. *Fgf8* is expressed in a narrow band encircling the neural tube at the isthmus located in the *Gbx2* positive region (Fig. 4) [45]. Mutant mice with reduced levels of Fgf8 activity [46] or with conditionally inactivated *Fgf8* in mid/hindbrain region [44] have deletions or severe defects of the midbrain and the cerebellum caused by ectopic cell death, further supporting important role of Fgf8 in development of mid/hindbrain region.

Other genes important for IsO formation and function

Wnt1 is another secreted molecule important for proper isthmus formation and function. At e9.5, *Wnt1* is expressed in narrow strip located within *Otx2* expression territory sharing a boundary with the *Fgf8* expression

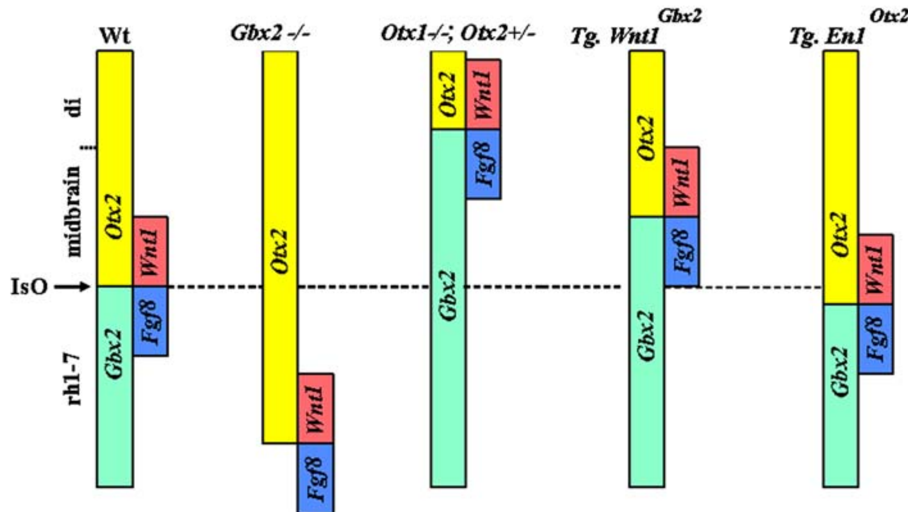

Fig. 4. The border between Gbx2 and Otx2 expression domains position IsO and determines anterior boundary of the midbrain and cerebellum. Expression domains of *Otx2*, *Gbx2*, *Wnt1*, and *Fgf8* are shown in yellow, green, red, and blue, respectively in e9.5 wild type (wt), *Gbx2*−/−, and *Otx1*−/−; *Otx2*+/− mouse embryos, as well as transgenic embryos expressing *Gbx2* under control of the *Wnt1* promoter (*Tg.* $Wnt1^{Gbx2}$), and *Otx2* under control of the *En1* promoter (*Tg.* $En1^{Otx2}$) manipulations. Arrow points to position of the isthmic organizer (IsO) in wild-type embryo. The new positions of the IsO in various genetic backgrounds are shown as the boundary between *Fgf8* and *Wnt1* expression domains, and are shifted relatively to the wild-type position of the IsO. di, the diencephalons; rh 1–7, rhombomeres 1–7. See text for further details. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

domain (Fig. 4) [47]. Loss of *Wnt1* results in severe deletions of both the midbrain and the cerebellum at early neural tube closure stages [48,49] indicating that Wnt1 is essential for development of these regions. Subsequent studies showed that Wnt1 is required to promote cellular proliferation [50] in addition to induction or maintenance of expression of several genes important for IsO development including *Fgf8* [51]. Interestingly, mice homozygous for a truncated allele of *Wnt1* (*swaying*) have a much less severe deletion phenotype with a failure of fusion of the anterior vermis and a loss of anterior folia [52].

A number of other genes that are important for establishing the IsO have also been identified. They include homeobox genes *En1* and *En2*, and the paired box genes *Pax2* and *Pax5*. These genes are expressed in broad domains across the IsO. Targeted inactivation of these genes causes varying deletions of midbrain and cerebellar structures [12]. In all cases, this phenotype can be traced back to defects in isthmus formation, maintenance, or both at early neural tube stages. Recent studies in zebrafish, *Xenopus* and chick suggest that another class of genes, genes of the Iroquois (*Iro*/*Irx*) family, may be also important for IsO development [53]. In mouse, six members of *Iro*/*Irx* family have been identified, but their role in IsO formation or maintenance is still unclear.

Formation of the posterior boundary of the cerebellar territory

To date, two studies have investigated the molecular mechanisms regulating the formation of the posterior boundary of the cerebellar anlage. Expression of the homeobox protein Hoxa2 in rhombomere 2 prevents caudal expansion of the cerebellum, since in *Hoxa2* targeted mutants, the cerebella are expanded at the expense of rhombomere 2 [54]. Interestingly, Fgf8, a major signaling molecule of the IsO (see above) has been reported to negatively regulate expression of *Hoxa2*, which positions the boundary between rhombomeres 1 and 2 [43]. Thus, the IsO not only directly positions the anterior boundary of the cerebellum but is also involved in determining its extension along the AP axis.

Establishment of the cerebellar territory along DV axis

Although transplantation studies have clearly demonstrated that the cerebellum arises from the dorsolateral domain of rhombomere 1 [14,20], little is known about the mechanisms involved in dorsoventral patterning of this region. This is in stark contrast to our understanding of the DV patterning mechanisms at the level of the spinal cord. In the developing spinal cord this process involves the action of two opposing signaling pathways. Peptides of the transforming growth factor-β (Tgfβ) and Wnt families, secreted from the nonneural ectoderm and roof plate, dorsalize neural progenitors converting them into several classes of sensory interneurons, while Sonic hedgehog (Shh) from the notochord and floor plate ventralizes neural tissue [55–57].

Recent evidence suggests that Bone morphogenetic proteins (Bmps) and Shh may also control dorsoventral

patterning of the cerebellar territory. It has been shown that *Bmp6*, *Bmp7*, and *Gdf7*, three members of Tgfβ family, expressed in the roof plate of the anterior hindbrain, play an important role in the specification of cerebellar granule neurons within the rhombic lip (see below for details) [58]. Further evidence supporting a critical role for the roof plate in cerebellar development has come from analysis of the spontaneous neurological mouse mutant *dreher*, which harbors mutations in the LIM homeodomain-encoding gene, *Lmx1a*. The adult *dreher* cerebellum lacks most of the vermis. Embryonically, the roof plate adjacent to the cerebellar territory is severely reduced in size. Since *Lmx1a* is specifically expressed in roof plate, the cerebellar defects observed in *dreher* mice are most likely due to indirect effects of loss of dorsalizing signals from the adjacent roof plate [59]. Ventral signaling mediated by Shh also has a role in normal development of the cerebellum since ectopic Shh in mid/hindbrain region ventralizes dorsal tissue and causes severe disruption of normal cerebellar patterning [60,61].

Initial specification of the cerebellar cell types

It is believed that the establishment of the identities of each cerebellar cell type depends on the activity of different sets of genes regulated by local signaling molecules. The first gene that has been identified in this regard is *Math1*, the mouse homologue of the *Drosophila* proneural gene *atonal*. *Math1* is expressed in germinal epithelium of the rhombic lip and many of its derivatives. A targeted mutation of *Math1* leads to a complete loss of several rhombic lip derivatives in adult mice including granule neurons of the cerebellar cortex and pontine nuclei of the precerebellar system. This phenotype can be traced back to early defects in rhombic lip region indicating that *Math1* is a critical gene for initial specification of granule neurons and pontine nuclei [62,63]. Another candidate gene that may control initial specification of granule cells is defined by the *meander tail* (*mea*) spontaneous mutation in mouse. This mutation causes a near-total depletion of granule cells in the anterior lobe of the cerebellum [64]. Chimeric analysis and transplantation experiments have established that the *mea* gene acts intrinsically to the granule cells or its progenitors to specify early steps in their development [65,66]. The identity of the *mea* gene remains unknown.

The search for inductive signals that direct early specification of the cerebellar cells identified members of Tgfβ family as candidate molecules. In particular, it has been shown that Bmp6, Bmp7, and Gdf7 induce the expression of *Math1* and other granule neuron markers in cultured explants of ventral tissue of rhombomere 1. Moreover, these BMP-treated neural cells formed mature granule neurons after transplantation into the early post-natal cerebellum, further supporting the role of BMPs in initiation of the program of granule cell specification [58].

The molecular mechanisms that underlie initial specification of other cerebellar cell types such as deep nuclei and Purkinje cells are unknown.

Regulation of neuronal number in the cerebellum

The cerebellum is a highly ordered structure composed of defined numbers of different cellular types. Numerous studies have demonstrated that during cerebellar development, the generation of the proper number of cells is achieved by tight regulation of programs that control proliferation, cell cycle withdrawal and differentiation, and apoptosis of cells of each cellular type. Recently, significant progress has been achieved in the understanding of the genetic regulation of these programs mostly through gene targeting and overexpression studies in mice. As with early specification of the cerebellar cell types, most of these studies have been concentrated on cerebellar granule neurons. In contrast to other cerebellar cell types, granule cell progenitors undergo a prolonged period of rapid proliferation after their birth resulting in the generation of millions of granule neurons. Previous work has identified several genes important for the clonal expansion of granule progenitors. These include *Ru49/Zipro1*, *Zic1*, and *Zic2*, which are expressed early in development of granule neuron lineage, at the time proliferating granule progenitors begin to migrate out from the rhombic lip [10,67]. Overexpression of *Ru49/Zipro1* in mouse embryos resulted in the over-production of cerebellar granule neurons indicating that proper copy number of this gene is important for regulation of proliferation capacity of this lineage [68]. *Zic1*—deficient (*Zic1−/−*) mice have hypoplastic cerebella with malformed folia. These abnormalities are associated with reduced proliferation of granule cells in the EGL [69]. Mice doubly heterozygous for mutations in both *Zic1* and *Zic2* (*Zic1+/−, Zic2+/kd*) showed cerebellar abnormalities similar to those found in mice homozygous for the *Zic1* mutation (*Zic1−/−*) indicating that mouse *Zic2* is also involved in the regulation of proliferation of cerebellar granule cells in cooperation with *Zic1* [70].

Cellular interactions are also important to maintain the proliferation of granule progenitors. Purkinje cells, in particular, are required for proliferation of granule cells in the EGL [71]. This regulation is mediated by the Shh signaling pathway. Gene expression studies determined that the secreted ligand Shh is expressed by Purkinje cells while granule cells express the Shh receptor Patched1 (Ptc1) [72,73]. In the absence of Shh, Ptc1 sends a signal to the nuclei of granule progenitors

repressing the transcription of genes that positively regulate cellular proliferation. When granule cells receive the Shh signal, the effect of Ptc1 is blocked and transcription of target genes is activated, stimulating proliferation. Mice heterozygous for *Ptc1* mutations (*Ptc1*+/−) have a high rate of medulloblastoma, a primitive neuroendocrine tumor that arises from granule cell progenitors [74]. Interestingly, human *Ptc* mutations are also associated with medulloblastoma [75].

The Notch signaling pathway has also been shown to control granule cell proliferation. *Notch2* is specifically expressed in proliferating granule progenitors in the EGL. Treatment of these cells with the soluble Notch ligand Jagged1, or overexpression of activated *Notch2* or its downstream target *Hes1*, maintains proliferation of granule progenitors. Of note, treatment of granule cell cultures with Shh leads to activation of the expression of *Hes1*, indicating that Hes1 may represent a point of cross-talk between the Notch2 and Shh signaling pathways [76].

Another group of genes critical for proper proliferation of the cerebellar cells are those encoding direct regulators of the cell cycle. One of these genes encodes a positive regulator of the cell cycle, cyclin D2. Targeted inactivation of *cyclin D2* in mouse causes reduction of the granule cell population because of decreased proliferation and increased apoptosis of granule progenitors, and results in cerebellar hypoplasia [77]. In contrast, inactivation of the negative regulator of the cell cycle, *p27Kip1* leads to an increased level of proliferation of cerebellar granule cells and formation of a larger cerebellum [78].

A recent study investigated the role of the *N-myc* protooncogene in cerebellar development by conditionally disrupting this gene in neuronal progenitor cells [79]. *N-myc* inactivation caused severe defects in proliferation of both granule progenitors and Purkinje cells. This is in contrast to many other genes mentioned above that specifically control proliferation of only one major cellular type in the cerebellum (e.g., granule neurons). Furthermore, in progenitor cell cultures derived from *N-myc* −/− embryonic brains, a dramatic increase in neuronal differentiation was observed compared with controls. Thus, *N-myc* simultaneously controls proliferation and differentiation of several classes of cerebellar progenitors most likely by regulating transcription of direct cell cycle regulators such as *cyclin D2* and *p27Kip1* [79].

Throughout the course of cerebellar development, a number of genes regulate neuronal number by promoting survival of granule neurons and Purkinje cells. For example, a basic helix-loop-helix transcription factor, *neuroD*, is highly expressed in the postmitotic premigratory granule cells regulating expression of genes important for the differentiation of this cellular type. Targeted inactivation of *neuroD* in the mouse cerebellum leads to extensive apoptosis of granule neurons [80] indicating that this gene is also important for the survival of postmitotic granule cells.

Analysis of mice with hypoplastic cerebellar neurodegenerative phenotypes, including *lurcher* and *hot-foot* (caused by mutation in *Grid2*, encoding δ2 glutamate receptor subunit), *tottering* (caused by mutation in α 1A calcium channel subunit gene), and *weaver* (caused by mutation in *Girk2*, encoding inward-rectifying K+ channel) have lead to the identification of several genes important for the survival of cerebellar cells during their differentiation. In all of these mutants, granule cell and Purkinje cell death occurs during differentiation as a result of mutations in genes encoding ional channel subunits [81,82]. This indicates that regulation of ionic homeostasis is critical for neuronal differentiation and survival. s*taggerer* is another spontaneous mutant mouse with a hypoplastic neurodegenerative cerebellar phenotype. This mutation is caused by the deletion of the retinoid-like orphan receptor α(*Ror*α) expressed in differentiating Purkinje cells [83]. In s*taggerer* mice, Purkinje cell development is initially normal, but Purkinje cells degenerate prior to completing terminal differentiation. This has been attributed to a block in their response to thyroid hormone [82].

Neuronal migration during cerebellar development

A number of molecular pathways have been implicated in migration of both Purkinje cells and granule neurons [84]. Analysis of the spontaneous neurological mouse mutant *rostral cerebellar malformation* (*rcm*) shed light on molecular mechanisms controlling early migration events in the embryonic cerebellum [85]. *rcm*/*rcm* animals have a hypoplastic cerebellar cortex and a reduction in the number of folia in the midline sagittal regions. This phenotype results from over-migration of granule cell progenitors and Purkinje cells during late embryogenesis. In the mutant, granule progenitors and Purkinje cells terminate their migration ectopically in the midbrain. Positional cloning revealed that the *rcm* locus encodes the mouse homologue of the *Caenorhabditis elegans unc-5* gene (*Unc5h3*), a receptor for mouse Netrin and established Unc5h3 and Netrin as the key regulators of the termination of migration of cerebellar cells *in vivo* [85,86]. Netrin is expressed in regions surrounding embryonic cerebellar territory. *Unc5h3* is expressed by both granule cells and Purkinje cells [87]. Chimeric studies and transplantation experiments, however, have shown that only *Unc5h3* expression in granule cells is necessary and sufficient to stop cellular migration at the anterior limit of the cerebellar territory. *Unc5h3* mutant granule progenitors that fail to correctly terminate their migration can non-cell autonomously attract wild-type Purkinje cells to ectopic positions [87,88]. This phenotype represents a dramatic example

of the interdependence of each cerebellar cell type to achieve proper development of the cerebellum.

Another gene important for proper migration of the cerebellar cells is *Pax6* [89]. *Pax6* is strongly expressed in the rhombic lip and in cells migrating away from it. Development of several rhombic lip derivatives is severely affected in *Pax6*−/− *(small eye)* mice. Although the initial differentiation and proliferation of the rhombic lip derived cells are not affected, cell migration and neurite extension are disrupted in *Pax6*−/− embryos. This causes abnormal formation of three out of five precerebellar nuclei and positioning of some granule cells in ectopic positions. Molecular analysis has revealed complete absence of *Unc5h3* expression in *Pax6*−/− granule cells [89] indicating that Pax6 activity, at least in part, is mediated through regulation of *Unc5h3*.

Several classes of ligand/receptor systems have been shown to regulate the migration of granule cells along radial glial fibers, as they migrate through the cerebellum to form the IGL. *Astrotactin* encodes a protein that contains epidermal growth factor (EGF) repeats and fibronectin type III domains. Antibodies against astrotactin have been shown to block migration of granule cells along astroglial fibers [90] and mice lacking *Astrotactin* show a reduction in granule cell migration and mild cerebellar hypoplasia [91]. While astrotactin has been identified as the neuronal component of a heterophilic neural–glial binding system for migration, its glial component remains unknown. Granule cells also express *Neuregulin*, while radial glia cells express its receptor, *erbB4.* When glial erbB receptors are blocked, granule cell migration along radial glial fibers is impaired [92]. Both the discoidin domain receptor 1 (Ddr1) and the mouse serine/threonine protein kinase, Unc5.1, have been implicated in initiation of granule cell axon extension, and thus, secondarily are also involved in regulation of migration of granule cells [93,94].

Migration of Purkinje cells from the ventricular zone depends on the Reelin pathway. The first member of this molecular pathway, Reelin, was uncovered by the fortuitous transgene insertion into the *reeler* locus [95]. Reelin is a large secreted protein that contains several EGF-like repeats [96]. In both spontaneous (*reeler*) and targeted *Reelin* mutant mice, Purkinje cells fail to migrate properly and remain largely situated in ectopic clusters beneath the granule cell layer resulting in a hypoplastic cerebellar phenotype [97]. Because *Reelin* is expressed in the marginal zone of the cerebellar anlage, above the site where Purkinje cells cease their migration, Reelin may function to terminate the initial migration of the immature Purkinje cells. Further analysis of this molecular pathway has demonstrated that the receptors for the reelin ligand, VLDLR and ApoER2 [98–100], and its downstream signaling transducer Dab1 [101,102] are also critical for the proper migration of Purkinje cells in the developing cerebellum. Analysis of *cerebellar deficient folia* (*cdf*) mice has revealed that αN-catenin, a protein linking cadherins to the neuronal cytoskeleton, is required intrinsically in Purkinje cells to properly direct their migration and cerebellar foliation [103–105]. It remains unknown, however if this gene acts within the reelin pathway.

Establishing cerebellar connectivity

The precise formation of the neuronal circuitry is the final step of cerebellar development and is necessary for the cerebellum to function as a coordination center. It has been well documented that establishing connectivity is closely associated with the terminal differentiation of cerebellar neurons [26], however, relatively little is known about the cellular and molecular mechanism that control this process.

Although the cerebellar circuitry described in Fig. 1C is reiterated across the entire cerebellum, the cerebellum is highly compartmentalized into distinct functional modules. Best described are longitudinal domains of corticonuclear and olivocerebellar projections onto Purkinje cells [106]. In both the developing and mature cerebellum, many genes expressed in Purkinje cells have longitudinal expression domains, which closely correlate with the afferent longitudinal domains. This has lead to the hypothesis that during development, a topographic map guides incoming cerebellar afferents by the matching of domain-specific labels between the incoming axons and Purkinje cells [107,108]. EphA receptors expressed in inferior olive axons and ephrin-A ligands expressed in Purkinje cell target domains have recently been demonstrated to be essential for correct axon targeting in the chick cerebellum [109]. It is hypothesized that a similar system operates in the mouse.

Purkinje cells receive input from climbing fibers from outside the cerebellum and granule cell axons from within the cerebellum. Purkinje cells are initially innervated by multiple climbing fibers. Elimination of supernumerary climbing fibers then occurs until each mature Purkinje cell is innervated by a single climbing fiber. Analysis of the spontaneous mutant, *hyperspiny Purkinje cell* (*hpc*) has revealed that this elimination process is controlled by a competition between climbing fibers translocating up from the base of the Purkinje cell body and parallel fibers from granule cells forming synapses at the apex of Purkinje cell dendrites [110]. The molecular identification of this locus is unknown, however, blockade of *N*-methyl-D-aspartate (NMDA) receptors during p15 and p16 results in a higher incidence of multiple climbing fiber innervation and causes loss of motor coordination [111], suggesting that

climbing fiber synapse elimination requires NMDA receptors during post-natal development. When the granule cell population is disrupted, Purkinje cell dendrites have abnormal morphology [71] providing additional evidence that granule cells directly influence Purkinje cell dendritic outgrowth. The molecular mechanisms of such interactions remain unknown. However, *Wnt3* probably participates in Purkinje cell dendrite development since it is expressed by Purkinje cells and its expression is modulated by interactions with granule cells [112].

Another member of Wnt family, *Wnt7a*, has been reported to have a role in contact formation between mossy fibers of the perecerebellar nuclei and granule neurons. Using both pharmacological and gene targeting experiments in mice it has been shown that granule cells secrete Wnt7a, which induces mossy fibers growth cone remodeling [113], indicating that Wnt7a can function as a synaptogenic factor.

Cerebellar development in mice and human cerebellar malformations

The delineation of the molecular and cellular mechanisms by which the cerebellum is formed is of interest from both a basic research and a clinical standpoint. Genetic and experimental manipulations in the mouse have greatly enhanced our knowledge regarding the mechanisms and molecules that drive cerebellar development. In contrast, our current understanding of the developmental basis of human cerebellar malformations is much less sophisticated, and their molecular basis remains largely unknown. Mutant analysis in the mouse, however, has identified multiple molecular mechanisms that cause phenotypes such as dysgenesis, hypoplasia, and/or developmental atrophy, phenotypes that are frequently observed in patients with cerebellar malformations. Until detailed pathological analyses of human malformations are available and their genetic loci identified, it is difficult to precisely determine which cell types and developmental processes are primarily affected. However, based on our knowledge of mouse cerebellar development, we can generate hypotheses as to which stages of cerebellar development are likely affected in some human cerebellar malformation disorders.

Small disruptions in isthmic formation and function can result in incorrect specification of medial cerebellar neurons, which can, in turn, lead to agenesis/digenesis of the inferior cerebellar vermis. Abnormal isthmic function is also associated with deletions and disruptions of the midbrain. These phenotypes are reminiscent of human molar tooth malformations (MTM) and suggest that MTM may be caused by abnormal AP patterning at the mid-hindbrain junction. Large mid-hindbrain deletions in the mouse are caused by complete loss of isthmus function such as the phenotypes associated with *Wnt1* and *Fgf8* null mutations. Equivalent phenotypes have never been described in humans and are most likely incompatible with life. Thus, if MTM loci do indeed have a role in isthmic formation and function, these loci are more likely to encode subtle modifiers of the isthmus rather than essential isthmic components.

Disruptions of the dorsal midline of the CNS can cause rhombic lip abnormalities, which may lead to aberrant granule cell specification and migration. Since the rhombic lip also gives rise to the neurons of the ventral pontine nuclei, pontine nuclei abnormalities are another likely outcome of dorsal midline disruptions. Other dorsal CNS midline structures can also be disrupted, including the corpus callosum and dorsal spinal cord. Interestingly, these abnormalities are often associated with Dandy–Walker malformation (DWM) and may indicate that the primary defect in DWM is a defect of the dorsal midline CNS development. A postulated etiology for the retrocerebellar cyst and hydrocephalous observed in DWM may be abnormal formation and/or function of the choroid plexus—the source of cerebral spinal fluid and a direct derivative of the CNS dorsal roof plate in the brain.

In the mouse, cerebellar hypoplasia is frequently associated with defects in proliferation, migration, and/or survival of cerebellar neurons. Interestingly, the general mechanisms regulating these processes are not restricted to the cerebellum but shared by other regions of the CNS. Thus, it is not surprising that cerebellar hypoplasia can also be associated with significant mental retardation and epilepsy in humans, where these symptoms are the most easily explained by aberrant cortical development and function.

Conclusion

Largely as a result of mouse mutant analysis, extensive progress has been made in understanding the cellular and molecular mechanisms directing cerebellar development. It is clear however, that much remains to be elucidated. Currently, numerous additional mouse mutants are being generated as a result of ongoing mouse mutagenesis programs worldwide. Molecular and phenotypic analysis of these and the many classical neurological mutants will continue to reveal important details of complex process of cerebellar development. Recent improvements in human brain imaging and malformation classification systems also provide an exciting opportunity to extend our genetic analysis to human cerebellar malformations. By combining the power of both the mouse and human genetic systems, the field of cerebellar development and neurogenetics promises to remain an exciting field of research.

Acknowledgments

We are grateful to Anne Lindgren, Inessa Grinberg, Ekaterina Steshina, and Melissa Parisi for helpful discussion of the manuscript. We also thank Phyllis Faust for the photograph included as Fig. 1B.

References

[1] M. Ito, The cerebellum and neural control, Raven, New York, 1984.
[2] J.H. Gao, L.M. Parsons, J.M. Bower, J. Xiong, J. Li, P.T. Fox, Cerebellum implicated in sensory acquisition and discrimination rather than motor control, Science 272 (1996) 545–547.
[3] J.A. Fiez, Cerebellar contributions to cognition, Neuron 16 (1996) 13–15.
[4] A. Bailey, P. Luthert, A. Dean, B. Harding, I. Janota, M. Montgomery, M. Rutter, P. Lantos, A clinicopathological study of autism, Brain 121 (1998) 889–905.
[5] G. Allen, E. Courchesne, Differential effects of developmental cerebellar abnormality on cognitive and motor functions in the cerebellum; an fMRI study of autism, Am. J. Psychiatry 160 (2003) 262–273.
[6] G. Allen, E. Courchesne, The cerebellum and non-motor function: clinical implications, Mol. Psychiatry 3 (1998) 207–210.
[7] M.A. Parisi, W.B. Dobyns, Human malformations of the mid-hindbrain, Mol. Genet. Metab. 80/1-2 (2003) 36–53.
[8] S. Patel, A.J. Barkovich, Analysis and classification of cerebellar malformations, Am. J. Neuroradiol. 23 (2002) 1074–1087.
[9] P. Demaerel, Abnormalities of cerebellar foliation and fissuration: classification, neurogenetics and clinicoradiological correlations, Neuroradiology 44 (2002) 639–646.
[10] V.Y. Wang, H.Y. Zoghbi, Genetic regulation of cerebellar development, Nat. Rev. Neurosci. 2 (2001) 484–491.
[11] C.Y. Saab, W.D. Willis, The cerebellum: organization, functions and its role in nociception, Brain Res. Brain Res. Rev. 42 (2003) 85–95.
[12] A.L. Joyner, Establishment of anterior–posterior and dorsal–ventral pattern in the early central nervous system, in: J. Rossant, P.P.L. Tam (Eds.), Mouse Development: Patterning, Morphogenesis and Organogenesis, Academic Press, San Diego, 2002, pp. 107–126.
[13] M.E. Hallonet, M.A. Teillet, N.M. Le Douarin, A new approach to the development of the cerebellum provided by the quail–chick marker system, Development 108 (1990) 19–31.
[14] M.E. Hallonet, N.M. Le Douarin, Tracing neuroepithelial cells of the mesencephalic and metencephalic alar plates during cerebellar ontogeny in quail-chick chimaeras, Eur. J. Neurosci. 5 (1993) 1145–1155.
[15] S. Millet, E. Bloch-Gallego, A. Simeone, R.M. Alvarado-Mallart, The caudal limit of Otx2 gene expression as a marker of the midbrain/hindbrain boundary: a study using in situ hybridisation and chick/quail homotopic grafts, Development 122 (1996) 3785–3797.
[16] R.J. Wingate, The rhombic lip and early cerebellar development, Curr. Opin. Neurobiol. 11 (2001) 82–88.
[17] R.M. Alvarado-Mallart, S. Martinez, C.C. Lance-Jones, Pluripotentiality of the 2-day-old avian germinative neuroepithelium, Dev. Biol. 139 (1990) 75–88.
[18] S. Martinez, M. Wassef, R.M. Alvarado-Mallart, Induction of a mesencephalic phenotype in the 2-day-old chick prosencephalon is preceded by the early expression of the homeobox gene en, Neuron 6 (1991) 971–981.
[19] F. Marin, L. Puelles, Patterning of the embryonic avian midbrain after experimental inversions: a polarizing activity from the isthmus, Dev. Biol. 163 (1994) 19–37.
[20] R.J. Wingate, M.E. Hatten, The role of the rhombic lip in avian cerebellum development, Development 126 (1999) 4395–4404.
[21] K.J. Millen, W. Wurst, K. Herrup, A.L. Joyner, Abnormal embryonic cerebellar development and patterning of postnatal foliation in two mouse Engrailed-2 mutants, Development 120 (1994) 695–706.
[22] J. Hanaway, Formation and differentiation of the external granular layer of the chick cerebellum, J. Comp. Neurol. 131 (1967) 1–14.
[23] J. Altman, S.A. Bayer, The Development of the Cerebellar System: In Relation in its Evolution, Structure and Function, CRC Press, New York, 1997.
[24] J. Alder, N.K. Cho, M.E. Hatten, Embryonic precursor cells from the rhombic lip are specified to a cerebellar granule neuron identity, Neuron 17 (1996) 389–399.
[25] J. Altman, S.A. Bayer, Embryonic development of the rat cerebellum. I. Delineation of the cerebellar primordium and early cell movements, J. Comp. Neurol. 231 (1985) 1–26.
[26] M.E. Hatten, N. Heintz, Mechanisms of neural patterning and specification in the developing cerebellum, Annu. Rev. Neurosci. 18 (1995) 385–408.
[27] M.E. Hatten, J. Alder, K. Zimmerman, N. Heintz, Genes involved in cerebellar cell specification and differentiation, Curr. Opin. Neurobiol. 7 (1997) 40–47.
[28] S.L. Baader, S. Sanlioglu, A.S. Berrebi, J. Parker-Thornburg, J. Oberdick, Ectopic overexpression of engrailed-2 in cerebellar Purkinje cells causes restricted cell loss and retarded external germinal layer development at lobule junctions, J. Neurosci. 18 (1998) 1763–1773.
[29] A. Simeone, Positioning the isthmic organizer where Otx2 and Gbx2 meet, Trends Genet. 16 (2000) 237–240.
[30] M. Rhinn, M. Brand, The midbrain–hindbrain boundary organizer, Curr. Opin. Neurobiol. 11 (2001) 34–42.
[31] W. Wurst, L. Bally-Cuif, Neural plate patterning: upstream and downstream of the isthmic organizer, Nat. Rev. Neurosci. 2 (2001) 99–108.
[32] A. Simeone, D. Acampora, M. Gulisano, A. Stornaiuolo, E. Boncinelli, Nested expression domains of four homeobox genes in developing rostral brain, Nature 358 (1992) 687–690.
[33] K.M. Wassarman, M. Lewandoski, K. Campbell, A.L. Joyner, J.L. Rubenstein, S. Martinez, G.R. Martin, Specification of the anterior hindbrain and establishment of a normal mid/hindbrain organizer is dependent on Gbx2 gene function, Development 124 (1997) 2923–2934.
[34] S. Millet, K. Campbell, D.J. Epstein, K. Losos, E. Harris, A.L. Joyner, A role for Gbx2 in repression of Otx2 and positioning the mid/hindbrain organizer, Nature 401 (1999) 161–164.
[35] D. Acampora, V. Avantaggiato, F. Tuorto, A. Simeone, Genetic control of brain morphogenesis through Otx gene dosage requirement, Development 124 (1997) 3639–3650.
[36] D. Acampora, V. Avantaggiato, F. Tuorto, P. Briata, G. Corte, A. Simeone, Visceral endoderm-restricted translation of Otx1 mediates recovery of Otx2 requirements for specification of anterior neural plate and normal gastrulation, Development 125 (1998) 5091–5104.
[37] Y. Suda, I. Matsuo, S. Aizawa, Cooperation between Otx1 and Otx2 genes in developmental patterning of rostral brain, Mech. Dev. 69 (1997) 125–141.
[38] M. Rhinn, A. Dierich, W. Shawlot, R.R. Behringer, M. Le Meur, S.L. Ang, Sequential roles for Otx2 in visceral endoderm and neuroectoderm for forebrain and midbrain induction and specification, Development 125 (1998) 845–856.

[39] V. Broccoli, E. Boncinelli, W. Wurst, The caudal limit of Otx2 expression positions the isthmic organizer, Nature 401 (1999) 164–168.
[40] P.H. Crossley, S. Martinez, G.R. Martin, Midbrain development induced by FGF8 in the chick embryo, Nature 380 (1996) 66–68.
[41] S. Martinez, P.H. Crossley, I. Cobos, J.L. Rubenstein, G.R. Martin, FGF8 induces formation of an ectopic isthmic organizer and isthmocerebellar development via a repressive effect on Otx2 expression, Development 126 (1999) 1189–1200.
[42] H. Shamim, R. Mahmood, C. Logan, P. Doherty, A. Lumsden, I. Mason, Sequential roles for Fgf4, En1 and Fgf8 in specification and regionalisation of the midbrain, Development 126 (1999) 945–959.
[43] C. Irving, I. Mason, Signalling by FGF8 from the isthmus patterns anterior hindbrain and establishes the anterior limit of Hox gene expression, Development 127 (2000) 177–186.
[44] A. Liu, K. Losos, A.L. Joyner, FGF8 can activate Gbx2 and transform regions of the rostral mouse brain into a hindbrain fate, Development 126 (1999) 4827–4838.
[45] P.H. Crossley, G.R. Martin, The mouse Fgf8 gene encodes a family of polypeptides and is expressed in regions that direct outgrowth and patterning in the developing embryo, Development 121 (1995) 439–451.
[46] E.N. Meyers, M. Lewandoski, G.R. Martin, An Fgf8 mutant allelic series generated by Cre- and Flp-mediated recombination, Nat. Genet. 18 (1998) 136–141.
[47] D.H. Rowitch, A.P. McMahon, Pax-2 expression in the murine neural plate precedes and encompasses the expression domains of Wnt-1 and En-1, Mech. Dev. 52 (1995) 3–8.
[48] A.P. McMahon, A. Bradley, The Wnt-1 (int-1) proto-oncogene is required for development of a large region of the mouse brain, Cell 62 (1990) 1073–1085.
[49] K.R. Thomas, M.R. Capecchi, Targeted disruption of the murine int-1 proto-oncogene resulting in severe abnormalities in midbrain and cerebellar development, Nature 346 (1990) 847–850.
[50] M.E. Dickinson, R. Krumlauf, A.P. McMahon, Evidence for a mitogenic effect of Wnt-1 in the developing mammalian central nervous system, Development 120 (1994) 1453–1471.
[51] E. Matsunaga, T. Katahira, H. Nakamura, Role of Lmx1b and Wnt1 in mesencephalon and metencephalon development, Development 129 (2002) 5269–5277.
[52] K.R. Thomas, T.S. Musci, P.E. Neumann, M.R. Capecchi, Swaying is a mutant allele of the proto-oncogene Wnt-1, Cell 67 (1991) 969–976.
[53] J.L. Gomez-Skarmeta, J. Modolell, Iroquois genes: genomic organization and function in vertebrate neural development, Curr. Opin. Genet. Dev. 12 (2002) 403–408.
[54] A. Gavalas, M. Davenne, A. Lumsden, P. Chambon, F.M. Rijli, Role of Hoxa-2 in axon pathfinding and rostral hindbrain patterning, Development 124 (1997) 3693–3702.
[55] K.J. Lee, T.M. Jessell, The specification of dorsal cell fates in the vertebrate central nervous system, Annu. Rev. Neurosci. 22 (1999) 261–294.
[56] A.W. Helms, J.E. Johnson, Specification of dorsal spinal cord interneurons, Curr. Opin. Neurobiol. 13 (2003) 42–49.
[57] R. Shirasaki, S.L. Pfaff, Transcriptional codes and the control of neuronal identity, Annu. Rev. Neurosci. 25 (2002) 251–281.
[58] J. Alder, K.J. Lee, T.M. Jessell, M.E. Hatten, Generation of cerebellar granule neurons in vivo by transplantation of BMP-treated neural progenitor cells, Nat. Neurosci. 2 (1999) 535–540.
[59] J.H. Millonig, K.J. Millen, M.E. Hatten, The mouse Dreher gene Lmx1a controls formation of the roof plate in the vertebrate CNS, Nature 403 (2000) 764–769.
[60] X.M. Zhang, E. Lin, X.J. Yang, Sonic hedgehog-mediated ventralization disrupts formation of the midbrain-hindbrain junction in the chick embryo, Dev. Neurosci. 22 (2000) 207–216.
[61] E. Belloni, M. Muenke, E. Roessler, G. Traverso, J. Siegel-Bartelt, A. Frumkin, H.F. Mitchell, H. Donis-Keller, C. Helms, A.V. Hing, H.H. Heng, B. Koop, D. Martindale, J.M. Rommens, L.C. Tsui, S.W. Scherer, Identification of Sonic hedgehog as a candidate gene responsible for holoprosencephaly, Nat. Genet. 14 (1996) 353–356.
[62] N. Ben-Arie, H.J. Bellen, D.L. Armstrong, A.E. McCall, P.R. Gordadze, Q. Guo, M.M. Matzuk, H.Y. Zoghbi, Math1 is essential for genesis of cerebellar granule neurons, Nature 390 (1997) 169–172.
[63] N. Ben-Arie, B.A. Hassan, N.A. Bermingham, D.M. Malicki, D. Armstrong, M. Matzuk, H.J. Bellen, H.Y. Zoghbi, Functional conservation of atonal and Math1 in the CNS and PNS, Development 127 (2000) 1039–1048.
[64] M.E. Ross, C. Fletcher, C.A. Mason, M.E. Hatten, N. Heintz, Meander tail reveals a discrete developmental unit in the mouse cerebellum, Proc. Natl. Acad. Sci. USA 87 (1990) 4189–4192.
[65] K.M. Hamre, D. Goldowitz, Meander tail acts intrinsic to granule cell precursors to disrupt cerebellar development: analysis of meander tail chimeric mice, Development 124 (1997) 4201–4212.
[66] C.M. Rosario, B.D. Yandava, B. Kosaras, D. Zurakowski, R.L. Sidman, E.Y. Snyder, Differentiation of engrafted multipotent neural progenitors towards replacement of missing granule neurons in meander tail cerebellum may help determine the locus of mutant gene action, Development 124 (1997) 4213–4224.
[67] K.J. Millen, J.H. Millonig, R.J. Wingate, J. Alder, M.E. Hatten, Neurogenetics of the cerebellar system, J. Child. Neurol. 14 (1999) 574–581.
[68] X.W. Yang, C. Wynder, M.L. Doughty, N. Heintz, BAC-mediated gene-dosage analysis reveals a role for Zipro1 (Ru49/Zfp38) in progenitor cell proliferation in cerebellum and skin, Nat. Genet. 22 (1999) 327–335.
[69] J. Aruga, O. Minowa, H. Yaginuma, J. Kuno, T. Nagai, T. Noda, K. Mikoshiba, Mouse Zic1 is involved in cerebellar development, J. Neurosci. 18 (1998) 284–293.
[70] J. Aruga, T. Inoue, J. Hoshino, K. Mikoshiba, Zic2 controls cerebellar development in cooperation with Zic1, J. Neurosci. 22 (2002) 218–225.
[71] D. Goldowitz, K. Hamre, The cells and molecules that make a cerebellum, Trends Neurosci. 21 (1998) 375–382.
[72] J. Oberdick, K. Schilling, R.J. Smeyne, J.G. Corbin, C. Bocchiaro, J.I. Morgan, Control of segment-like patterns of gene expression in the mouse cerebellum, Neuron 10 (1993) 1007–1018.
[73] R.J. Smeyne, T. Chu, A. Lewin, F. Bian, S.C. Kunsch, S.A. Lira, J. Oberdick, Local control of granule cell generation by cerebellar Purkinje cells, Mol. Cell. Neurosci. 6 (1995) 230–251.
[74] R.B. Corcoran, M.P. Scott, A mouse model for medulloblastoma and basal cell nevus syndrome, J. Neurooncol. 53 (2001) 307–318.
[75] W.A. Weiss, Genetics of brain tumors, Curr. Opin. Pediatr. 12 (2000) 543–548.
[76] D.J. Solecki, X.L. Liu, T. Tomoda, Y. Fang, M.E. Hatten, Activated Notch2 signaling inhibits differentiation of cerebellar granule neuron precursors by maintaining proliferation, Neuron 31 (2001) 557–568.
[77] J.M. Huard, C.C. Forster, M.L. Carter, P. Sicinski, M.E. Ross, Cerebellar histogenesis is disturbed in mice lacking cyclin D2, Development 126 (1999) 1927–1935.
[78] K. Miyazawa, T. Himi, V. Garcia, H. Yamagishi, S. Sato, Y. Ishizaki, A role for p27/Kip1 in the control of cerebellar granule cell precursor proliferation, J. Neurosci. 20 (2000) 5756–5763.
[79] P.S. Knoepfler, P.F. Cheng, R.N. Eisenman, N-myc is essential during neurogenesis for the rapid expansion of progenitor cell

populations and the inhibition of neuronal differentiation, Genes Dev. 16 (2002) 2699–2712.
[80] T. Miyata, T. Maeda, J.E. Lee, NeuroD is required for differentiation of the granule cells in the cerebellum and hippocampus, Genes Dev. 13 (1999) 1647–1652.
[81] N. Heintz, H.Y. Zoghbi, Insights from mouse models into the molecular basis of neurodegeneration, Annu. Rev. Physiol. 62 (2000) 779–802.
[82] R. Lalonde, C. Strazielle, Motor performance and regional brain metabolism of spontaneous murine mutations with cerebellar atrophy, Behav. Brain Res. 125 (2001) 103–108.
[83] B.A. Hamilton, W.N. Frankel, A.W. Kerrebrock, T.L. Hawkins, W. FitzHugh, K. Kusumi, L.B. Russell, K.L. Mueller, V. van Berkel, B.W. Birren, L. Kruglyak, E.S. Lander, Disruption of the nuclear hormone receptor RORalpha in staggerer mice, Nature 379 (1996) 736–739.
[84] M.E. Hatten, Central nervous system neuronal migration, Annu. Rev. Neurosci. 22 (1999) 511–539.
[85] S.L. Ackerman, L.P. Kozak, S.A. Przyborski, L.A. Rund, B.B. Boyer, B.B. Knowles, The mouse rostral cerebellar malformation gene encodes an UNC-5-like protein, Nature 386 (1997) 838–842.
[86] E.D. Leonardo, L. Hinck, M. Masu, K. Keino-Masu, S.L. Ackerman, M. Tessier-Lavigne, Vertebrate homologues of *C. elegans* UNC-5 are candidate netrin receptors, Nature 386 (1997) 833–838.
[87] S.A. Przyborski, B.B. Knowles, S.L. Ackerman, Embryonic phenotype of Unc5h3 mutant mice suggests chemorepulsion during the formation of the rostral cerebellar boundary, Development 125 (1998) 41–50.
[88] D. Goldowitz, K.M. Hamre, S.A. Przyborski, S.L. Ackerman, Granule cells and cerebellar boundaries: analysis of Unc5h3 mutant chimeras, J. Neurosci. 20 (2000) 4129–4137.
[89] D. Engelkamp, P. Rashbass, A. Seawright, V. van Heyningen, Role of Pax6 in development of the cerebellar system, Development 126 (1999) 3585–3596.
[90] C. Zheng, N. Heintz, M.E. Hatten, CNS gene encoding astrotactin, which supports neuronal migration along glial fibers, Science 272 (1996) 417–419.
[91] N.C. Adams, T. Tomoda, M. Cooper, G. Dietz, M.E. Hatten, Mice that lack astrotactin have slowed neuronal migration, Development 129 (2002) 965–972.
[92] C. Rio, H.I. Rieff, P. Qi, T.S. Khurana, G. Corfas, Neuregulin and erbB receptors play a critical role in neuronal migration, Neuron 19 (1997) 39–50.
[93] R.S. Bhatt, T. Tomoda, Y. Fang, M.E. Hatten, Discoidin domain receptor 1 functions in axon extension of cerebellar granule neurons, Genes Dev. 14 (2000) 2216–2228.
[94] T. Tomoda, R.S. Bhatt, H. Kuroyanagi, T. Shirasawa, M.E. Hatten, A mouse serine/threonine kinase homologous to *C. elegans* UNC51 functions in parallel fiber formation of cerebellar granule neurons, Neuron 24 (1999) 833–846.
[95] G. D'Arcangelo, G.G. Miao, S.C. Chen, H.D. Soares, J.I. Morgan, T. Curran, A protein related to extracellular matrix proteins deleted in the mouse mutant reeler, Nature 374 (1995) 719–723.
[96] G. D'Arcangelo, K. Nakajima, T. Miyata, M. Ogawa, K. Mikoshiba, T. Curran, Reelin is a secreted glycoprotein recognized by the CR-50 monoclonal antibody, J. Neurosci. 17 (1997) 23–31.
[97] D.S. Rice, T. Curran, Mutant mice with scrambled brains: understanding the signaling pathways that control cell positioning in the CNS, Genes Dev. 13 (1999) 2758–2773.
[98] G. D'Arcangelo, R. Homayouni, L. Keshvara, D.S. Rice, M. Sheldon, T. Curran, Reelin is a ligand for lipoprotein receptors, Neuron 24 (1999) 471–479.
[99] T. Hiesberger, M. Trommsdorff, B.W. Howell, A. Goffinet, M.C. Mumby, J.A. Cooper, J. Herz, Direct binding of Reelin to VLDL receptor and ApoE receptor 2 induces tyrosine phosphorylation of disabled-1 and modulates tau phosphorylation, Neuron 24 (1999) 481–489.
[100] M. Trommsdorff, M. Gotthardt, T. Hiesberger, J. Shelton, W. Stockinger, J. Nimpf, R.E. Hammer, J.A. Richardson, J. Herz, Reeler/Disabled-like disruption of neuronal migration in knockout mice lacking the VLDL receptor and ApoE receptor 2, Cell 97 (1999) 689–701.
[101] M. Sheldon, D. Rice, G. D'Arcangelo, H. Yoneshima, K. Nakajima, K. Mikoshiba, B. Howell, J. Cooper, D. Goldowitz, T. Curran, Scrambler and yotari disrupt the disabled gene and produce a reeler-like phenotype in mice, Nature 389 (1997) 730–733.
[102] B. Howell, R. Hawkes, P. Soriano, J. Cooper, Neuronal position in the developing brain is regulated by mouse disabled-1, Nature 389 (1997) 733–737.
[103] C. Park, W. Falls, J.H. Finger, C.M. Longo-Guess, S.L. Ackerman, Deletion in Catna2, encoding alpha N-catenin, causes cerebellar and hippocampal lamination defects and impaired startle modulation, Nat. Genet. 31 (2002) 279–284.
[104] C. Park, J.H. Finger, J.A. Cooper, S.L. Ackerman, The cerebellar deficient folia (cdf) gene acts intrinsically in Purkinje cell migrations, Genesis 32 (2002) 32–41.
[105] E. Beierbach, C. Park, S.L. Ackerman, D. Goldowitz, R. Hawkes, Abnormal dispersion of a purkinje cell subset in the mouse mutant cerebellar deficient folia (cdf), J. Comp. Neurol. 436 (2001) 42–51.
[106] J. Voogd, M. Glickstein, The anatomy of the cerebellum, Trends Neurosci. 21 (1998) 370–375.
[107] M. Wassef, B. Cholley, C.W. Heizmann, C. Sotelo, Development of the olivocerebellar projection in the rat: II. Matching of the developmental compartmentations of the cerebellum and inferior olive through the projection map, J. Comp. Neurol. 323 (1992) 537–550.
[108] C. Sotelo, A. Chedotal, Development of the olivocerebellar projection, Perspect Dev. Neurobiol. 5 (1997) 57–67.
[109] K. Nishida, J.G. Flanagan, M. Nakamoto, Domain-specific olivocerebellar projection regulated by the EphA-ephrin- A interaction, Development 129 (2002) 5647–5658.
[110] C. Sotelo, Cerebellar synaptogenesis: what we can learn from mutant mice, J. Exp. Biol. 153 (1990) 225–249.
[111] S. Kakizawa, M. Yamasaki, M. Watanabe, M. Kano, Critical period for activity-dependent synapse elimination in developing cerebellum, J. Neurosci. 20 (2000) 4954–4961.
[112] P.C. Salinas, C. Fletcher, N.G. Copeland, N.A. Jenkins, R. Nusse, Maintenance of Wnt-3 expression in Purkinje cells of the mouse cerebellum depends on interactions with granule cells, Development 120 (1994) 1277–1286.
[113] A.C. Hall, F.R. Lucas, P.C. Salinas, Axonal remodeling and synaptic differentiation in the cerebellum is regulated by WNT-7a signaling, Cell 100 (2000) 525–535.