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Review Article

Development and evolution of cerebellar neural circuits

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The cerebellum controls smooth and skillful movements and it is also involved in higher cognitive and emotional functions. The cerebellum is derived from the dorsal part of the anterior hindbrain and contains two groups of cerebellar neurons: glutamatergic and gamma-aminobutyric acid (GABA)ergic neurons. Purkinje cells are GABAergic and granule cells are glutamatergic. Granule and Purkinje cells receive input from outside of the cerebellum from mossy and climbing fibers. Genetic analysis of mice and zebrafish has revealed genetic cascades that control the development of the cerebellum and cerebellar neural circuits. During early neurogenesis, rostrocaudal patterning by intrinsic and extrinsic factors, such as *Otx2*, *Gbx2* and *Fgf8*, plays an important role in the positioning and formation of the cerebellar primordium. The cerebellar glutamatergic neurons are derived from progenitors in the cerebellar rhombic lip, which express the proneural gene *Atoh1*. The GABAergic neurons are derived from progenitors in the ventricular zone, which express the proneural gene *Ptf1a*. The mossy and climbing fiber neurons originate from progenitors in the hindbrain rhombic lip that express *Atoh1* or *Ptf1a*. Purkinje cells exhibit mediolateral compartmentalization determined on the birthdate of Purkinje cells, and linked to the precise neural circuitry formation. Recent studies have shown that anatomy and development of the cerebellum is conserved between mammals and bony fish (teleost species). In this review, we describe the development of cerebellar neurons and neural circuitry, and discuss their evolution by comparing developmental processes of mammalian and teleost cerebellum.

Key words: cerebellum, compartmentalization, evolution, granule cells, Purkinje cells.

Introduction

The cerebellum, a structure derived from the dorsal part of the most anterior hindbrain, functions in the control of smooth and skillful movements. It is also implicated in higher cognitive and emotional functions (Ito 2008). The cerebellum integrates sensory and predictive inputs, which include proprioception and information associated with motor commands, to elicit precise motor control and modulate higher cognitive/emotional functions (Ito 2002a,b, 2006, 2008). The functions of the cerebellum rely on its well organized and evolutionarily conserved structure and circuitry.

The cerebellum contains several different types of neurons, which are categorized according to their function as excitatory or inhibitory neurons (Butler & Hodos 1996; Altman & Bayer 1997) (Fig. 1). The excitatory neurons use glutamate as their major neurotransmitter (glutamatergic neurons). They include the granule cells (GCs), unipolar brush cells (UBC), and excitatory projection neurons; that is, large neurons in the deep cerebellar nuclei (DCN) in mammals or eurydendroid cells in teleosts. The inhibitory neurons use gamma-aminobutyric acid (GABA) and/or glycine (GABAergic neurons), and include Purkinje cells (PCs), Golgi cells, Lugaro cells, candelabrum cells, basket cells, stellate cells, and small neurons in the DCN (Laine & Axelrad 1994; Butler & Hodos 1996; Altman & Bayer 1997; Voogd & Glickstein 1998; Sillitoe & Joyner 2007). Additionally, there are astrocytes, Bergmann glia (a specific type of astrocyte in the cerebellum), and oligodendrocytes in the cerebellum. These neurons and glia are arranged in a three-layer structure in the cerebellum, from

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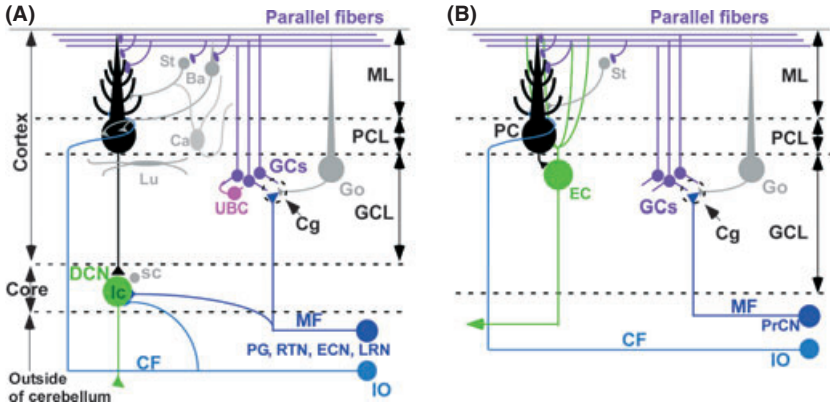

Fig. 1. Structure of cerebellar neural circuits in mouse and zebrafish. Schematic representation of cerebellar neurons and neural circuits in the mouse (A) and zebrafish (B) cerebellum. Ba, basket cell; Ca, candelabrum cell; CF, climbing fiber; Cg, cerebellar glomeruli; DCN, deep cerebellar nuclei (lc, large cell; sc, small cell); EC, eurydendroid cell; ECN, external cuneate nuclei; GCs, granule cells; GCL, granule cell layer; Go, Golgi cell; IO, inferior olive nuclei; Lu, Lugaro cell; LRN, lateral reticular nuclei; MF, mossy fiber; ML, molecular layer; PC, Purkinje cell; PCL, Purkinje cell layer; PG, pontine gray nuclei; PrCN, precerebellar nuclei (except IO); RTN, reticulotegmental nuclei; St, stellate cells; UBC, unipolar brush cell.

superficial to deep: the molecular layer (ML), Purkinje cell layer (PCL), and granule cell layer (GCL) (Figs 1, 3). These layers are located over the inner core composed of the white matter and three pairs of the DCN (there is no white matter or DCN in the teleost cerebellum). The ML contains the dendrites of PCs, axons of GCs (parallel fibers), and basket and stellate interneurons in addition to the fibers of Bergmann glia. The PCL contains the somata of PCs, Bergmann glia, and candelabrum interneurons. The GCL contains numerous small GCs, and the somata of Golgi cells, Lugaro cells, and UBCs. Many of these cerebellar neurons and glia are known to be conserved among mammalian species, although the appearance of candelabrum and basket cells in the teleost cerebellum has not been reported (Butler & Hodos 1996; Altman & Bayer 1997). The eurydendroid cells in the teleost cerebellum are located in the GCL near the PCs, and they receive the axons projected from PCs (Murakami & Morita 1987; Ikenaga *et al.* 2005). Unlike the DCN, the eurydendroid cells extend their dendrites to the ML to receive inputs from GCs via parallel fibers.

The cerebellum of higher vertebrates, including mammalian and avian species, has 10 lobules (I–X), and each lobule contains the three-layer structure (Butler & Hodos 1996; Altman & Bayer 1997). In contrast, the teleost cerebellum is composed of three major lobular structures: the valvula cerebelli (Va, anterior lobe), the corpus cerebelli (CCe, main lobe), and the vestibulo-lateral lobe (caudo-lateral lobe), which consists of the eminentia granularis (EG) and the lobus caudalis cerebelli (LCa) (Butler & Hodos 1996; Wullimann *et al.* 1996; Altman & Bayer 1997; Bae *et al.* 2009) (Fig. 3A,B). The anterior and main lobes have the same three-layer structure, whereas the caudo-lateral lobe contains only a GCL in the teleost cerebellum.

The cerebellar neurons receive excitatory input from neurons in the precerebellar nuclei outside the cerebellum. There are two main types of afferent inputs, the climbing fibers (CFs) and mossy fibers (MFs). The CFs originate exclusively from the contralateral side of the inferior olive nuclei (IO) in the caudoventral hindbrain, and innervate the proximal domain of PC dendrites in the ML. The MFs originate from neurons in the precerebellar nuclei, including pontine gray matter nuclei (PG), reticulotegmental nuclei (RTN), the external cuneate nuclei (ECN), and lateral reticulate nuclei (LRN), and synapse with GC dendrites that are in contact with the axons of Golgi cells, to form the cerebellar glomeruli. Information from MFs is conveyed to the dendrites of PCs by the axons of GCs. The information from CFs and MFs is integrated by the PCs. Neural activity of CFs suppresses synaptic transmission from parallel fibers, when the PCs receive these inputs simultaneously, by a mechanism called long-term depression (LTD). LTD is known to play an important role in motor learning (Ito 2002a,b, 2006). PCs send their axons to neurons in the DCN in mammals and eurydendroid cells in teleosts (also to adjacent PCs, at least at early stages) (Alonso *et al.* 1992; Meek *et al.* 1992; Butler & Hodos 1996; Altman & Bayer 1997). These projection neurons (DCN and eurydendroid cells) send their axons to other regions of the brain.

Although the cerebellar neural circuits are basically conserved between mammalian and teleost species, there are some differences. The location and cell morphology of the projection neurons (DCN vs. eurydendroid cells) are different (Fig. 1). Furthermore, all parallel

fibers of GCs contact the PC dendrites in the mammalian cerebellum, whereas GCs in the caudo-lateral lobe of the teleost cerebellum extend their parallel fibers to the dorsal hindbrain (crista cerebellaris, CC), where the parallel fibers contact the dendrites of crest cells, whose somata are located in the medial octavolateralis nucleus (MON) (Mikami *et al.* 2004; Bell *et al.* 2008; Bae *et al.* 2009) (Fig. 3B,C). This circuit functions as part of the cerebellum (or "cerebellum-like structure"), similar to that found in the flocculonodular lobe in the mammalian cerebellum (Altman & Bayer 1997; Bell *et al.* 2008).

A cerebellar structure is found in cartilaginous fish, although it is slightly different from the teleost cerebellum. Among agnathans, the lamprey cerebellum contains GCs and cells that are reminiscent of PCs, and the cerebellum appears as a simple bridge of gray matter between the right and left sides of the anterior hindbrain (Nieuwenhuys 1967; Butler & Hodos 1996; Altman & Bayer 1997). Therefore, the cerebellum is an ancient component of the vertebrate brain.

Regionalization during early development: a set location of the cerebellum on the neural tube

The neuroanatomic topography of the developing cerebellum reveals the formation of distinct structural patterns along its dorsoventral and mediolateral (M-L) axes. The complex formation of the central nervous system begins from the formation of the neural tube in the embryo. A portion of this neural tube is specialized during development, and begins to secrete signaling elements along the antero-posterior and dorso-ventral axes of the neural tube. The secretory elements specify positional information along the neural tube. From this nascent positional information, the neural tube establishes the neurodevelopmental regions along the antero-posterior and dorso-ventral axes. For instance, the hindbrain is compartmentalized into rhombomeres, and the telencephalon and diencephalon are compartmentalized into prosomeres. These early compartments ultimately develop into the complex brain. The cerebellum also goes through the process of regionalization during development (Zervas *et al.* 2005).

Genetic manipulations in mice and analysis of zebrafish mutants have revealed the molecular mechanisms of cerebellar morphogenesis (Wilson *et al.* 2002; Raible & Brand 2004; Zervas *et al.* 2005). During cerebellar development, several key molecules are expressed in restricted regions of the embryonic mouse brain and define the midbrain-hindbrain boundary (MHB). First, two homeobox genes, *Otx2* and *Gbx2*, are independently expressed in the neural plate early in development. The expression of *Otx2* and *Gbx2* is observed in the anterior and posterior regions of the neural plate, respectively (Simeone *et al.* 1992, 2002; Joyner *et al.* 2000; Simeone 2000; Liu & Joyner 2001; Wurst & Bally-Cuif 2001; Nakamura *et al.* 2005). The boundaries of *Otx2* and *Gbx2* gene expression become clearer by embryonic day (E) 8.5 in mice. They define the future position of the MHB (Bally-Cuif & Wassef 1994; Acampora *et al.* 1997). In zebrafish, *gbx1* is expressed earlier than *gbx2*, and plays a more important role in positioning the MHB (Rhinn *et al.* 2009). Subsequently, a set of MHB genes, including *Pax2* (*pax2a* in zebrafish), *Engrailed (En) 1/2* (*eng2a/2b* in zebrafish), and *Wnt1* and *Fgf8* are expressed in the midbrain and hindbrain domains. *Wnt1* and *Fgf8* are expressed in the *Otx2*-positive (+) region (blue region in Fig. 2A) and the $Gbx2^{+}$ region (green region in Fig. 2A), respectively. In mice, the patterns of *Wnt1* gene (red region in Fig. 2A) and *Fgf8* gene (yellow region in Fig. 2A) expression tightens at E9.5 when the neural plate closes and forms the neural tube. *Wnt1* gene expression (red region in Fig. 2A) is localized to the caudal edge of the mesencephalon (mes). In contrast, *Fgf8* gene expression (yellow region in Fig. 2A) is localized to the rostral edge of rhombomere 1 (r1). In addition, *Pax5*, *Pax8*, and *Fgf17* genes are activated in the MHB, and this activation is dependent on the function of Pax2 (Pax2a in zebrafish) and Fgf8 (Lun & Brand 1998; Pfeffer *et al.* 1998; Reifers *et al.* 1998, 2000; Bouchard *et al.* 2000; Xu *et al.* 2000). *Otx2*-driven *Wnt1* gene expression and *Gbx2*-driven *Fgf8* gene expression provide positional information in the neural tube along the antero-posterior axis and facilitates regional specification. Consequently, the localized expression of *Wnt1* and *Fgf8* define the boundary between midbrain and hindbrain. *Wnt1* expression probably controls the expression of *En 1/2* in the MHB and r1 and the proliferation of the midbrain and anterior hindbrain regions (Dickinson *et al.* 1994; Danielian & McMahon 1996). In zebrafish, in addition to *wnt1*, *wnt10b* and *wnt3a* are also expressed in overlapping domains across the MHB, and these *wnt* genes are required for the formation of the MHB constriction and for preventing apoptosis in the MHB region (Buckles *et al.* 2004). The $Otx2^{+}$ region (blue region in Fig. 2A) is the future forebrain and midbrain, and the $Gbx2^{+}$ region (green region in Fig. 2A) is the future hindbrain and spinal cord. Furthermore, the MHB arises from the signaling between *Otx2*/*Wnt1* and *Gbx2*/*Fgf8* gene expression. The $Fgf8^{+}$ region curves toward the inside of the brain and forms a neck identified as the isthmus. *Fgf8* is required for the formation of the isthmic structure and expression of MHB-specific genes (Crossley *et al.* 1996; Reifers *et al.* 1998; Liu *et al.* 1999; Marti-

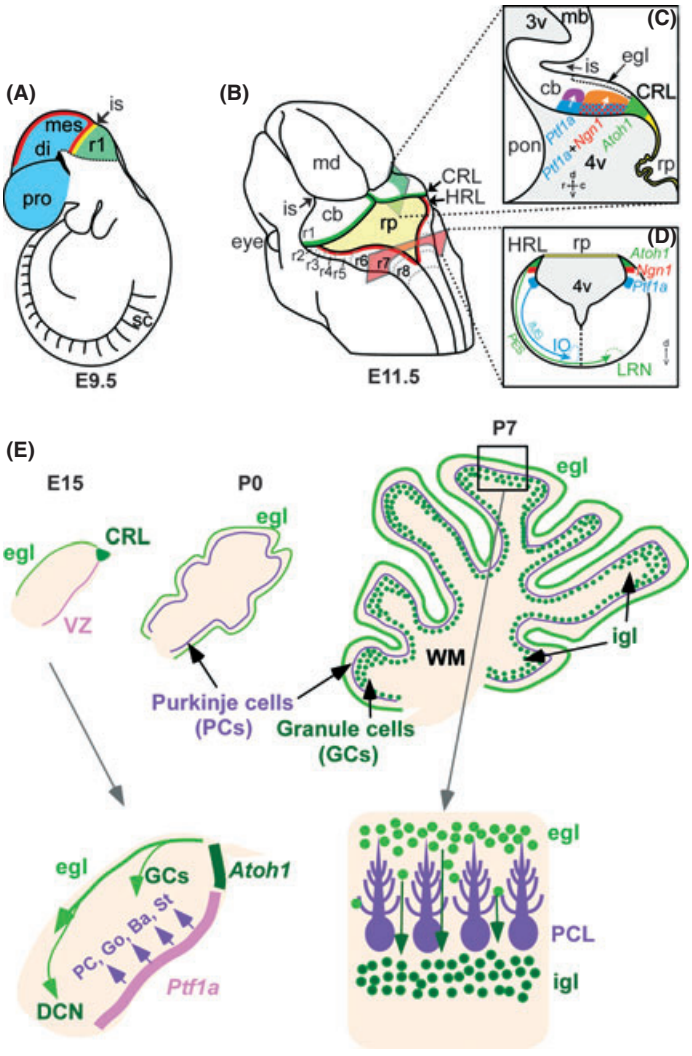

Fig. 2. Cerebellar and precerebellar nuclei development in mice. (A) A schematic view of the mouse embryo at E9.5. The regional expression of transcription factor genes *Otx2* (blue), *Wnt1* (red), *Fgf8* (yellow), and *Gbx2* (green) in the E9.5 mouse embryo is shown. The spatial and temporal expression of these transcription factors and morphogens defines the midbrain–hindbrain boundary and induces the isthmus (is) to become the organizing center for early development of the midbrain and hindbrain. Secretory factors from the isthmus induce the formation of the cerebellum (cb) from rhombomere 1 (r1). (B) A schematic view of the posterolateral side of the mouse embryo at E11.5. The cerebellar rhombic lip (CRL) is located at the caudal edge of the cerebellum, the hindbrain rhombic lip (HRL) is located at the dorsal edge of the hindbrain in r2–r8, and the roof plate (rp) is located between CRL and HRL colored with green, red, and yellow, respectively. (C) A schematic view of a sagittal section of the cb along the green plane shown in (B). The ventricular zone (VZ) of the cerebellum, CRL, and rp are colored blue, green, and yellow, respectively. The cerebellum is regionalized into the *Ptf1a*$^+$ VZ of the cb (blue region) and the *Atoh1*$^+$ CRL (green region). *Ptf1a*$^+$ and *Atoh1*$^+$ regions generate inhibitory (such as PCs, cerebellar interneurons) and excitatory neurons (such as GCs) in the cerebellum, respectively. *Ptf1a*$^+$ region is further divided into two regions by a pattern of *Neurogenin1* (*Ngn1*) expression (red dotted region). Progenitor cells in the caudal cerebellar VZ express *Ngn1*, not those in the rostral cerebellar VZ. Furthermore, the *Ptf1a*$^+$ and *Ngn1*$^+$ VZ generates Corl2$^+$ PCs (orange region). In contrast, the *Ptf1a*$^+$ and *Ngn1*$^-$ VZ generates *Pax2*$^+$ cerebellar interneurons (purple region). The transcription factor *Lmx1a* is locally expressed on the rp, and the transforming growth factor (TGF)*β* family proteins, Gdf7, BMP6, and BMP7 are secreted from the rp. (D) A schematic view of a transversal section of the hindbrain in r7 along the red plane shown in B. HRL is also regionalized into the *Atoh1*$^+$ (green region), the *Ngn1*$^+$ (red region), and the *Ptf1a*$^+$ (blue region) regions. The neurons of the lateral reticular nucleus (LRN) and external cuneate nucleus (ECN) are generated from the *Atoh1*$^+$ dorsal HRL and migrate from HRL to LRN and ECN along the posterior extramural stream (PES). The inferior olive (IO) neurons are generated from the *Ptf1a*$^+$ ventral HRL and migrate from HRL to IO nuclei along the intramural migratory stream (IMS). (E) A schematic view of a sagittal section of the mouse cb at E15, P0, and P7. 3v, third ventricle; 4v, fourth ventricle; di, diencephalon; egl, external granular cell layer; hb, hindbrain; igl, internal granular cell layer; mb, midbrain; mes, mesencephalon; pon, pons; pro, prosencephalon; sc, spinal cord; WM, white matter; d, dorsal; v, ventral; r, rostral; c, caudal. Other abbreviations are described in the legend of Figure 1.

nez *et al.* 1999; Picker *et al.* 1999). The isthmus acts as the organizing center for early development of the midbrain and hindbrain (Brand *et al.* 1996; Liu & Joyner 2001; Wurst & Bally-Cuif 2001; Sato *et al.* 2004; Nakamura *et al.* 2005). The secretory factors from the isthmus regulate morphogenesis of the midbrain and r1 along the anterior-posterior axis and induce cerebellar formation from r1. Consequently, the cerebellar territory in the neural tube is established by the spatial and temporal expression of transcription factors and secreted molecules by E9.5 (Fig. 2A).

Zebrafish *fgf8a* mutants (*acerebellar* mutants) lack an MHB organizer and cerebellum (Reifers *et al.* 1998; Picker *et al.* 1999), indicating that Fgf8 is a major mediator of the isthmic organizing activity in zebrafish. Zebrafish *spiel ohne grenzen* (*spg*) mutants, which have a defective *pou5f1* gene, lack the isthmic structure and a cerebellum (Belting *et al.* 2001; Burgess *et al.* 2002). The POU domain-containing transcription factor Pou5f1, which is the orthologue of mammalian Oct3/4, confers competence to respond to Fgf8 (Belting *et al.* 2001; Burgess *et al.* 2002; Reim & Brand 2002). The importance of Fgf8 signaling in the MHB and cerebellum formation is supported by the identification of Canopy1, which functions as a positive feedback regulator of Fgf signaling involved in MHB formation (Hirate & Okamoto 2006). The roles of Pou5f1 and Canopy1 in MHB formation and in the formation of the mammalian cerebellum await further investigation. Although Fgf8 defects lead to loss of the cerebellum, the co-inhibition of the *otx1b/otx2* (which may play an equivalent role to mouse *Otx2* gene) and *fgf8* genes in zebrafish restores the generation of PCs and GCs to some extent, suggesting that Fgf8 also functions to maintain the cerebellum region by repressing *Otx* expression (Foucher *et al.* 2006).

In addition to the patterning genes, the hairy and *enhancer of split*-related basic helix-loop-helix (bHLH) gene *her5* is expressed in the MHB region before MHB establishment in zebrafish, and Her5 with a close homologue, Her11 functions in this region to prevent neuronal differentiation and promote cell proliferation (Geling *et al.* 2003, 2004; Ninkovic *et al.* 2005). These genes may be required for the maintenance of the isthmic organizer activity. This is consistent with the finding that the expression of *Hes1* and *Hes3*, which are homologues of *her5* and *her11*, in the mouse MHB region is required for the isthmic organizer activity (Hirata *et al.* 2001).

The regional expression of transcription factors in the cerebellar rhombic lip and ventricular zone shapes the cerebellum

After E10.5 in mice, the isthmic region narrows, the cerebellar primordium expands laterally, and the wing-like cerebellar shape becomes clearly distinguishable (Fig. 1B). Additionally, the hindbrain that is contiguous to the cerebellum separates into two lateral parts along the dorsal midline, and a thin layer of non-neuronal epithelial cells occupies the area between the cerebellum and the bilaterally separated hindbrain, which is called the roof plate (rp) and the future choroid plexus (yellow region in Fig. 2B–D). The formation of the rp plays an important role in the regionalization of the cerebellum and hindbrain. The rp expresses the LIM homeobox transcription factor *Lmx1a* and secretes the transforming growth factor (TGF)β family proteins Gdf7, BMP6, and BMP7. These proteins induce the expression of the proneural bHLH factor *Atoh1* (mammalian homologue of *atonal*) in a restricted region adjacent to the rp in the cerebellum (cerebellar rhombic lip: CRL; green region in Fig. 2B) and the hindbrain (dorsal part of hindbrain rhombic lip: HRL; red region in Fig. 2B) (Alder *et al.* 1999; Chizhikov & Millen 2005; Landsberg *et al.* 2005; Chizhikov *et al.* 2006).

Neurons in the cerebellum are generated from two germinal zones: the cerebellar ventricular zone (VZ), which is located at the roof of the fourth ventricle (blue region in Fig. 1C), and the CRL, which is located at the caudal edge of the cerebellar primordium (green region in Fig. 2C,E) (Altman & Bayer 1997; Wingate & Hatten 1999; Wingate 2001; Zervas *et al.* 2004). The cerebellar VZ adjacent to the *Atoh1*$^+$ CRL begins to express the proneural gene *Ptf1a* from E10.5 to E14.5 in mice (Hoshino *et al.* 2005; Hoshino 2006; Fujiyama *et al.* 2009). However, it is not clear what molecule induces the *Ptf1a*$^+$ cerebellar VZ. In addition to *Ptf1a*, another proneural gene *Ascl1*, which is a homologue of *achaete-scute* complex genes, is also expressed in the cerebellar VZ (Grimaldi *et al.* 2009; Sudarov *et al.* 2011). The neuroepithelial cells in the *Ptf1a*$^+$ and *Ascl1*$^+$ cerebellar VZ produce GABAergic neurons including PCs, Golgi cells, basket cells, stellate cells, candelabrum cells and small neurons in the DCN (Fig. 2E) (Hashimoto & Mikoshiba 2004; Hoshino *et al.* 2005; Hoshino 2006; Leto *et al.* 2006, 2009; Grimaldi *et al.* 2009; Sudarov *et al.* 2011). Likewise, the neuroepithelial cells from the *Atoh1*$^+$ CRL give rise to the glutamatergic neurons including cerebellar GCs and large neurons in the DCN (Fig. 2E) (Machold & Fishell 2005; Wang *et al.* 2005). Accordingly, as the differentially located expression of *Ptf1a* and *Atoh1* structurally regionalizes the cerebellum, *Ptf1a*$^+$ and *Atoh1*$^+$ regions generate inhibitory neurons and excitatory neurons, respectively (Fig. 2C,E). PCs are generated from *Ptf1a*$^+$ neuroepithelial cells from E10.5 to E12.5 in mice (Hashimoto & Mikoshiba 2003; Hoshino *et al.* 2005). Immature and mature PCs can be distinguished by Corl2 expression (Minaki *et al.* 2008). The other

interneurons including Golgi cells, basket cells, stellate cells, and small neurons in the DCN are generated from *Ptf1a*$^+$ neuroepithelial cells after E13.5 (Leto *et al.* 2006, 2009). The interneurons are recognized by Pax2 expression (Maricich & Herrup 1999; Weisheit *et al.* 2006). The *Ptf1a*$^+$ region is further divided into the caudal and rostral regions (Zordan *et al.* 2008). In the *Ptf1a*$^+$ VZ of E12.5 mice, *Neurogenin1* (*Ngn1*) is expressed in the caudal region (red dotted region in Fig. 2C), but not in the rostral region. Furthermore, the *Ptf1*$^+$ and *Ngn1*-negative (–) region (blue region in Fig. 2C) is adjacent to the *Pax2*$^+$ region (purple region in Fig. 2C). The cell fate mapping of *Ngn1*$^+$ progenitor cells has been conducted using the BAC-EGFP reporter transgenic mouse (Ngn1-EGFP) (Lundell *et al.* 2009). The *Ngn1*$^+$ progenitor cells in a mouse cerebellum generate PCs and Pax2$^+$ cerebellar interneurons excluding small inhibitory neurons in DCN (Golgi, basket, and stellate cells). Similarly, Mizuhara *et al.* (2010) indicates that the *Ptf1a*$^+$ VZ in E12.5 mouse cerebellum is divided into two regions by a pattern of E-cadherin (E-cad) expression. E-cad is strongly expressed in the caudal region, which is called the c2d region, whereas E-cad is weakly expressed in the rostral region, which is called the c2v region. The authors suggest that the c2d region generates Corl2$^+$ PCs (orange region in Fig. 2C), whereas the c2v region generates small inhibitory neurons in DCN (Minaki *et al.* 2008; Mizuhara *et al.* 2010). The region that expresses E-cad weakly (c2v region) in E12.5 mouse cerebellum seems to be identical to the *Ptf1a*$^+$ and *Ngn1*$^-$ region (blue region, Fig. 2C), because these regions are similarly adjacent to the Pax2$^+$ region (purple region in Fig. 2C). Furthermore, the c2d region corresponds with the *Ptf1a*- and *Ngn1*-double positive region (red dotted region in Fig. 2C), since both of them are the origin of PC's (orange region in Fig. 2C). The cell fate of each subtype of cerebellar GABAergic neuron is individually controlled by the expression of particular molecules.

The PCs migrate from the ventricular side to the surface of the cerebellum and form the PCL (Fig. 2E). Excitatory neurons of the DCN are first generated from *Atoh1*$^+$ neuroepithelial cells in CRL between E10 and E12 in mice. These cells initially migrate along the outer surface of the cerebellum, and then, eventually migrate into the central part of the cerebellum, which is the location of the DCN (Machold & Fishell 2005; Wang *et al.* 2005; Fink *et al.* 2006). Following generation of excitatory neurons of the DCN, *Atoh1*$^+$ CRL gives rise to the cerebellar GC precursors (GCPs) after E12.5 in mice. The GCPs migrate tangentially over the entire surface of the cerebellum, using a similar route as the excitatory neurons of the DCN, and then form the external granular layer (egl; Fig. 2C,E) (Machold & Fishell 2005; Wang *et al.* 2005). Thereafter, the GCPs in the egl radially move from egl to the inner granule cell layer (igl) and differentiate into GCs. The cerebellar glial cells, Bergmann glia, astrocytes, and oligodendrocytes are generated from VZ progenitors (Zhang & Goldman 1996a,b; Milosevic & Goldman 2002; Hoshino *et al.* 2005; Sudarov *et al.* 2011).

The layer formation of the cerebellum is controlled by various signaling molecules, such as semaphorin (Kerjan *et al.* 2005; Renaud *et al.* 2008; Maier *et al.* 2011) and reelin (Terashima *et al.* 1985; Miyata *et al.* 1997, 2010; Takayama *et al.* 1997). Semaphorin 4C, 4G, and 6A control the radial migration of GCPs (Kerjan *et al.* 2005; Renaud *et al.* 2008; Maier *et al.* 2011). Reelin is highly expressed in GCPs in the egl (Herrup 2000), and it controls positioning of PCs and architectural organization of the cerebellar layers in mice (Goffinet 1983; Terashima *et al.* 1985; Miyata *et al.* 1997). PCs are thought to provide trophic support to GCPs in the mammalian cerebellum (Wetts & Herrup 1983; Smeyne *et al.* 1995). PCs express Sonic hedgehog (Shh), and promote the expansion of GCPs, and thereby control the foliation of the cerebellum in mammals (Dahmane & Ruiz I Altaba 1999; Wallace 1999; Wechsler-Reya & Scott 1999; Lewis *et al.* 2004; Corrales *et al.* 2006). Aberrant activation of Shh signaling is known to result in the formation of the malignant tumor, medulloblastoma in human patients (Hatten & Roussel 2011; Roussel & Hatten 2011).

Development of cerebellar neurons in zebrafish

Developmental processes of cerebellar neurons are conserved in teleost species, including zebrafish (Bae *et al.* 2009; Kani *et al.* 2010; Hibi & Shimizu 2011). PCs are derived from progenitors in the VZ that express *ptf1a*, while most of the glutamatergic neurons originate from the *atoh1*-expressing CRL progenitors (Kani *et al.* 2010) (Fig. 3D). However, there are teleost-specific features. In contrast to the mouse, the zebrafish has three *atoh1* genes, *atoh1a/b/c*, which show overlapping and distinct expression patterns, and might mark different progenitor populations in the CRL (Kani *et al.* 2010). Lineage tracing with transgenic zebrafish and birthdate analysis reveal that *atoh1*-expressing CRL progenitor cells also give rise to GCs in zebrafish (Kani *et al.* 2010; Wullimann *et al.* 2011). The anterior CRL progenitors, which preferentially express *atoh1a* and/or *atoh1b*, generate GCs in the anterior lobe (Va) and the main lobe (CCe), whereas the caudal and lateral CRL progenitors expressing *atoh1b* and/or *atoh1c* give rise to GCs in the caudo-lateral lobe (LCa and EG, Fig. 3A,B,

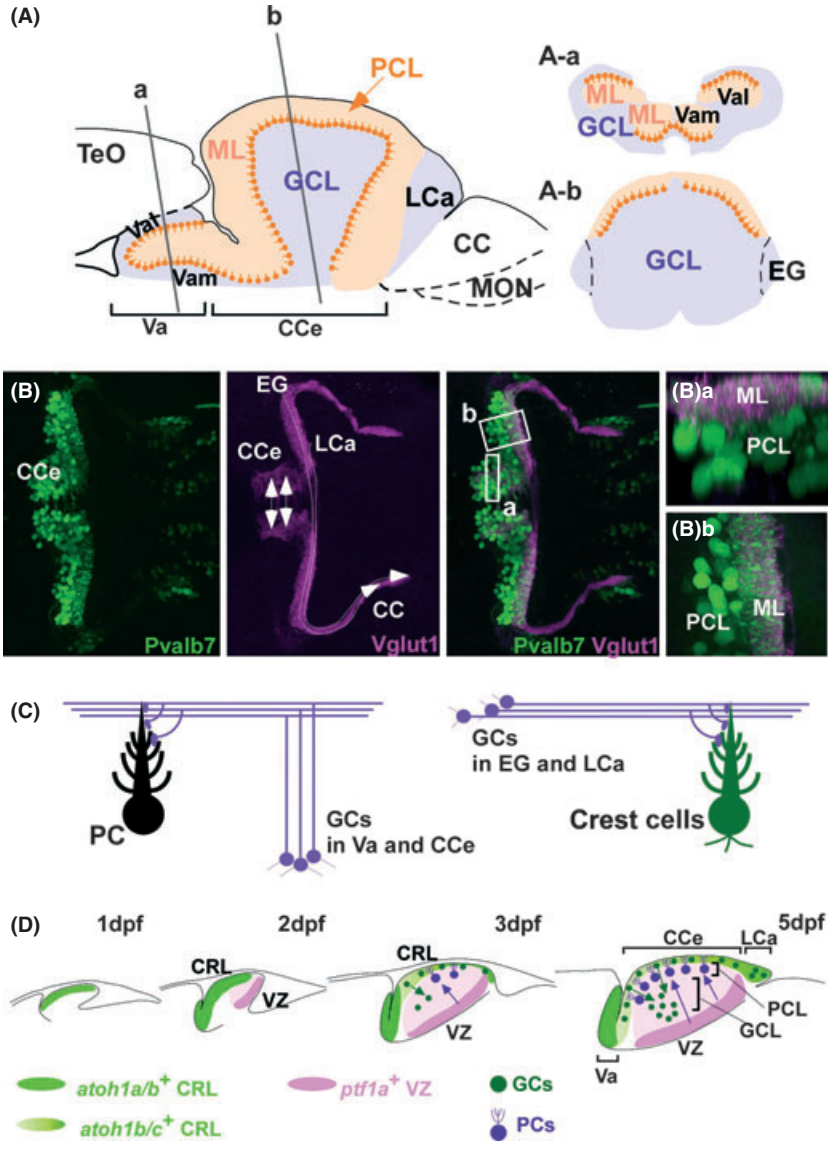

Fig. 3. Anatomy and development of zebrafish cerebellum. (A) Schematic representation of adult zebrafish cerebellar structure. A sagittal section image. (A-a, b) Cross section images at the levels (a, valvula cerebelli, Va; b, corpus cerebelli, CCe) shown in (A). (B) Structure of larval cerebellum (at 5 days postfertilization, dpf). Staining with anti-parvalbumin 7 (Pvalb7, green) and anti-Vglut1 (magenta) antibodies, which recognizes PCs and the axons of GCs, respectively. Dorsal views with anterior to the left. (B-a) Transverse-section image of box a in the merged image. (B-b) High magnification view of box b in the merged image. (C) Schematic representation of parallel fibers in the anterior (Va) and main (CCe) lobes (left panel), and in the caudo-lateral lobes (EG, eminentia granularis and LCa, lobus caudalis cerebelli) and the crista cerebellaris (CC). Parallel fibers of GCs in Va and CCe contact the PC dendrites, whereas parallel fibers in EG and LCa contact the dendrites of crest cells (direction of axons are indicated by arrows in B). (D) Schematic representation of the development of GCs and PCs. MON, medial octavolateralis nucleus; TeO, optic tectum; Vam, medial division of valvula cerebelli; Val, lateral division of the valvula cerebelli. The other abbreviations are described in Figures 1 and 2. This figure is modified from (Bae *et al.* 2009; Kani *et al.* 2010; Hibi & Shimizu 2011).

D). As seen in mice, the *atoh1*-expressing cells are initially located in the superficial layer, which likely corresponds to the egl, and migrate to the inside to form the GCL in the anterior and main lobes (Kani *et al.* 2010) (Fig. 3D). It is, however, argued that the *atoh1*-expressing progenitors migrate quickly and do not exhibit a transit amplification of GCs in zebrafish. Furthermore, the *atoh1*-expressing cells in the caudo-lateral lobe stay and do not migrate inside (Volkmann *et al.* 2008; Kani *et al.* 2010). Thus, the zebrafish cerebellum may not have a typical egl (Chaplin *et al.* 2010). Furthermore, in the mammalian cerebellum, the GCPs in the egl

proliferate in response to Shh, which is produced by PCs (Dahmane & Ruiz I Altaba 1999; Wallace 1999; Wechsler-Reya & Scott 1999), whereas PCs in the zebrafish do not express Shh, or GCPs (or immature GCs) do not respond to Shh signaling (Chaplin *et al.* 2010). Therefore, the *atoh1*-expressing cells in the superficial domain in zebrafish may not be identical to the cells in the egl. Nevertheless, the *atoh1a/b/c*-expressing CRL progenitors constitute a population of proliferating cells that are quite similar to the egl progenitor population in mammals. As described above, reelin plays an important role in layer formation in the mouse cerebellum, and is also expressed in both the immature and mature GCs of the zebrafish cerebellum, similar to that in the mouse cerebellum (Carletti *et al.* 2008). However, it has not been shown whether reelin controls layer formation in the zebrafish cerebellum. During early development of the zebrafish cerebellum, descendant cells from *atoh1*$^+$ and *ptf1a*$^+$ progenitors are adjacent to each other (Kani *et al.* 2010). Deletion of the *ptf1a*$^+$ lineage led to a strong reduction in the amount of GCs (M. Hibi, unpubl. data, 2008), suggesting that the generation of GCs depends on the presence of the *ptf1a*$^+$ lineage, such as PCs. Although zebrafish GCPs do not respond to Shh signaling (Chaplin *et al.* 2010), a trophic role for PCs in GC proliferation may be conserved in zebrafish. Future studies are required to identify the molecules responsible for the interaction between GCs and PCs in teleost species.

In addition to the GCs and DCN neurons, the *Atoh1*-expressing CRL progenitors generate tegmental nuclei, including the parabrachial nuclei and parabigeminal nuclei in mice (Machold & Fishell 2005; Wang *et al.* 2005). The CRL progenitors in zebrafish give rise to the secondary gustatory/viscerosensory nuclei, the nuclei isthmi, and superior reticular nuclei (Kani *et al.* 2010; Volkmann *et al.* 2010). The secondary gustatory/viscerosensory nucleus is considered to be homologous to the parabrachial nucleus of mammals (Morita *et al.* 1980, 1983; Finger 1983, 1987), and the nucleus isthmi is thought to be the homologue of the parabigeminal nucleus in mammals (Clark 1933; Sakamoto *et al.* 1981; Ito *et al.* 1982; Volkmann *et al.* 2010). Thus, the tegmental nuclei from *atoh1*-expressing progenitors likely have conserved functions in vertebrates.

Eurydendroid cells are teleost-specific cerebellar projection neurons that transmit information from PCs to extracerebellar domains (Ikenaga *et al.* 2005, 2006; Bae *et al.* 2009). Although the function of the eurydendroid cells is thought to be equivalent to that of neurons in the DCN of the mammalian cerebellum, the origin of eurydendroid cells has been perplexing. In the mouse cerebellum, there are glutamatergic and GABAergic neurons in the DCN; these neurons originate from *Atoh1*$^+$ CRL and *Ptf1a*$^+$ VZ progenitors, respectively (Hoshino *et al.* 2005; Machold & Fishell 2005; Wang *et al.* 2005). An analysis of eurydendroid cells in the goldfish cerebellum suggests that there is no GABAergic eurydendroid cell population (Ikenaga *et al.* 2005). Marker analysis and retrograde labeling experiments with zebrafish revealed two types of eurydendroid cells, *olig2*-expressing eurydendroid cells and calretinin-immunoreactive (Cr-ir$^+$) eurydendroid cells, in the zebrafish cerebellum (Bae *et al.* 2009). Lineage tracing with transgenic zebrafish indicated that the majority of the *olig2*$^+$ eurydendroid cells are derived from *ptf1a*$^+$ VZ progenitors, and some are derived from *atoh1*$^+$ CRL progenitors (Bae *et al.* 2009; Kani *et al.* 2010). Although *Ptf1a*$^+$ VZ progenitors generate only GABAergic neurons in the mouse DCN, the *olig2*$^+$ eurydendroid cells from both *ptf1a*$^+$ VZ and *atoh1*$^+$ CRL progenitors are glutamatergic neurons (Bae *et al.* 2009; Kani *et al.* 2010). Thus, the development of the *olig2*$^+$ eurydendroid cells may be different from that of the DCN neurons. Future studies are needed to reveal whether the Cr-ir$^+$ and *olig2*$^+$ eurydendroid cells have different origins and functions, and whether there are *Olig2*$^+$ neurons in the mammalian cerebellum.

In contrast to the mammalian cerebellum, the zebrafish cerebellum can be regenerated after its ablation, at least at the early embryonic stage (Koster & Fraser 2006). This regeneration is accompanied by repatterning of the anterior hindbrain, in which the *otx2*$^-$ and *hox*$^-$ r1 identity is re-established, and requires Fgf signaling (Koster & Fraser 2006). Furthermore, cerebellum neurons and glial cells are continuously generated throughout the adult stage (Zupanc *et al.* 2005; Kaslin *et al.* 2009; Kani *et al.* 2010). *atoh1a/b/c* and *ptf1a* are expressed in the ML and the VZ, respectively, of adult cerebellum, and the most *atoh1a*, *atoh1b*, and/or *atoh1c*-expressing cells and some *ptf1a*-expressing cells proliferate in the adult cerebellum (Kani *et al.* 2010). Bergmann glial cells also proliferate, and Nestin/Sox2/Musashi$^+$ proliferating cell populations have also been found in the adult cerebellum (Kaslin *et al.* 2009; Kani *et al.* 2010). These data suggest that there are distinct progenitor populations in the adult cerebellum: proneural gene(s)-expressing progenitors, Bergman glial cells, and Nestin/Sox2/Musashi$^+$ cells. Considering the function of proneural genes, it is likely that *atoh1*$^+$ or *ptf1a*$^+$ progenitors generate glutamatergic and GABAergic neurons in the adult cerebellum, as they do in the embryonic/larval stages. It is not yet clear whether Bergmann glial cells or Nestin/Sox2/Musashi$^+$ cells serve as neural stem cells to generate both neurons and glia. Further studies are required to clarify this issue. Although there are different types of

proliferating cells in the zebrafish adult cerebellum, the major population is derived from the GC lineage (Zupanc *et al.* 2005; Kaslin *et al.* 2009; Kani *et al.* 2010). Immature GCs in the ML proliferate and migrate rapidly into the GCL within a week. In contrast, turnover of PCs is very slow in the adult cerebellum, indicating that GCs play more important roles in remodeling cerebellar neural circuits in adult zebrafish.

The precerebellar nuclei are generated from the hindbrain rhombic lip at rhombomeres 6 to 8

The cerebellar cortex is innervated by two major afferent inputs, CFs and MFs, which originate from the precerebellar nuclei IO and LRN/ECN/RTN/PG, respectively, in mice (the MF neurons in the hindbrain are not well studied in zebrafish). During development, neurons of the precerebellar nuclei are generated from neuroepithelial cells in HRL, which is located at the dorsal edge of the hindbrain (red region in Fig. 2B) (Bourrat & Sotelo 1988, 1990b; Altman & Bayer 1997; Wingate 2001). In mice, neurons of precerebellar nuclei are generated from HRL in the following order: IO neurons during E10.5–E11.5, LRN and ECN neurons during E11.5–E13.5, and RTN and PG neurons after E12.5. Newborn IO neurons first extend long leading processes to the floor plate (FP) along the surface of the hindbrain, and then, the cell bodies of IO neurons ventrally migrate from the HRL to the FP through the intramural migratory stream (IMS; Fig. 2D) (Bourrat & Sotelo 1990b; Altman & Bayer 1997). The long leading processes of IO neurons (which later become CFs) go through the FP, whereas the cell bodies of IO neurons cease migrating before the FP and form immature IO nuclei in the caudoventral hindbrain at E14.5 in mice (Bourrat & Sotelo 1988, 1990a, b; Altman & Bayer 1997). Consequently, IO neurons develop a specific projection only to the contralateral side of the cerebellar cortex. The arrest of IO neurons before the FP is essential for establishment of the contralateral olivocerebellar projection. LCN and ECN neurons ventrally migrate from HRL to their destinations through the posterior extramural stream (PES; Fig. 2D), which is more superficial than the IMS (Bourrat & Sotelo 1990b; Altman & Bayer 1997). In contrast to IO neurons, LCN and ECN neurons cross the midline of the FP, and therefore settle in the contralateral side to their origin in HRL and project their axons to the ipsilateral side of the cerebellum as the MFs. Therefore, IO neurons and LCN/ECN neurons are different in their developmental processes and neuronal projections. IO, LCN, and ECN neurons express the Netrin receptor DCC (Deleted in Colorectal Cancer) and Slit receptor roundabout (Robo)1/2/3, and respond to Netrin-1 and Slit1/2/3 generated by the FP, which act as short- and long-range chemoattractants and chemorepellents (Bloch-Gallego *et al.* 1999; Causeret *et al.* 2002; Marillat *et al.* 2004; Di Meglio *et al.* 2008). In addition, IO neurons express a tyrosine kinase receptor, EphA4, whereas the FP expresses EphrinB3, which is a ligand for EphA4. The Netrin/DCC, Slit/Robo, and Ephrin/Eph signaling between the FP and IO/LCN/ECN neurons is involved in the guidance of neuronal migration and controls the midline crossing of these neurons (Bloch-Gallego *et al.* 1999; Causeret *et al.* 2002; Marillat *et al.* 2004; Di Meglio *et al.* 2008; Hashimoto *et al.* 2011).

Previous studies indicate that neurons of precerebellar nuclei are derived from the neuroepithelial cells in HRL at rhombomeres 6 to 8 (Cambronero & Puelles 2000; Landsberg *et al.* 2005; Farago *et al.* 2006; Kawauchi *et al.* 2006; Yamada *et al.* 2007; Liu *et al.* 2008; Ray & Dymecki 2009). Furthermore, genetic studies indicate that HRL in rhombomeres 6–8 is regionalized by the patterns of *Atoh1*, bHLH gene *Neurogenin1* (*Ngn1*), and *Ptf1a* expression (Fig. 2D), as well as the cerebellum. The *Atoh1*$^+$ region is located on the dorsal part of HRL (green region in Fig. 2D), and the *Ngn1*$^+$ region is contiguous to this *Atoh1*$^+$ region (red region in Fig. 2D) (Landsberg *et al.* 2005). The *Ptf1a*$^+$ region is located on the ventral part of HRL (blue region in Fig. 2D). In addition, *Wnt1* and bHLH gene *Olig3* are expressed in the entire HRL in a dorsoventral gradient (Rodriguez & Dymecki 2000; Landsberg *et al.* 2005; Liu *et al.* 2008; Ray & Dymecki 2009; Storm *et al.* 2009). Using Cre-*loxP*, Flp-*FTR*, and ligand-inducible Cre recombinase (CreER) systems, temporal fate maps of the *Atoh1*-, *Ptf1a*-, *Wnt1*-, and *Olig3*$^+$ neuroepithelial cells in HRL have been constructed (Rodriguez & Dymecki 2000; Hoshino *et al.* 2005; Landsberg *et al.* 2005; Yamada *et al.* 2007; Liu *et al.* 2008; Ray & Dymecki 2009; Storm *et al.* 2009). The *Atoh1*$^+$ neuroepithelial cells in HRL at rhombomeres 6–8 generate neurons of PG, LRN, and ECN (Rodriguez & Dymecki 2000; Landsberg *et al.* 2005). In contrast, the *Ptf1a*$^+$ neuroepithelial cells in HRL at rhombomeres 6–8 generate IO neurons in mice (Hoshino *et al.* 2005; Yamada *et al.* 2007) as well as in zebrafish (Bae *et al.* 2009). Accordingly, the differentially located expression of *Ptf1a* and *Atoh1* in HRL at rhombomeres 6–8 is important to determine the cell fate of precerebellar nuclei. Furthermore, the gradual expression of *Olig3* in HRL is involved in the regional specification in HRL because *Olig3* knockout mice are associated with a reduction of the *Atoh1*$^+$ and *Ngn1*$^+$ domains in HRL, and dorsal expansion of the *Ptf1a*$^+$ domain (Liu *et al.* 2008; Storm *et al.* 2009). However, both *Olig3* and *Ptf1a* are required for the generation of

IO neurons (Yamada *et al.* 2007; Liu *et al.* 2008; Storm *et al.* 2009). Consequently, the neurogenesis of precerebellar nuclei is closely regulated by the spatial and temporal expression of *Atoh1*, *Ptf1a*, and *Olig3* in HRL (Fig. 2D).

Mediolateral compartmentalization of the mammalian cerebellum

The mammalian cerebellar cortex is organized into a series of longitudinal compartments along the M-L axis (for reviews, see Apps & Garwicz 2005; Apps & Hawkes 2009; Ito 2001, 2005; Voogd & Glickstein 1998). PCs in each compartment are innervated by a specific subarea of IO through CFs (olivocerebellar projections) and project their axons to a specific region in the DCN or vestibular nuclei (corticonuclear projections). Therefore, the olivo-cortico-nuclear circuit shows modular formation and compartmentalizes the cerebellum along the M-L axis. Remarkably, CFs projected from the subset of neurons in a sub-nucleus of the IO form specific striped compartments in the cerebellum, which show a bilateral symmetrical distribution (Fig. 4).

Molecular studies indicate that the cerebellar compartments are identified by the expression of a variety of marker genes (for reviews, see Hawkes & Eisenman 1997; Herrup & Kuemerle 1997; Oberdick *et al.* 1998; Larouche & Hawkes 2006; Sillitoe & Joyner 2007; Apps & Hawkes 2009). For instance, gene expression

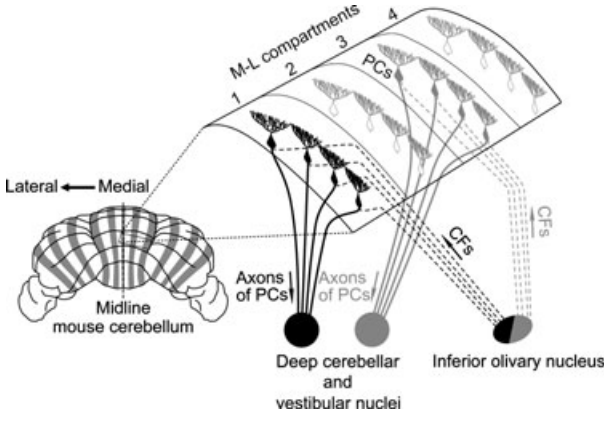

Fig. 4. Mediolateral compartmentalization of the cerebellar neural projections. A specific subnucleus of inferior olive nuclei (IO) nuclei projects its axons (climbing fibers [CFs]) into the contralateral cerebellar cortex. CFs organize into striped, compartmentalized regions within the cerebellum along the mediolateral (M-L) axis (M-L compartments 1–4). Additionally, the Purkinje cells (PCs) within each M-L compartment project their axons into a particular region of the deep cerebellar and vestibular nuclei. The olivo-cortico-nuclear circuit shows modular formation and compartmentalizes the cerebellum along the M-L axis.

of *L7/pcp2* (a genetic marker of cerebellar PCs), *En1*, *En2*, *Pax2,* and *Wnt7B* appears in specific striped-regions. In addition, the expression of zebrin II (aldolase C) also shows a specific striped-pattern in the adult cerebellum. Interestingly, *En2*-deficient mice show an abnormal morphology of the cerebellum (Kuemerle *et al.* 1997), and the striped pattern of zebrin II is closely correlated to the striped region formed by the olivocerebellar and corticonuclear projections (Gravel *et al.*, 1987, Gravel & Hawkes, 1990, Voogd *et al.*, 2003, Sugihara & Shinoda, 2004, Voogd & Ruigrok, 2004, Odeh *et al.*, 2005, Sugihara & Quy 2007; Ruigrok *et al.*, 2008), and to physiological activity in the adult cerebellum (Chen *et al.* 1996; Apps & Garwicz 2005; Gao *et al.* 2006; Sugihara & Quy 2007; Pijpers *et al.* 2008). However, the striped pattern of expression is not stable. The striped-patterns of *L7/pcp2*, *En1*, *En2*, *Pax2,* and *Wnt7B* gene expression initially appear from E15.5, but then disappear shortly after birth; therefore, the striped-pattern of gene expression is referred to as an early-onset pattern. In addition, all PCs express zebrin II by the first week after birth, but the expression of zebrin II gradually changes to a striped-pattern within the cerebellum until 20 days after birth; thus, the striped-pattern of zebrin II expression is referred to as a late-onset pattern (Leclerc *et al.* 1988). Furthermore, the striped pattern of zebrin II (late-onset) is entirely different from the *L7/pcp2*, *En1*, *En2*, *Pax2*, and *Wnt7B* gene expression patterns (early-onset). Consequently, the difference between the embryonic and adult compartments and the transient disappearance of the cerebellar compartments have made it difficult to determine how striped compartments are established, and how the embryonic compartments (early-onset pattern) and the adult compartments (late-onset pattern) are related, despite several studies having addressed this issue (Larouche & Hawkes 2006; Marzban *et al.* 2007; Sillitoe & Joyner 2007). In any case, the striped cerebellar compartments are thought to be the basic modular structures from which cerebellar functions are performed, because the striped compartments are observed not only at the neural circuit and neurophysiologic level, but also at the cellular and molecular levels.

In a teleost cerebellum, zebrin II is expressed in most if not all PCs and does not show compartment-specific expression (Brochu *et al.* 1990; Lannoo *et al.* 1991a,b; Meek *et al.* 1992; Bae *et al.* 2009). For instance, in zebrafish cerebellum, anti-zebrin II antibody stains the same population of PCs as the anti-parvalbumin and anti-carbonic anhydrase 8 (Ca8) antibodies (Bae *et al.* 2009). Parvalbumin and Ca8 are expressed in all PCs of the mammalian cerebellum (Celio & Heizmann 1981; Celio 1990; Kato 1990; Nogradi *et al.* 1997; Hirota

et al. 2003). These data indicate that zebrin II, parvalbumin, and Ca8-expressing PCs are the sole PCs in the zebrafish cerebellum. Currently, there is no compartment-specific expression of any PC genes reported for the teleost cerebellum (Nieuwenhuys & Nicholson 1967; Meek 1992; Altman & Bayer 1997; Meek *et al.* 2008). Thus, the teleost cerebellum does not exhibit clear M-L compartmentalization marked by the gene expression that distinguishes the compartments in the mammalian cerebellum. M-L compartmentalization may have arisen during evolution as the cerebellar neural circuits became more complex. The present data, however, do not exclude the possibility that the M-L compartmentalization of the cerebellum is present in teleosts and may be detected by different genes from mammals.

Close correlation between the birthdate of PCs and the mediolateral compartmentalization of the cerebellum

Using replication-defective adenoviral vectors, we have successfully performed a "pulse gene transfer" approach to deliver an exogenous gene into restricted sub-populations of neuronal progenitor cells in a birthdate specific manner (Hashimoto & Mikoshiba 2004). The adenoviral vector system is very useful for the examination of neuronal development and function because we can genetically manipulate each subset of neurons that share the same neuronal birthdate and can examine the native behavior of each such neuronal subset.

The timed adenovirus gene transfer approach can be applied to the study of cerebellar development and regionalization (Hashimoto & Mikoshiba 2003). When AdexCAG-NL-LacZ, a viral vector designed for nucleus-targeted β-galactosidase (βgal) expression, is injected into the midbrain ventricle of the mouse embryo, it also infects the progenitor cells on the surface of the fourth ventricle and effectively transfers the nuclear-targeted βgal into the progenitor cells. Interestingly, the injection of AdexCAG-NL-LacZ into embryos at E10.5, E11.5, and E12.5 reveals that successfully infected βgal^+ progenitor cells develop normally and differentiate into cerebellar PCs. This result is consistent with a report indicating that cerebellar PCs are generated from *Ptf1a*$^+$ progenitor cells (blue region in Fig. 1C) on the surface of the fourth ventricle (Hoshino *et al.* 2005). By using adenoviral vectors, we can efficiently transfer a foreign gene into the progenitor cells of PCs in a neuronal birthdate-specific manner and observe the native behavior of each cohort of PCs that share the same neuronal birthdate. Remarkably, PCs that shared the same birthdate formed specific subsets of M-L compartments in the cerebellum (Fig. 5). PCs born on E10.5 (Fig. 5B,E), E11.5 (Fig. 5C,F), and E12.5 (Fig. 5D,G) are respectively labeled with nuclear-targeted βgal by the injection of the adenoviral vector AdexCAG-NL-LacZ into the midbrain ventricle of

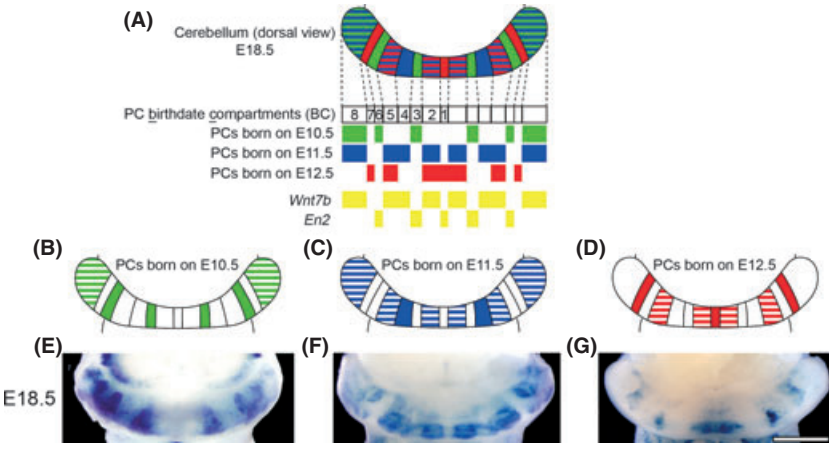

Fig. 5. Mediolateral compartments formed by the birthdate-related Purkinje cells (PCs) in E18.5. (A) The distribution of PCs born on E10.5 (green), E11.5 (blue), and E12.5 (red) in E18.5 cerebella are illustrated. At E18.5, cohorts of PCs born on E10.5, E11.5, and E12.5 form eight longitudinal compartments along the mediolateral axis (designated PC birthdate compartments, BC1–BC8). Each cohort of birthdate-related PCs is located to a specific subset of compartments displaying nested and, in part, mutual complementarity. PCs born on E10.5 form BC3, BC6, and BC8 (green, A, B), PCs born on E11.5 form BC2, BC4, BC5, and BC8 (blue, A, C), and PCs born on E12.5 form BC1, BC2, BC5, and BC7 (red, A, D). AdexCAG-NL-LacZ was injected into embryos at E10.5, E11.5, and E12.5, and then each cerebellum was whole mount stained for β-gal (blue color) at E18.5. The dorsal views of the E18.5 cerebella are shown (E–G). In (A), expression patterns of *Wnt7b* and *En2* (Millen *et al.* 1995; Hashimoto & Mikoshiba 2003), early-onset markers, are also shown. Scale bar, 1 mm.

mouse embryos at E10.5, E11.5, and E12.5. We found that PCs that are generated at E10.5, E11.5, and E12.5 form distinct subsets of compartments, which are arranged mediolaterally (designated PC birthdate compartments, BC1–BC8) in the E18.5 cerebellum (Fig. 5). The PC precursors that share the same birthdate seem to migrate tangentially from the cerebellar VZ to their fated position (Hashimoto & Mikoshiba 2003). However, details about the developmental processes involved in forming the PC birthdate compartments are still unclear. The patterns of PC birthdate compartments in the embryonic cerebellum are similar to the expression patterns of several early-onset markers (Fig. 5A, *En2*, *Wnt7b*) (Hashimoto & Mikoshiba 2003). In contrast to these early- but also to late-onset markers such as zebrin II, the adenoviral labeling of PCs is stable from embryo until adult (at least until 1.5 years of age, data not shown). Furthermore, there is a close correlation between the patterns of PC birthdate compartments and the striped expression of zebrin II in the adult mouse cerebellum (Namba *et al.* 2011). Consequently, PCs are already predestined to form specific subsets of M-L compartments on their neuronal birthdates between E10.5 and E12.5, and the M-L compartments determined by the birthdate of PCs are unchangeable structures from embryo until adult. This is supported by another study showing a correlation between birthdates of PCs, as determined by using BrdU and striped expression of zebrin II in the adult cerebellum (Larouche & Hawkes 2006). It is possible that each subset of PCs that share the same birthdate acquires distinct characteristics by expressing particular genes based on PC birthdate. For instance, early B-cell factor 2 (*Ebf2*), a member of the atypical helix-loop-helix transcription factor family Collier/Olf1/EBF, is a candidate for cell fate determination of PCs (Croci *et al.* 2006; Chung *et al.* 2008). The expression of *Ebf2* shows a striped-pattern in the adult mouse cerebellum (Croci *et al.* 2006). Deficiency of *Ebf2* results in a small cerebellum and abnormal foliation, particularly in the anterior vermis. Additionally, zebrin II-immunonegative stripes disappear in *Ebf2* knockout mice (Croci *et al.* 2006). Detailed analysis of gene expression in *Ebf2* knockout mice suggests that *Ebf2* contributes to cell fate determination of the zebrin II-immunopositive PC population (Chung *et al.* 2008). The striped expression of *Ebf2* appears to be similar to the striped distribution of E11.5-born PCs in the adult mouse cerebellum, but the relationship between them is unclear.

Perspectives

Studies have revealed that development of cerebellar neural circuits is mainly controlled by molecular mechanisms that are conserved among vertebrates. However, many questions still remain to be elucidated. Glutamatergic and GABAergic cerebellar neurons are generated from *Atoh1* and *Ptf1a* (*Ascl1a*)-expressing neuronal progenitors. It is not known what mechanisms underlie generation of a diverse set of glutamatergic and GABAergic neurons. Which molecules control differentiation and migration of cerebellar neurons? What mechanisms regulate and coordinate the formation of axons and dendrites of cerebellar neurons and CF/MF neurons?

There are variations in the structure of the cerebellum and cerebellar neural circuits between mammals and teleosts (e.g. mice vs. zebrafish), and among the same species (e.g. the cerebellum of mormyrid fish has a more complex structure than that of the zebrafish). What accounts for such differences? The teleost cerebellum contains eurydendroid cells, and the location, morphology, and possibly the origin of eurydendroid cells are different from that of DCN neurons. It would be intriguing to know how eurydendroid cells and DCN neurons arise during evolution. The teleost cerebellum exhibits adult neurogenesis; GCs are generated during remodeling of adult cerebellar neural circuits. How neuronal progenitors and neural stem cells are maintained in the adult teleost cerebellum is not currently known. Knowledge on adult neurogenesis and regeneration in teleost species may potentially lead to treatments of disorders of the cerebellum, such as spinocerebellar ataxia.

M-L compartmentalization is a prerequisite for formation and function of cerebellar neural circuits as it is linked to formation of the topographic map of olivocerebellar and corticonuclear connections. The birthdate of PCs determines which compartment individual PCs belong to. It is not known what mechanism gives PCs different identities during differentiation; how PCs having the same birthdate are segregated into compartments, and how PCs in each compartment control the formation of the topographic map is also unknown. What is the physiological meaning of the compartmentalization of the cerebellum? Future studies with transgenic and mutant mice and zebrafish are needed to shed light on the development and function of the cerebellum.

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