Specification of Cerebellar and Precerebellar Neurons

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Abstract

The cerebellum is thought to participate in the regulation of movement and is comprised of various types of neurons in the cerebellar cortex and nuclei. Each type of neurons has morphologically, immunohistochemically, and electrophysiologically distinct characteristics. In addition, there are two precerebellar afferent systems, the mossy fiber (MF) system and the climbing fiber (CF) system. MF neurons are located in various nuclei throughout the brainstem and send their axons to cerebellar granule cells, whereas CF neurons reside exclusively in the

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inferior olivary nucleus (ION) and project to Purkinje cells. Recently developed genetic lineage-tracing methods as well as gene-transfer technologies have accelerated the studies on the molecular machinery to specify neuronal subtypes in the cerebellum and the precerebellar systems.

Keywords

Cerebellum · bHLH · Transcription factor · Atoh1 · Ptf1a · Ngn1 · Ascl1 · Olig3 · Pax6 · Rhombomere · Neuroepithelium · Roof plate · Rhombic lip (RL) · Ventricular zone (VZ) · Glutamatergic neuron · GABAergic neuron · Cerebellar nucleus (CN) · CN-Glu neuron · CN-GABA-ION neuron · CN-GABA-interneuron · Lineage trace · In utero electroporation · Null mutant · Precerebellar systems · Climbing fiber (CF) neuron · Mossy fiber (MF) neuron · Hindbrain · Pontine gray nucleus (PGN) · Reticulotegmental nucleus (RTN) · Lateral reticular nucleus (LRN) · External cuneate nucleus (ECN) · Inferior olive nucleus (ION) · Wnt-1 · BMP · Specification · Subtype · Cochlear nucleus · Dorsal · Grafting study · Mitotic · Postmitotic · Granule cell · Purkinje cell · Golgi cell · Stellate cell · Basket cell

Specification of Cerebellar Neurons

The cerebellum consists of three parts: cortex, white matter, and nucleus. The cerebellar cortex contains Purkinje, Golgi, Lugaro, stellate, basket, granule, and unipolar brush cells. The latter two cell types are glutamatergic excitatory neurons, while the others are all GABAergic inhibitory neurons. The cerebellar nucleus (CN) includes three types of neurons: large glutamatergic projection neurons (CN-Glu neurons), midsized GABAergic inhibitory projection neurons (CN-GABA-ION neurons), and small GABAergic interneurons (CN-GABA interneurons). CN-GABA-ION neurons extend their axons to the inferior olivary nucleus (ION) (Carletti and Rossi 2008), while CN-Glu neurons send their axons to nuclei outside the cerebellum, including the red nucleus and the thalamus.

It is believed that all types of cerebellar neurons are generated from the neuroepithelium of the alar plate of rhombomere 1 (r1) during development (Millet et al. 1996; Wingate and Hatten 1999; Chizhikov and Millen 2003; Zervas et al. 2004). The dorsal-most part of the r1 neuroepithelium, that is, the roof plate, does not produce neurons but cells of the choroid plexus (Chizhikov et al. 2006). Neuroepithelium that produces cerebellar neurons can be divided into two regions: the rhombic lip (RL) and the ventricular zone (VZ). These two regions can be morphologically discriminated by a notch located on the border.

Although the history of studies on the cerebellum is very long (Ramón y Cajal 1911), the molecular machinery underlying cerebellar neuron development is still unclear. In 1997, Ben-Arie et al. reported that a basic-helix-loop-helix type (bHLH) transcription factor, Atoh1 (also called Math1), is expressed in the rhombic lip and involved in producing cerebellar granule cells (Ben-Arie et al. 1997). However, the

development of the other types of neurons in the cerebellum remained elusive until three breakthrough papers were published in 2005.

While generating certain transgenic lines, Hoshino et al. found a novel mutant mouse line, *cerebelless*, which lacked the entire cerebellar cortex. In this mutant, all types of GABAergic neurons are not produced in the cerebellum, which leads to the secondary loss of glutamatergic granule cells and, eventually, the entire cerebellar cortex (Hoshino et al. 2005). The responsible gene was identified as *pancreatic transcription factor 1a* (*Ptf1a*), which was known to participate in pancreatic development and to encode a bHLH transcription factor. This gene is expressed in the neuroepithelium of the VZ but not of the RL, and its expression is lost in the *cerebelless* mutants. Cre-loxP recombination-based lineage-tracing analysis revealed that all types of cerebellar GABAergic neurons are derived from Ptf1a-expressing cells, but glutamatergic neurons, such as granule cells and CN-Glu neurons, are not. Loss of *Ptf1a* expression in *cerebelless* as well as *Ptf1a*-knock out mice resulted in inhibition of the production of GABAergic neurons in the cerebellar primordium. Furthermore, ectopic introduction of Ptf1a by means of in utero electroporation resulted in the abnormal production of neurons with GABAergic characteristics from the dorsal telencephalon that should only produce glutamatergic neurons under normal conditions. In addition, Pascual et al. reported that, in the *Ptf1a*-null mutants, the fate of neurons produced from the VZ is changed to that of granule cells (Pascual et al. 2007). These observations suggested that Ptf1a, expressed in the cerebellar VZ, determines GABAergic neuronal fate in the cerebellum. *PTF1A* was also identified as a causative gene for a human disease that exhibits permanent neonatal diabetes mellitus and cerebellar agenesis (Sellick et al. 2004).

On the other hand, two other groups revealed a molecular fate map of the derivatives of Atoh1-expressing neuroepithelial cells in the cerebellar RL (Machold and Fishell 2005; Wang et al. 2005). They showed that not only granule cells but also, at least in part, some neurons in the CN are derived from the RL, although they did not discriminate between glutamatergic and GABAergic subtypes in the CN. In their studies, the development of RL-derived CN neurons was shown to be disrupted in the *Atoh1*-null mice. Because Hoshino et al. reported that GABAergic but not glutamatergic CN neurons are derived from Ptf1a-expressing neuroepithelial cells in the VZ (Hoshino et al. 2005), their findings suggest that cerebellar glutamatergic neurons such as granule cells and CN-Glu neurons are derived from the RL. Accordingly, unipolar brush cells, which are glutamatergic, were also shown to emerge from the RL (Englund et al. 2006).

Together, these studies indicate the presence of two molecularly defined neuroepithelial areas in the cerebellum, the Atoh1-expressing RL and the Ptf1a-expressing VZ, which generate glutamatergic and GABAergic neurons, respectively (Hoshino 2006). Correspondingly, if the expression of Atoh1 and Ptf1a is spatially switched, the Ptf1a-expressing RL and the Atoh1-expressing VZ produce GABAergic and glutamatergic neurons, respectively (Yamada et al. 2014). This suggests a hypothesis that the bHLH transcription factors Atoh1 and Ptf1a give distinct spatial identities to the RL and VZ to generate glutamatergic and GABAergic neurons. In the telencephalon, similar regulation by bHLH transcription

factors takes place. Neurogenin 1/2 (Ngn 1/2) and Ascl1 are involved in producing glutamatergic and GABAergic neurons from ventral and dorsal neuroepithelium, respectively (Wilson and Rubenstein 2000).

How are these spatially distinct neuroepithelial areas formed? In general, the roof plate can affect the dorsal structure of the neural tube (Lee et al. 2000; Millonig et al. 2000). Chizhikov et al. revealed that the roof plate plays an important role in the formation of the cerebellar dorsoventral domain formation by analyzing cerebellar mutants that lack the roof plate (Chizhikov et al. 2006). Moreover, it has been suggested that bone morphogenetic proteins (BMPs) secreted from the roof plate as well as Notch signaling are involved in the formation of the RL and the VZ (Machold et al. 2007). A recent study that induced Purkinje cells from ES cells suggested that loss of sonic hedgehog signaling may give the dorsoventral spatial information of the cerebellar VZ to the cerebellar neuroepithelium which eventually leads to the expression of Ptf1a (Muguruma et al. 2010).

Birth-dating studies using ^{3}H-thymidine and BrdU (Chan-Palay et al. 1977; Batini et al. 1992; De Zeeuw and Berrebi 1995; Sultan et al. 2003; Leto et al. 2006) as well as adenovirus (Hashimoto and Mikoshiba 2003) revealed that each type of neuron is generated at distinct developmental stages. As to GABAergic neurons, Purkinje cells are produced at an early stage (embryonic day (E) 10.5–13.5 in mice), Golgi cells at middle stages (E14.5~), and stellate/basket cells at a late stage (Perinatal~). Regarding glutamatergic neurons, in addition to the experiment above, molecule-based lineage-tracing analyses (Machold and Fishell 2005; Wang et al. 2005; Englund et al. 2006) have clarified that CN-Glu neurons leave the cerebellar RL at early stages (E10.5–12.5) and granule cells and unipolar brush cells at middle to late stages (granule cell:E12.5~, ubc:E12.5–E18.5). In addition, somatic recombination-based clonal analyses suggested that Purkinje, Golgi, and basket/stellate cells as well as some CN neurons (probably GABAergic) belong to the same lineage (Mathis et al. 1997; Mathis and Nicolas 2003). These data indicate that some temporal information in the neuroepithelium may be involved in specification of neuronal types in the RL and VZ, respectively.

Some scientists tried to divide the structure of the cerebellar primordium into several domains. Chizhikov et al. defined four cellular populations (denoted c1-c4 domains) in the cerebellar primordium by the expression of a few transcription factors (Chizhikov et al. 2006). c1 corresponds to the Atoh1-expressing RL, and c2 is located just above the Ptf1a-expressing VZ (denoted pc2), indicating that c2 cells mainly consist of GABAergic inhibitory neurons. Although c3 and c4 express Lmx1a and Lhx1/5, respectively, their neuronal subtypes remain to be determined. This subdomain structure is disrupted when the roof plate was removed (Chizhikov et al. 2006). Furthermore, at the early neurogenesis stage (e.g., E12.5 in mice), Minaki et al. subdivided the c2 domain into dorsally (c2d) and ventrally (c2v) located subdomains that express corl2 and Pax2, respectively (Minaki et al. 2008). While corl2 is exclusively expressed in immature and mature Purkinje cells (Minaki et al. 2008), Pax2 is expressed in GABAergic interneurons (e.g., Golgi, stellate, basket, CN-GABA neurons) in the cerebellum (Maricich and Herrup 1999; Weisheit et al. 2006). They also subdivided the Ptf1a-expressing neuroepithelial domain (pc2)

into pc2d and pc2v, which strongly and weakly express E-cadherin, respectively. From the positions of the neuroepithelial and neuronal subdomains, they suggested that the pc2d neuroepithelial subdomain produces cells in the c2d domain which give rise to Purkinje cells, and pc2v subdomain generates cells in the c2v that become GABAergic interneurons (Mizuhara et al. 2010). As development proceeds, pc2d and pc2v subdomains become smaller and larger, respectively, and by E14.5 in mice, the Ptf1a-expressing pc2 domain is comprised only by the pc2v subdomain which expresses E-cadherin weakly. This correlates with the fact that, at E14.5 in mice, Ptf1a-expressing neuroepithelium does not produce Purkinje cells but Pax2-positive interneurons (Pax2+ INs) (Maricich and Herrup 1999; Hashimoto and Mikoshiba 2003).

Seto et al. reported that there are two types of Ptf1a-positive progenitors in the VZ: Olig2-expressing Purkinje cell-producing progenitors (PCPs) and Gsx1 (also called Gsh1)-expressing Pax2+ IN-producing progenitors (PIPs) (Fig. 1, Seto et al. 2014). At the early stages (E10.5, E11.5), only a small number of PIPs are located at the ventral-most region within the VZ, and a large number of PCPs occupy the remaining regions in the VZ. As development proceeds, PCPs gradually transit to become PIPs starting from ventral to dorsal regions. This temporal identity transition of cerebellar GABAergic neuron progenitor causes the loss of PCPs in the VZ by E14.5, correlating with the observations that Purkinje cells are produced only at early neurogenesis stages (E10.5–E13.5). Deducing from their position and

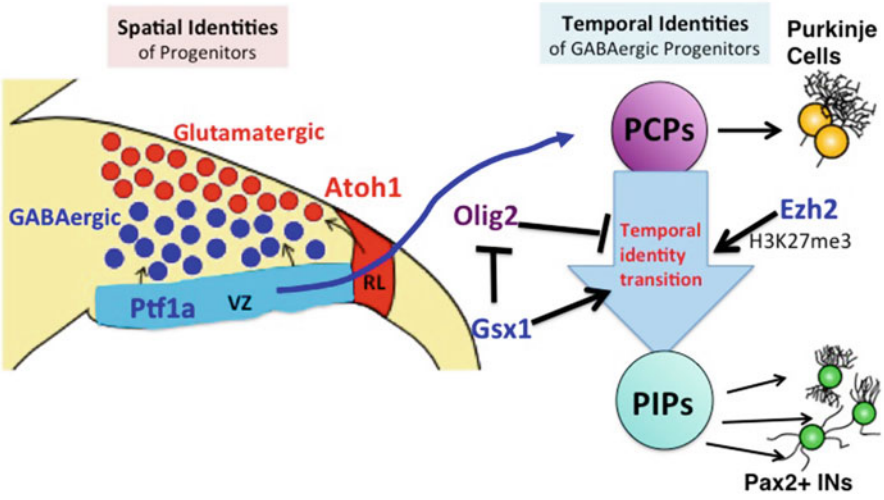

Fig. 1 Specification of cerebellar neurons in the neuroepithelium. The two bHLH transcription factors, Atoh1 and Ptf1a, confer spatial identities of the RL and the VZ to produce glutamatergic and GABAergic neurons, respectively. Within the VZ, temporal identity transition of GABAergic progenitors from PCPs to PIPs gradually takes place. The speed of the temporal identity transition is negatively regulated by Olig2 and positively by Gsx1 and Ezh2. As Ezh2 is involved in histone methylation, the temporal identity transition seems to be regulated by epigenetic modification

dynamics, PCPs and PIPs may correspond to GABAergic progenitors in Pc2d and pc2v presented by Chizhikov et al. (2006).

The temporal identity transition of cerebellar GABAergic neuron progenitors from PCPs to PIPs is negatively regulated by Olig2 and positively by Gsx1, which may contribute to proper numbers of Purkinje cells and Pax2+ INs being produced (Fig. 1, Seto et al. 2014), while Olig2 is also reported to participate in Purkinje cell differentiation (Ju et al. 2017). The temporal identity transition may also be regulated by epigenetic modification. Ezh2 is a catalytic subunit of polycomb complex 2 for histone tri-methylation (Young 2011). Targeted disruption of Ezh2 resulted in reduction of total H3K27 tri-methylation (H3K27me3) as well as reduced number of Pax2+ INs and increased number of Purkinje cells in the cerebellum (Feng et al. 2016), which can be explained by delayed temporal identity transition. This suggests that the temporal identity transition from PCPs to PIPs is regulated by epigenetic histone methylation.

Zordan et al. reported the expression profiles of proneural bHLH transcription factors, such as Ngn1, Ngn2, and Ascl1 in the cerebellar VZ (Zordan et al. 2008). Pax2+ INs, but not Purkinje cells, are reduced in the *Ascl1*-null cerebellum (Grimaldi et al. 2009), while Purkinje cells are reduced in *Ngn1*-null mice (Lundell et al. 2009). These findings suggest that proneural factors, such as Ascl1 and Ngn1, may also regulate the temporal identity transition from PCPs to PIPs.

In addition, several factors have been reported to participate in the development of a certain type of cerebellar neurons. Double knockout of transcription factors, *Lhx1* and *Lhx5*, as well as the targeted disruption of their cofactor *Ldb1* resulted in lack of Purkinje cell production in the cerebellum although Pax2+ INs did not seem to be affected. Because Lhx1 and Lhx5 are expressed in postmitotic cells, this suggests that Lhx1, Lhx5, and Ldb1 are postmitotically involved in Purkinje cell specification (Zhao et al. 2007). Moreover, targeted disruption of Frizzled co-receptors Lrp5/6 caused misexpression of tyrosine hydroxylase in Purkinje cells, suggesting that canonical WNT signaling plays an important role in proper differentiation of Purkinje cells (Huang et al. 2016). Targeted disruption of *cyclin D2* caused loss of stellate cells in the cerebellar molecular layer, suggesting its involvement in the development of stellate cells (Huard et al. 1999). Transcriptional factor AP-2 family Tfap2a/2b are downstream targets of Ptf1a and involved in specification of interneurons (Jin et al. 2015; Zainolabidin et al. 2017).

From the RL, several types of glutamatergic neurons, such as CN-Glu neurons, granule cells, and unipolar brush cells, are generated. CN-Glu neurons leave the RL at early neurogenesis stages. Some transcription factors, such as Tbr1, Irx3, Meis2, Lhx2, and Lhx9, have been found to be expressed in postmitotic progenitors of CN-Glu neurons, but their roles have not been clarified (Morales and Hatten 2006). Other molecules, such as Zic1 (Aruga et al. 1998), have been reported to play important roles in the migration, maturation, and survival of granule cells, but the molecular machinery underlying the specification of granule cell identity is unknown. It is reported that a transcription factor Meis1 plays crucial roles in granule cell development by regulating Pax6 transcription, BMP signaling, and Atoh1 degradation, but its involvement in granule cell specification is unclear (Owa et al. 2018). Although unipolar brush cells strongly express Tbr2, its function is also

elusive. In addition, Pax6 and WNT signaling are indicated to be important for the survival and/or specification of UBCs (Yeung et al. 2014; Yeung and Goldowitz 2017). In Ezh2 KO mice, granule cell precursors are severely reduced at late embryonic stages (Feng et al. 2016). This indicates epigenetic modification of histone methylation may also underlie the development of excitatory neurons produced from the RL.

In addition to genetic analyses, heterotopic and heterochronic transplantation studies have also provided important clues to understanding cerebellar development (Carletti and Rossi 2008). When tissues from embryonic and postnatal cerebella were mixed and transplanted to the fourth ventricle of an adult mouse, the postnatal-derived cells differentiated only into interneurons such as granule, basket, and stellate cells, but not projection neurons, such as Purkinje cells, whereas the embryonic-derived cells were capable of becoming all types of cerebellar neurons (Jankovski et al. 1996). In addition, it was shown that dissociated cells taken from cerebellar primordium at early neurogenesis stages could differentiate into all major types of cerebellar neurons, but those from postnatal cerebellum differentiated only to Pax2-positive interneurons (Carletti et al. 2002). These findings suggest that the differentiation competence of cerebellar progenitors becomes restricted as development proceeds. Probably, epigenetic regulation such as histone modification may underlie this fate restriction, as was suggested by the phenotype of Ezh2 KO mice (Feng et al. 2016).

Interestingly, Leto et al. suggested that pax2+ INs are derived from same progenitor pool and that extrinsic instructive cues in the microenvironment may affect the terminal neuronal type commitment (Leto et al. 2006, 2009). One candidate for the cue may be sonic hedgehog (SHH) (Fleming et al. 2013; De Luca et al. 2015).

Specification of Precerebellar Neurons

There are two types of precerebellar afferent systems: mossy fiber (MF) and climbing fiber (CF) systems. MF neurons are located in several nuclei throughout the brain stem and extend their glutamatergic projections to granule cells conveying peripheral and cortical information to the cerebellum. Four major nuclei containing MF neurons are the pontine gray nucleus (PGN), the reticulotegmental nucleus (RTN), the lateral reticular nucleus (LRN), and the external cuneate nucleus (ECN) in the hindbrain (Altman and Bayer 1987). In addition, some MF neurons are also located in the spinal trigeminal nucleus (Sp5) in the hindbrain and Clarke's column in the spinal cord. In contrast, CF neurons reside exclusively in the inferior olive nucleus (ION), which receive input from the cerebral cortex, the red nucleus, spinal cord, and other brain stem nuclei and send glutamatergic projections to Purkinje cells (Ruigrok et al. 1995). Both types of precerebellar neurons also send branch axons to the neurons in the cerebellar nucleus. These precerebellar systems are thought to transmit the external and internal information to the cerebellar cortex to modulate cerebellar function, including regulation of animal movement.

Previous birth-dating studies in mice revealed that CF neurons are generated at relatively early neurogenesis stages (E9.5–11.5) and MF neurons are produced at slightly later stages (E10.5–16.5) (Pierce 1973). Along the rostrocaudal axis, both

MF and CF neurons in the hindbrain are generated from the caudal hindbrain, around rhombomeres 6–8 (r6–r8), as suggested by avian grafting studies as well as mammalian fate map analyses (Ambrosiani et al. 1996; Cambronero and Puelles 2000; Farago et al. 2006; Kawauchi et al. 2006). By contrast, MF neurons in the Clarke's nucleus are generated in the spinal cord (Bermingham et al. 2001). Classic anatomical and immunohistochemical studies have suggested that these precerebellar nuclei neurons in the hindbrain emerge from the dorsal part of the hindbrain and migrate tangentially or circumferentially to their final loci (Bloch-Gallego et al. 1999; Yee et al. 1999; Kyriakopoulou et al. 2002). However, they take slightly different paths from each other; MF and CF neurons move extramurally and intramurally, respectively. Introduction of a GFP-expressing vector into the embryonic dorsal hindbrain allowed the dramatic visualization of migrating precerebellar nuclei neurons during development (Kawauchi et al. 2006; Okada et al. 2007; Nishida et al. 2011; Shinohara et al. 2013; Kobayashi et al. 2013, 2015; Hatanaka et al. 2016).

Many groups have reported transcription factors that are expressed within the dorsal neuroepithelium of the caudal (r6–8) hindbrain during embryonic development, trying to define domains along the dorsoventral axis. The dorsal-most part expressing Lmx1a corresponds to the roof plate which gives rise to the choroid plexus (Chizhikov et al. 2006). Other than the roof plate, the dorsal neuroepithelium can be divided into six domains (dP1–dP6) according to the expression pattern of the transcription factors, such as Atoh1, Ngn1, Ascl1, Ptf1a, and Olig3 (Fig. 2). As to the precerebellar nuclei neurons, a series of studies have tried to clarify the precise origins of MF and CF neurons by genetic lineage-tracing methods.

By analyzing genetically engineered mice that express *lacZ* or *Cre recombinase* under the control of the endogenous or exogenous *Atoh1* promoter, MF neurons of PGN, RTN, LRN, and ECN were shown to emerge from the Atoh1-expressing

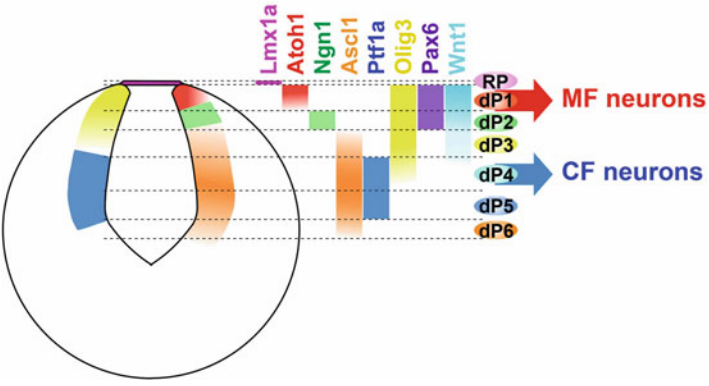

Fig. 2 Neuroepithelial domain structure in the caudal hindbrain. In the caudal hindbrain (r6–8), several transcription factors are expressed within the dorsal neuroepithelium during embryonic development. The dorsal-most part, the roof plate (RP), expresses Lmx1a. Other than the roof plate, the dorsal neuroepithelium can be divided into six domains (dP1–dP6) according to the expression pattern of transcription factors such as Atoh1, Ngn1, Pax6, Ascl1, Ptf1a, and Olig3. While mossy fiber (MF) neurons are derived from the dP1 domain expressing Atoh1, climbing fiber (CF) neurons are generated from the dP4 domain expressing Ptf1a and Olig3

neuroepithelial domain (dP1, Ben-Arie et al. 2000; Rodriguez and Dymecki 2000; Landsberg et al. 2005; Wang et al. 2005). Targeted disruption of the *Atoh1* gene resulted in the loss of these MF neurons, suggesting an involvement of Atoh1 in the MF neuron development.

Atoh1 regulates the expression of the transcription factor Barhl1 (Mbh2) that is expressed in MF neurons. Loss of Barhl1 expression resulted in a decrease of MF neurons, leading to a decrease in the size of MF precerebellar nuclei (Li et al. 2004). In addition, Flora et al. reported that one of the E-proteins, Tcf4, interacts with Atoh1 and regulates differentiation of a specific subset (PGN, RTN) of MF neurons (Flora et al. 2007).

Landsberg et al. also performed lineage trace analysis by using two variants of FLP (Flipperase recombinase) with different recombinase activities that were expressed under the control of the *Wnt-1* promoter whose strength is the highest at the dorsal-most part and gradually decreases ventrally. They demonstrated that CF neurons are derived from the neuroepithelial region where *Wnt-1* is very weakly expressed, whereas MF neurons emerge from the strongly Wnt1-expressing region (Landsberg et al. 2005). In addition, Nichols and Bruce generated transgenic mice carrying a *Wnt-1*-enhancer/lacZ transgene and observed that MF neurons but not CF neurons were labeled by β-gal in those mice (Nichols and Bruce 2006). These findings suggested that CF neurons are generated from the neuroepithelial region ventral to the Atoh1-expressing domain.

By Cre-loxP-based lineage trace analysis, Yamada et al. showed that all CF neurons in the ION are derived from the Ptf1a-expressing neuroepithelial region (Yamada et al. 2007). Loss of the *Ptf1a* gene resulted in the fate change of some CF neurons to MF neurons, suggesting that Ptf1a plays a critical role in fate determination of CF neurons. They also showed an involvement of Ptf1a in migration, differentiation, and survival of CF neurons. Storm et al. reported that not only MF neurons but also CF neurons are derived from the Olig3-expressing neuroepithelial region that broadly expands within the dorsal hindbrain (Storm et al. 2009) by Cre-loxP-based linage tracing. Targeted disruption of the *Olig3* gene caused the disorganized development of MF neurons and complete loss of CF neurons (Liu et al. 2008; Storm et al. 2009). Moreover, the ectopic co-expression of Olig3 and Ptf1a induced cells expressing a CF neuron marker in chick embryos (Storm et al. 2009). These findings suggest that CF neurons emerge from the Ptf1a-/Olig3-expressing neuroepithelial domain (dP4) and that Ptf1a and Olig3 are cooperatively involved in the development of CF neurons. Domain structure of the dorsal neuroepithelium in the caudal hindbrain region is shown in Fig. 2.

Conclusions and Future Directions

Various types of neurons are generated from the dorsal hindbrain. As described above, the dorsal neuroepithelium of the rostral hindbrain (r1) produces all types of cerebellar neurons, while the dorsal regions of the caudal hindbrain (r6–r8) generate neurons that include the precerebellar system neurons, such as MF and CF neurons. In addition, histological observations suggested that the dorsal part of the middle

hindbrain produces neurons of the cochlear nucleus, where auditory information is processed and relayed to the brain (Pierce 1967; Ivanova and Yuasa 1998). More directly, genetic fate-mapping studies using transgenic mice confirmed that neurons of the cochlear nucleus are derived from the dorsal part of r2–r5 in mice (Farago et al. 2006), although in avians, they were shown to emerge from a broader part (r3–r8) by grafting studies (Tan and Le Douarin 1991; Cambronero and Puelles 2000; Cramer et al. 2000). As to neuronal subtypes, Fujiyama et al. identified origins of inhibitory and excitatory neurons of the cochlear nucleus; inhibitory (glycinergic and GABAergic) and excitatory (glutamatergic) neurons are derived from Ptf1a- and Atoh1-expressing neuroepithelial regions, respectively (Fujiyama et al. 2009), and their development is dependent on the corresponding bHLH proteins.

In the hindbrain from r1 to r8, there are dorsoventral domain structures defined by several transcription factors, which are longitudinally expressed throughout the hindbrain. Especially, two bHLH transcription factors, Atoh1 and Ptf1a, seem to play important roles in specifying distinct neuronal subtypes. These two proteins are expressed in different neuroepithelial regions throughout the hindbrain (Fig. 3). In both the rostral (r1) and middle hindbrain (r2–r5 in mice), Atoh1 and Ptf1a participate in generating excitatory and inhibitory neurons, respectively. However, this

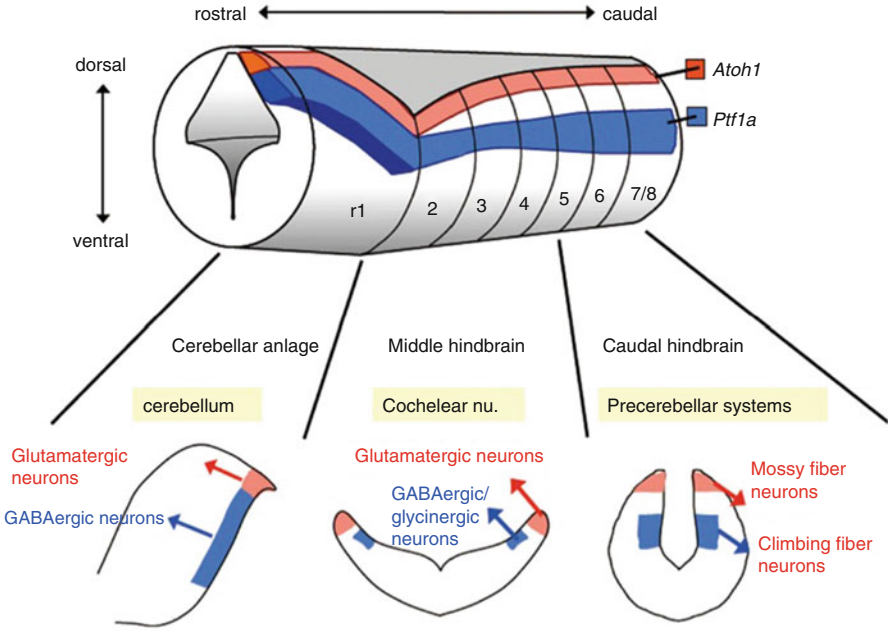

Fig. 3 Basic HLH proteins and neurons produced from the dorsal hindbrain. Atoh1 and Ptf1a are expressed in distinct neuroepithelial regions throughout the rhombomeres 1–8 (r1–8). Each number represents the rhombomeric number. Upper side is dorsal, and lower is ventral. Left side is rostral, and right side is caudal. Neuronal subtypes generated from the dorsal neuroepithelium of the rostral, middle, and caudal hindbrain regions are shown

rule is not applicable to the caudal hindbrain. The Ptf1a neuroepithelial domain in the caudal hindbrain (r6–r8 in mice) produces not only inhibitory neurons (local circuit neurons) but also glutamatergic neurons (CF neurons, Yamada et al. 2007), while the Atoh1 domain generates glutamatergic MF neurons. This raises the possibility that the rostral/middle (r1–r5) and caudal (r6–r8) hindbrain subregions have distinct characteristics. Overall, throughout the hindbrain regions, transcription factors, such as Atoh1 and Ptf1a, seem to define neuroepithelial domains along the dorsoventral axis and participate in specifying distinct neuronal subtypes according to the rostrocaudal spatial information (Fig. 3).

Cross-References

▶ Development of Cerebellar Nuclei
▶ Development of Glutamatergic and GABAergic Synapses

References

Altman J, Bayer SA (1987) Development of the precerebellar nuclei in the rat. I–IV. J Comp Neurol 257:477–552

Ambrosiani J, Armengol JA, Martinez S, Puelles L (1996) The avian inferior olive derives from the alar neuroepithelium of the rhombomeres 7 and 8: an analysis by using chick-quail chimeric embryos. Neuroreport 7:1285–1288

Aruga J, Minowa O, Yaginuma H, Kuno J, Nagai T, Noda T, Mikoshiba K (1998) Mouse Zic1 is involved in cerebellar development. J Neurosci 18:284–293

Batini C, Compoint C, Buisseret-Delmas C, Daniel H, Guegan M (1992) Cerebellar nuclei and the nucleocortical projections in the rat: retrograde tracing coupled to GABA and glutamate immunohistochemistry. J Comp Neurol 315:74–84

Ben-Arie N, Bellen HJ, Armstrong DL, McCall AE, Gordadze PR, Guo Q, Matzuk MM, Zoghbi HY (1997) Math1 is essential for genesis of cerebellar granule neurons. Nature 390:169–172

Ben-Arie N, Hassan BA, Bermingham NA, Malicki DM, Armstrong D, Matzuk M, Bellen HJ, Zoghbi HY (2000) Functional conservation of atonal and Math1 in the CNS and PNS. Development 127:1039–1048

Bermingham NA, Hassan BA, Wang VY, Fernandez M, Banfi S, Bellen HJ, Fritzsch B, Zoghbi HY (2001) Proprioceptor pathway development is dependent on Math1. Neuron 30:411–422

Bloch-Gallego E, Ezan F, Tessier-Lavigne M, Sotelo C (1999) Floor plate and netrin-1 are involved in the migration and survival of inferior olivary neurons. J Neurosci 19:4407–4420

Cambronero F, Puelles L (2000) Rostrocaudal nuclear relationships in the avian medulla oblongata: a fate map with quail chick chimeras. J Comp Neurol 427:522–545

Carletti B, Rossi F (2008) Neurogenesis in the cerebellum. Neuroscientist 14:91–100

Carletti B, Grimaldi P, Magrassi L, Rossi F (2002) Specification of cerebellar progenitors after heterotopic-heterochronic transplantation to the embryonic CNS in vivo and in vitro. J Neurosci 22:7132–7146

Chan-Palay V, Palay SL, Brown JT, Van Itallie C (1977) Sagittal organization of olivocerebellar and reticulocerebellar projections: autoradiographic studies with 35S-methionine. Exp Brain Res 30:561–576

Chizhikov V, Millen KJ (2003) Development and malformations of the cerebellum in mice. Mol Genet Metab 80:54–65

Chizhikov VV, Lindgren AG, Currle DS, Rose MF, Monuki ES, Millen KJ (2006) The roof plate regulates cerebellar cell-type specification and proliferation. Development 133:2793–2804

Cramer KS, Fraser SE, Rubel EW (2000) Embryonic origins of auditory brain-stem nuclei in the chick hindbrain. Dev Biol 224:138–151

De Luca A, Parmigiani E, Tosatto G, Martire S, Hoshino M, Buffor A, Leto K, Rossi F (2015) Exogenous Sonic Hedgehog modulates the pool of GABAergic interneurons during cerebellar development. Cerebellum 14:72–85

De Zeeuw CI, Berrebi AS (1995) Postsynaptic targets of Purkinje cell terminals in the cerebellar and vestibular nuclei of the rat. Eur J Neurosci 7:2322–2333

Englund C, Kowalczyk T, Daza RA, Dagan A, Lau C, Rose MF, Hevner RF (2006) Unipolar brush cells of the cerebellum are produced in the rhombic lip and migrate through developing white matter. J Neurosci 26:9184–9195

Farago AF, Awatramani RB, Dymecki SM (2006) Assembly of the brainstem cochlear nuclear complex is revealed by intersectional and subtractive genetic fate maps. Neuron 50:205–218

Feng X, Juan AH, Wang HA, Ko KD, Zare H, Sartorelli V (2016) Polycomb Ezh2 controls the fate of GABAergic neurons in the embryonic cerebellum. Development 143:1971–1980

Fleming JT, He W, Hao C, Ketova T, Pan FC, Wright CC, Litingtung Y, Chaing C (2013) The Purkinje neuron act as a central regulator of spatially and functionally distinct cerebellar precursors. Dev Cell 27:278–292

Flora A, Garcia JJ, Thaller C, Zoghbi HY (2007) The E-protein Tcf4 interacts with Math1 to regulate differentiation of a specific subset of neuronal progenitors. Proc Natl Acad Sci U S A 104:15382–15387

Fujiyama T, Yamada M, Terao M, Terashima T, Hioki H, Inoue YU, Inoue T, Masuyama N, Obata K, Yanagawa Y, Kawaguchi Y, Nabeshima Y, Hoshino M (2009) Inhibitory and excitatory subtypes of cochlear nucleus neurons are defined by distinct bHLH transcription factors, Ptf1a and Atoh1. Development 136:2049–2058

Grimaldi P, Parras C, Guillemot F, Rossi F, Wassef M (2009) Origins and control of the differentiation of inhibitory interneurons and glia in the cerebellum. Dev Biol 328:422–433

Hashimoto M, Mikoshiba K (2003) Mediolateral compartmentalization of the cerebellum is determined on the "birth date" of Purkinje cells. J Neurosci 23:11342–11351

Hatanaka Y, Zhu Y, Torigoe M, Kita Y, Murakami F (2016) From migration to settlement: the pathways, migration modes and dynamics of neurons in the developing brain. Proc Jpn Acad Ser B Phys Biol Sci 92:1–19

Hoshino M (2006) Molecular machinery governing GABAergic neuron specification in the cerebellum. Cerebellum 5:193–198

Hoshino M, Nakamura S, Mori K, Kawauchi T, Terao M, Nishimura YV, Fukuda A, Fuse T, Matsuo N, Sone M, Watanabe M, Bito H, Terashima T, Wright CV, Kawaguchi Y, Nakao K, Nabeshima Y (2005) *Ptf1a*, a bHLH transcriptional gene, defines GABAergic neuronal fates in cerebellum. Neuron 47:201–213

Huang Y, Zhang Q, Song NN, Zhang L, Sun YL, Hu L, Chen JY, Zhu W, Li J, Ding YQ (2016) Lrp5/6 are required for cerebellar development and for suppressing TH expression in Purkinje cells via β-catenin. Mol Brain 9:7

Huard JM, Forster CC, Carter ML, Sicinski P, Ross ME (1999) Cerebellar histogenesis is disturbed in mice lacking cyclin D2. Development 126:1927–1935

Ivanova A, Yuasa S (1998) Neuronal migration and differentiation in the development of the mouse dorsal cochlear nucleus. Dev Neurosci 20:495–511

Jankovski A, Rossi F, Sotelo C (1996) Neuronal precursors in the postnatal mouse cerebellum are fully committed cells: evidence from heterochronic transplantations. Eur J Neurosci 8:2308–2319

Jin K, Jiang H, Xiao D, Zou M, Zhu J, Xiang M (2015) Tfap2a and 2b act downstream of Ptf1a to promote amacrine cell differentiation during retinogenesis. Mol Brain 8:28

Ju J, Liu Q, Zhang Y, Liu Y, Jiang M, Zhang L, He X, Peng C, Zheng T, Lu QR, Li H (2017) Olig2 regulates Purkinje cell generation in the early developing mouse cerebellum. Sci Rep 6:30711

Kawauchi D, Taniguchi H, Watanabe H, Saito T, Murakami F (2006) Direct visualization of nucleogenesis by precerebellar neurons: involvement of ventricle-directed, radial fibre-associated migration. Development 133:1113–1123

Kobayashi H, Kawauchi D, Hashimoto Y, Ogata T, Murakami F (2013) The control of precerebellar neuron migration by RNA-binding protein Csde1. Neuroscience 253:292–303

Kobayashi H, Saragai S, Naito A, Ichio K, Kawauchi D, Murakami F (2015) Calm1 signaling pathway is essential for the migration of mouse precerebellar neurons. Development 142:375–384

Kyriakopoulou K, de Diego I, Wassef M, Karagogeos D (2002) A combination of chain and neurophilic migration involving the adhesion molecule TAG-1 in the caudal medulla. Development 129:287–296

Landsberg RL, Awatramani RB, Hunter NL, Farago AF, DiPietrantonio HJ, Rodriguez CI, Dymecki SM (2005) Hindbrain rhombic lip is comprised of discrete progenitor cell populations allocated by Pax6. Neuron 48:933–947

Lee KJ, Dietrich P, Jessell TM (2000) Genetic ablation reveals that the roof plate is essential for dorsal interneuron specification. Nature 403:734–740

Leto K, Carletti B, Williams IM, Magrassi L, Rossi F (2006) Different types of cerebellar GABAergic interneurons originate from a common pool of multipotent progenitor cells. J Neurosci 26:11682–11694

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

Li S, Qiu F, Xu A, Price SM, Xiang M (2004) Barhl1 regulates migration and survival of cerebellar granule cells by controlling expression of the neurotrophin-3 gene. J Neurosci 24:3104–3114

Liu Z, Li H, Hu X, Yu L, Liu H, Han R, Colella R, Mower GD, Chen Y, Qiu M (2008) Control of precerebellar neuron development by Olig3 bHLH transcription factor. J Neurosci 28:10124–10133

Lundell TG, Zhou Q, Doughty ML (2009) Neurogenin1 expression in cell lineages of the cerebellar cortex in embryonic and postnatal mice. Dev Dyn 238:3310–3325

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

Machold RP, Kittell DJ, Fishell GJ (2007) Antagonism between Notch and bone morphogenetic protein receptor signaling regulates neurogenesis in the cerebellar rhombic lip. Neural Dev 2:5

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

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

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

Millet S, Bloch-Gallego E, Simeone A, Alvarado-Mallart RM (1996) 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 122:3785–3797

Millonig JH, Millen KJ, Hatten ME (2000) The mouse Dreher gene Lmx1a controls formation of the roof plate in the vertebrate CNS. Nature 403:764–769

Minaki Y, Nakatani T, Mizuhara E, Inoue T, Ono Y (2008) Identification of a novel transcriptional corepressor, Corl2, as a cerebellar Purkinje cell-selective marker. Gene Expr Patterns 8:418–423

Mizuhara E, Minaki Y, Nakatani T, Kumai M, Inoue T, Muguruma K, Sasai Y, Ono Y (2010) Purkinje cells originate from cerebellar ventricular zone progenitors positive for Neph3 and E-cadherin. Dev Biol 338:202–214

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

Muguruma K, Nishiyama A, Ono Y, Miyawaki H, Mizuhara E, Hori S, Kakizuka A, Obata K, Yanagawa Y, Hirano T, Sasai Y (2010) Ontogeny-recapitulating generation and tissue integration of ES cell-derived Purkinje cells. Nat Neurosci 13:1171–1180

Nichols DH, Bruce LL (2006) Migratory routes and fates of cells transcribing the Wnt-1 gene in the murine hindbrain. Dev Dyn 235:285–300

Nishida K, Nakayama K, Yoshimura S, Murakami F (2011) Role of Neph2 in pontine nuclei formation in the developing hindbrain. Mol Cell Neurosci 46:662–670

Okada T, Keino-Masu K, Masu M (2007) Migration and nucleogenesis of mouse precerebellar neurons visualized by in utero electroporation of a green fluorescent protein gene. Neurosci Res 57:40–49

Owa T, Taya S, Miyashita S, Yamashita M, Adachi T, Yamada K, Yokoyama M, Aida S, Nishioka T, Yukiko UI, Goitsuka R, Nakamura T, Inoue T, Kaibuchi K, Hoshino M (2018) Meis1 coordinates cerebellar granule cell development by regulating Pax6 transcription, BMP signaling and Atoh1 degradation. J Neurosci 38:1277–1294

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

Pierce ET (1967) Histogenesis of the dorsal and ventral cochlear nuclei in the mouse. An autoradiographic study. J Comp Neurol 131:27–54

Pierce ET (1973) Time of origin of neurons in the brain stem of the mouse. Prog Brain Res 40:53–65

Ramon y Cajal S (1911) Histologie du Systeme Nerveux de l'Homme et des Vertebres. Maloine, Paris

Rodriguez CI, Dymecki SM (2000) Origin of the precerebellar system. Neuron 27:475–486

Ruigrok TJ, Cella F, Voogd J (1995) Connections of the lateral reticular nucleus to the lateral vestibular nucleus in the rat. An anterograde tracing study with *Phaseolus vulgaris* leucoagglutinin. Eur J Neurosci 7:1410–1413

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

Seto Y, Nakatani T, Masuyama N, Taya S, Kumai M, Minaki Y, Hamaguchi A, Inoue YU, Inoue T, Miyashita S, Fujiyama T, Yamada M, Chapman H, Campbell K, Magnuson MA, Wright CV, Kawaguchi Y, Ikenaka K, Takebayashi H, Ishiwata S, Ono Y, Hoshino M (2014) Temporal identity transition from Purkinje cell progenitors to GABAergic interneuron progenitors in the cerebellum. Nat Commun 5:3337

Shinohara M, Zhu Y, Murakami F (2013) Four-dimensional analysis of nucleogenesis of the pontine nucleus in the hindbrain. J Comp Neurol 521:3340–3357

Storm R, Cholewa-Waclaw J, Reuter K, Brohl D, Sieber M, Treier M, Muller T, Birchmeier C (2009) The bHLH transcription factor Olig3 marks the dorsal neuroepithelium of the hindbrain and is essential for the development of brainstem nuclei. Development 136:295–305

Sultan F, Czubayko U, Thier P (2003) Morphological classification of the rat lateral cerebellar nuclear neurons by principal component analysis. J Comp Neurol 455:139–155

Tan K, Le Douarin NM (1991) Development of the nuclei and cell migration in the medulla oblongata. Application of the quail-chick chimera system. Anat Embryol (Berl) 183:321–343

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

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

Wilson SW, Rubenstein JL (2000) Induction and dorsoventral patterning of the telencephalon. Neuron 28:641–651

Wingate RJ, Hatten ME (1999) The role of the rhombic lip in avian cerebellum development. Development 126:4395–4404

Yamada M, Terao M, Terashima T, Fujiyama T, Kawaguchi Y, Nabeshima Y, Hoshino M (2007) Origin of climbing fiber neurons and their developmental dependence on Ptf1a. J Neurosci 27:10924–10934

Yamada M, Seto Y, Taya S, Owa T, Inoue YU, Inoue T, Kawaguchi Y, Nabeshima Y, Hoshino M (2014) Specification of spatial identities of cerebellar neuron progenitors by Ptf1a and Atoh1 for proper production of GABAergic and glutamatergic neurons. J Neurosci 34:4786–4800

Yee KT, Simon HH, Tessier-Lavigne M, O'Leary DM (1999) Extension of long leading processes and neuronal migration in the mammalian brain directed by the chemoattractant netrin-1. Neuron 24:607–622

Yeung J, Goldowitz D (2017) Wls expression in the rhombic lip orchestrates the embryonic development of the mouse cerebellum. Neuroscience 354:30–42

Yeung J, Ha TJ, Swanson DJ, Choi K, Tong Y, Goldowitz D (2014) Wls provides a new compartmental view of the rhombic lip in mouse cerebellar development. J Neurosci 34:12527–37

Young RA (2011) Control of the embryonic stem cell state. Cell 144:940–954

Zainolabidin N, Kamath SP, Thanawalla AR, Chen AI (2017) Distinct activities of Tfap2A and Tfap2B in the specification of GABAergic interneurons in the developing cerebellum. Front Mol Neurosci 10:281

Zervas M, Millet S, Ahn S, Joyner AL (2004) Cell behaviors and genetic lineages of the mesencephalon and rhombomere 1. Neuron 43:345–357

Zhao Y, Kwan KM, Mailloux CM, Lee WK, Grinberg A, Wurst W, Behringer RR, Westphal H (2007) LIM-homeodomain proteins Lhx1 and Lhx5, and their cofactor Ldb1, control Purkinje cell differentiation in the developing cerebellum. Proc Natl Acad Sci U S A 104:13182–13186

Zordan P, Croci L, Hawkes R, Consalez GG (2008) Comparative analysis of proneural gene expression in the embryonic cerebellum. Dev Dyn 237:1726–1735