Chapter 15
Specification of Cerebellar Neurons

Mikio Hoshino

Abstract The cerebellum consists of about ten types of neurons that have distinct characteristics in terms of their location, morphology, immunoreactivity and physiology. They can be categorized into two groups; glutamatergic excitatory and GABAergic inhibitory neurons. Excitatory neurons are comprised of glutamatergic deep cerebellar nuclei (DCN) neurons, granule cells and unipolar brush cells (UBCs). Inhibitory neurons include GABAergic DCN neurons, Purkinje cells, Golgi cells, Lugaro cells, basket cells, and stellate cells. GABAergic DCN neurons are interneurons that contribute to local circuitry and projection neurons that extend axons towards the inferior olivary nucleus. As all cerebellar GABAergic interneurons express Pax2 (Maricich and Herrup 1999), they are called Pax2+ interneurons (Pax2+ INs). Recent studies have partly uncovered the molecular machinery for neuronal subtype specification in the cerebellum.

Keywords Neural progenitor • Rhombic lip • Ventricular zone • Glutamatergic • GABAergic • bHLH • Transcription factor • Spatiotemporal regulation

15.1 Birthplaces and Birthdates of Cerebellar Neurons

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M. Hoshino (✉)
Department of Biochemistry and Cellular Biology, National Institute of Neuroscience,
National Center of Neurology and Psychiatry,
4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-8502, Japan
e-mail: hoshino@ncnp.go.jp

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interneurons that contribute to local circuitry and projection neurons that extend axons towards the inferior olivary nucleus. As all cerebellar GABAergic interneurons express Pax2 (Maricich and Herrup 1999), they are called Pax2+ interneurons (Pax2+ INs).

All neurons in the cerebellum emerge from the cerebellar neuroepithelium that includes the dorsally located rhombic lip (RL) and the ventrally located ventricular zone (VZ). In the cerebellum, the RL produces all glutamatergic neurons (Ben-Arie et al. 1997; Machold and Fishell 2005; Wang et al. 2005). The VZ generates all GABAergic neurons (Hoshino et al. 2005), although postnatally produced neurons, such as stellate and basket cells, do not directly emerge from the VZ but from the prospective white matter (PWM) thought to be derived from the VZ (Leto et al. 2009).

Birthdates of GABAergic neurons can be estimated by BrdU or tritium incorporation studies or adenoviral infection studies (Chan-Palay et al. 1977; Batini et al. 1992; De Zeeuw and Berrebi 1995; Sultan et al. 2003; Leto et al. 2006; Hashimoto and Mikoshiba 2003). In mice, Purkinje cells are generated at embryonic day (E) 10.5–E13.5, GABAergic DCN neurons at E10.5–E11.5, and Golgi cells at approximately E13.5~ postnatal (peak around E13.5–E15.5). Late born GABAergic neurons, including stellate and basket cells, emerge from GABAergic progenitor cells in the PWM at later stages (approximately E13.5~ perinatal, peak around E17.5~ perinatal). Somatic recombination-based clonal analyses have revealed that Purkinje, Golgi and basket/stellate cells belong to the same lineage (Mathis et al. 1997; Mathis and Nicolas 2003). This suggests that not only Purkinje and Golgi cells but also GABAergic progenitors in the PWM are derived from the VZ. As to glutamatergic neurons, glutamatergic DCN neurons leave the RL at early stages (E10.5–12.5) and granule cells and UBCs at middle to late stages (granule cells; E13.5~, UBCs; E13.5–E18.5) (Machold and Fishell 2005; Wang et al. 2005; Englund et al. 2006).

15.2 Molecular Machinery to Specify Distinct Types of Cerebellar Neurons

Two basic-helix-loop-helix proteins are involved in specification of glutamatergic vs. GABAergic neurons. Atoh1 (also called Math1) is expressed in the RL and involved in producing glutamatergic neurons (Ben-Arie et al. 1997; Machold and Fishell 2005; Wang et al. 2005). Ptf1a is expressed in the VZ and specifies the GABAergic neuron lineage (Hoshino et al. 2005; Pascual et al. 2007). When the expression of Atoh1 and Ptf1a is switched, the RL and the VZ produce GABAergic and glutamatergic neurons, respectively (Yamada et al. 2014), suggesting that these two bHLH proteins are sufficient to specify glutamatergic and GABAergic fates. These observations imply that neural progenitors in the RL and the VZ have spatially-regulated distinct identities to produce glutamatergic and GABAergic neurons, respectively.

At early neurogenesis stages such as E11.5, there are two types of GABAergic neuron progenitors in the VZ that express Ptf1a; Pax2+ IN-producing progenitors (PIPs) and Purkinje cell-producing progenitors (PCPs). PIPs and PCPs express transcription factors, Gsx1 (also called Gsh1) and Olig2, respectively. At the early stages, only a small number of PIPs are located at the ventralmost 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). 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 distinct subtypes of neurons being produced (Seto et al. 2014). However, whether GABAergic projection neurons in the DCN are derived from PCPs or PIPs is unknown.

Considering the birthdates of distinct Pax2+ INs, PIPs may first produce GABAergic interneurons in the DCN (E10.5~), and then generate Golgi cells (E13.5~). PIPs at late neurogenesis stages may give rise to progenitor cells in the PWM that eventually generate stellate and basket cells. Previous transplantation studies suggested that distinct Pax2+ INs are derived from the same progenitor pool and that extrinsic instructive cues in the microenvironment may affect the terminal neuronal type commitment (Leto 2006, 2009). One candidate for the cue may be sonic hedgehog (SHH) (Fleming et al. 2013; De Luca et al. 2015). In addition, Lhx1 and Lhx5 as well as their cofactor Lbd1 are known to postmitotically participate in Purkinje cell differentiation (Zhao et al. 2007).

In contrast to GABAergic neurons, the machinery to specify each cerebellar glutamatergic neuron subtype remains elusive. Some transcription factors, such as Tbr1, Irx3, Meis2, Lhx2, Lhx9 and Olig2 are expressed in subsets of postmitotic progenitors of glutamatergic DCN neurons, but their function is still unclear (Morales and Hatten 2006; Seto et al. 2014). As to granule cells, it is known that intrinsic and extrinsic molecules such as Zic1 and SHH play important roles in cell migration, maturation and survival (Aruga et al. 1998; Dahmane and Ruiz-i-Altaba 1999; Lewis et al. 2004; Wallace 1999; Wechsler-Reya and Scott 1999), but the cell-type specification machinery remains to be identified. Although UBCs strongly express Tbr2 (Englund et al. 2006), its function remains elusive.

The molecular machinery to specify cerebellar neuronal cell types is summarized in Fig. 15.1. Neuronal cell types seem to be defined according to the spatiotemporal identities of neural progenitors in the neuroepithelium. The bHLH transcription factors, Atoh1 and Ptf1a, confer the spatial identities of the RL and the VZ on neural progenitors, letting them produce glutamatergic and GABAergic neurons, respectively. Glutamatergic and GABAergic neuron progenitors in the RL and the VZ change their temporal identities to produce distinct types of neurons during development. As to GABAergic neurons, PCPs at early stages gradually change their temporal identity to become PIPs, and this temporal identity transition is negatively and positively regulated by Olig2 and Gsx1. PCPs may produce distinct

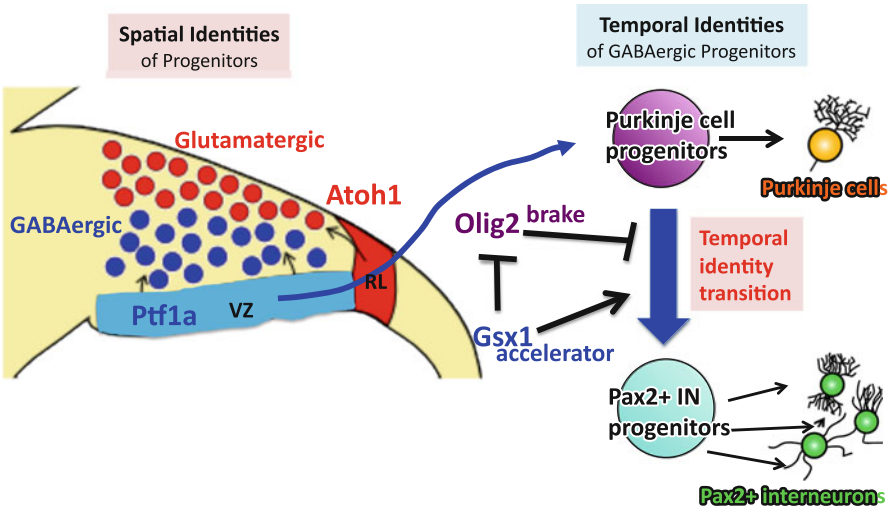

Fig. 15.1 Specification of cerebellar neurons by spatiotemporal regulation of neural progenitor identities

types of Pax2+ INs according to extrinsic instructive cues. Temporal identities of glutamatergic neuron progenitors remain unclarified, and, further investigation will be required to fully understand the mechanisms underlying cerebellar neuronal type specification.

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