Chapter 7
Precerebellar Nuclei

Mayumi Yamada and Mikio Hoshino

Abstract The cerebellar cortex receives several inputs from the surrounding nuclei, the precerebellar systems. Two major types of precerebellar systems are known; mossy fiber (MF) and climbing fiber (CF) systems. MF neurons are found in several nuclei in the brain stem. Four major nuclei in the hindbrain contain MF neurons; the pontine gray nucleus (PGN), the reticulotegmental nucleus (RTN), the lateral reticular nucleus (LRN) and the external cuneate nucleus (ECN). In addition, MF neurons also reside in the spinal trigeminal nucleus (Sp5) in the hindbrain and Clarke's column (CC) in the spinal cord. MF neurons extend their glutamatergic projection to granule cells conveying peripheral and cortical information to the cerebellum. In contrast, CF neurons are located mainly in the inferior olive nucleus (ION), which receive inputs from the cerebral cortex, the red nucleus, spinal cord and other brain stem nuclei, and extend their glutamatergic projection to Purkinje cells. Both types of precerebellar neurons also project to neurons in the cerebellar nuclei. It is thought that these precerebellar systems transmit the external and internal information to the cerebellar cortex to modulate cerebellar function, including regulation of animal movement.

Keywords Precerebellar system • Mossy fiber neuron • Climbing fiber neuron • PGN • RTN • LRN • ECN • ION • Cerebellum • Transcription factor • Rhombomere • Neuroepithelial domain

M. Yamada
Graduate School of Medicine, Kyoto University,
Shogoin-Kawahara, Sakyo-ku, Kyoto 606-8507, Japan

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

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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7.1 Anatomy and Histology of the Precerebellar Nuclei

The cerebellar cortex receives several inputs from the surrounding nuclei, the precerebellar systems. Two major types of precerebellar systems are known; mossy fiber (MF) and climbing fiber (CF) systems. MF neurons are found in several nuclei in the brain stem. Four major nuclei in the hindbrain contain MF neurons; the pontine gray nucleus (PGN), the reticulotegmental nucleus (RTN), the lateral reticular nucleus (LRN) and the external cuneate nucleus (ECN) (Altman and Bayer 1987) (Fig. 7.1a–c). In addition, MF neurons also reside in the spinal trigeminal nucleus (Sp5) in the hindbrain and Clarke's column (CC) in the spinal cord (Fig. 7.1a–d). MF neurons extend their glutamatergic projection to granule cells conveying peripheral and cortical information to the cerebellum. In contrast, CF neurons are located mainly in the inferior olive nucleus (ION) (Fig. 7.1a, c), which receive inputs from the cerebral cortex, the red nucleus, spinal cord and other brain stem nuclei, and extend their glutamatergic projection to Purkinje cells (Ruigrok et al. 1995). Both types of precerebellar neurons also project to neurons in the cerebellar nuclei. It is thought that these precerebellar systems transmit the external and internal information to the cerebellar cortex to modulate cerebellar function, including regulation of animal movement.

7.2 Specification of Precerebellar Nuclei Neurons

Birthdating studies using ^{3}H-thymidine and BrdU in mice showed that CF neurons are produced at relatively early neurogenesis stages (embryonic day (E) 9.5–11.5) and MF neurons are generated at slightly later stages (E10.5–16.5) (Pierce 1973). Avian grafting studies as well as mammalian fate map analyses have revealed that in the hindbrain, both MF and CF neurons are generated from the caudal part, around rhombomeres 6–8 (Fig. 7.1e) (Ambrosiani et al. 1996; Cambronero and Puelles 2000; Farago et al. 2006; Kawauchi et al. 2006). In contrast, MF neurons in the Clarke's nucleus are produced in the spinal cord (Bermingham et al. 2001). Classic anatomical and immunohistochemical studies have suggested that these precerebellar nuclei neurons in the hindbrain are generated from the dorsal part of the rhombomere and migrate circumferentially to their final loci (Bloch-Gallego et al. 1999; Yee et al. 1999; Kyriakopoulou et al. 2002). However, they take slightly different pathways; MF and CF neurons migrate extramurally and intramurally, respectively (Fig. 7.1e). Introduction of a GFP-expressing vector into the embryonic dorsal hindbrain enabled the dramatic visualization of migrating precerebellar nuclei neurons during development (Kawauchi et al. 2006; Okada et al. 2007; Shinohara et al. 2013).

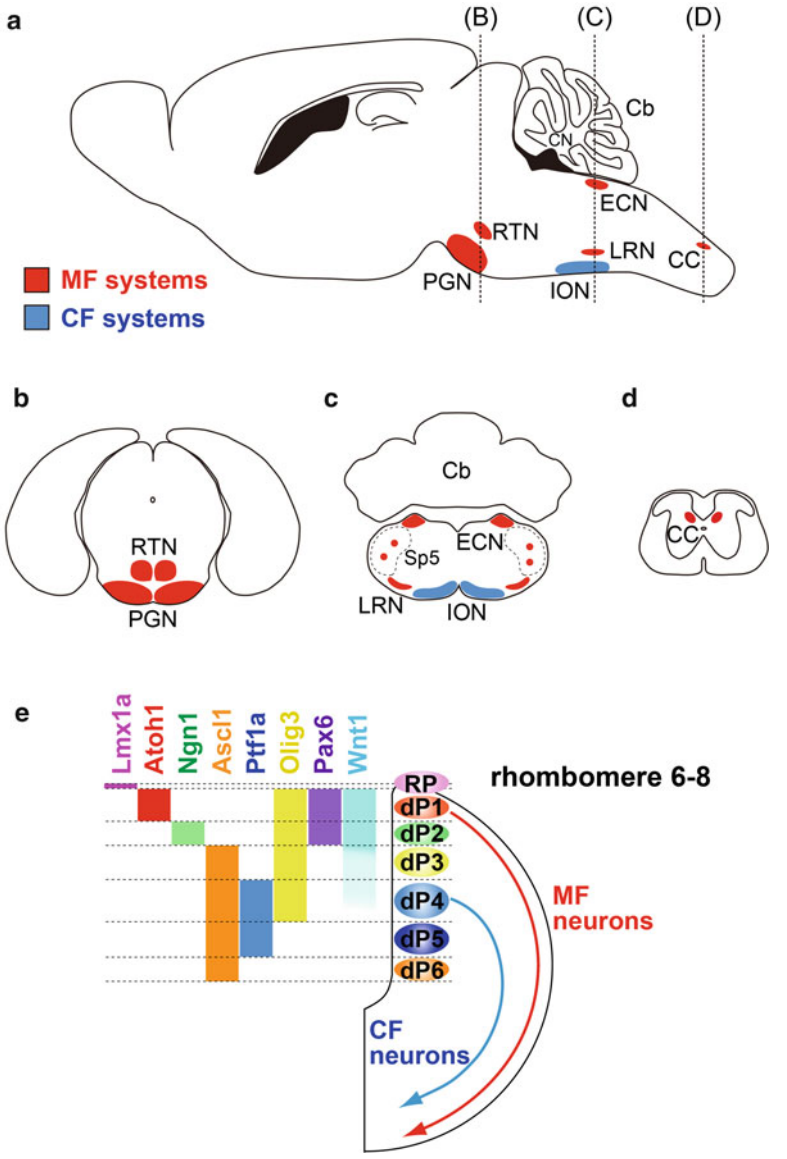

Fig. 7.1 Precerebellar systems in the brain stem. (**a**–**d**) Two types of precerebellar afferent systems; MF (*red*) and CF (*blue*) systems. *Cb* cerebellum, *CN* cerebellar nucleus. (**e**) In the caudal hindbrain (r6-8), the dorsal neuroepithelium can be divided into six domains (dP1 ~ dP6) according to the expression pattern of transcription factors during embryonic development. While MF neurons (*red*) are derived from the dP1 domain, CF neurons (*blue*) are generated from the dP4 domain

Several transcription factors are reportedly expressed within the dorsal neuroepithelium of the caudal rhombomeres 6–8 during embryonic development, and have been used to try to define domains along the dorso-ventral axis. Lmx1a is expressed in the roof plate, the dorsal-most part of rhombomere, which gives rise to the choroid plexus (Chizhikov et al. 2006). Other transcription factors are expressed in the dorsal neuroepithelium, which can be divided into six domains (dP1 ~ dP6) according to the pattern of transcription factors, such as Atoh1, Ngn1, Ascl1, Ptf1a, Pax6 and Olig3 (Fig. 7.1e). Using genetic lineage tracing methods, a series of studies have tried to clarify the precise origins of MF and CF neurons.

Analyses of genetically engineered mice that express *lacZ* or *Cre recombinase* under the control of the endogeous or exogenous *Atoh1* promoter revealed that MF neurons of PGN, RTN, LRN and ECN were generated from the Atoh1-expressing neuroepithelial domain (dP1, Ben-Arie et al. 2000; Rodriguez and Dymecki 2000; Landsberg et al. 2005; Wang et al. 2005). Loss of the *Atoh1* gene resulted in a defect in production of these MF neurons, suggesting the involvement of Atoh1 in MF neuron development.

Landsberg et al. also performed lineage tracing 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 decreases ventrally. They observed that CF neurons are generated from the neuroepithelial region where *Wnt-1* is very weakly expressed, whereas MF neurons are derived from the strong *Wnt1*-expressing region (Landsberg et al. 2005). In addition, Nichols and Bruce showed that in mice carrying a *Wnt-1*-enhancer/lacZ transgene, MF neurons but not CF neurons were labeled by β-gal (Nichols and Bruce 2006). These findings suggested that CF neurons are derived from the neuroepithelial region ventral to the Atoh1-expressing domain.

Yamada et al. performed Cre-loxP-based lineage trace analysis and showed that all CF neurons in the ION are generated from the Ptf1a-expressing neuroepithelial domain (Yamada et al. 2007). Targeted disruption of the *Ptf1a* gene caused defects in the production of these CF neurons and in 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 that Ptf1a is important for migration, differentiation and survival of CF neurons. Storm et al. used Cre-loxP-based linage tracing to show that not only MF neurons but also CF neurons are generated from the Olig3-expressing neuroepithelial region that broadly expands within the dorsal hindbrain (Storm et al. 2009). Loss of the *Olig3* gene resulted in the disorganized development of MF neurons and complete loss of CF neurons (Liu et al. 2008; Storm et al. 2009). Moreover, ectopic co-expression of Olig3 and Ptf1a induced the expression of a CF neuron marker in chick embryos (Storm et al. 2009). These findings suggest that CF neurons are derived from the Ptf1a/Olig3-expressing neuroepithelial domain (dP4) and that Ptf1a and Olig3 cooperatively regulate the development of CF neurons. The domain structure of the dorsal neuroepithelium in the caudal hindbrain is shown in Fig. 7.1e.

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