Chapter 18
Synaptogenesis and Synapse Elimination in Developing Cerebellum

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Abstract Purkinje cells (PCs) are the sole output neurons of the cerebellar cortex and play pivotal roles in coordination, control, and learning of movements. In the adult cerebellum, they receive two distinctive excitatory synaptic inputs from parallel fibers (PFs), the axons of granule cells (GCs), and climbing fibers (CFs) arising from the inferior olivary nucleus in the medulla oblongata. Each PC receives functionally weak but numerous (c.a. 100,000 in mice) PF synapses, on spines of distal dendrites. In contrast, most PCs are innervated by single but functionally very strong CFs on stubby spines of their proximal dendrites. PCs receive GABAergic inhibitory synaptic inputs from basket and stellate cells (BCs and SCs) in the molecular layer. These synaptic organizations are established mostly during the first 3 weeks of rodent's life. In this article, we briefly review how these microcircuits around PCs are organized, maintained and modified during postnatal development.

Keywords Purkinje cell • Basket cell • Stellate cell • Granule cell • Parallel fiber • Climbing fiber • Synaptogenesis • Synapse elimination • Cerebellum

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18.1 Synaptogenensis and Refinement of CF to PC Synapses

18.1.1 Synaptogenesis of CFs to Immature PCs

Olivo-cerebellar axons reach the immature cerebellum around E18 (Hashimoto and Kano 2013; Watanabe and Kano 2011). They start to form synapses just after their arrival, but do not have the typical "climbing" morphology at this stage. Immature olivo-cerebellar axons extensively ramify in the white matter and the GC layer, and give rise to many collaterals around PCs (creeper stage) (Chedotal and Sotelo 1993). Since immature PCs are devoid of large primary dendrites, CFs mainly form terminals on abundant perisomatic protrusions and thorns emerging from PC somata.

18.1.2 Postnatal Refinement of CF to PC Synapses

While most PCs are innervated by single CFs in the adult cerebellum, each PC receives synaptic inputs from multiple CFs at birth. Adult-like mono innervation is gradually established during postnatal development by elimination of surplus CFs, which proceeds in at least four distinct phases (Hashimoto and Kano 2013; Watanabe and Kano 2011).

Around P2–P3, individual multiply-innervating CFs form synapses with relatively similar strengths (Fig. 18.1). During the first postnatal week, a single CF is selectively strengthened on the soma of each PC both functionally and morphologically (termed "functional differentiation"). Mice deficient in Cav2.1, the α-subunit of the P/Q-type voltage-dependent Ca^{2+} channel (VDCC), show impairment in the selective strengthening of a single CF, suggesting that activity-dependent Ca^{2+} influx through VDCCs is crucial for establishing a single "winner" CF in each PC.

Then, the strongest CF extends its innervation territory from the soma to dendrites, which is known as "CF translocation" (Fig. 18.1). As mentioned above, CFs initially establish synaptic contacts on the fine processes emerging from the soma, and form a plexus on the lower part of the PC somata ("pericellular nest" stage) (Ramon y Cajal 1911). While the stem dendrite of PCs starts to grow into the molecular layer from around P6, multiple CFs continue to innervate PC somata until P9. After the functional differentiation of CFs, only the strongest (winner) CF extends its innervation territory from the soma to stem dendrites from P9 ("capuchin" stage) (Ramon y Cajal 1911). In the "dendritic" stage (Ramon y Cajal 1911), CF synapses progressively translocate to growing PC dendrites. On the other hand, weaker (loser) CFs remain around the soma, and are eventually eliminated in two distinct phases (the "early and late phases of CF elimination") mediated by distinct mechanisms (Hashimoto and Kano 2013; Watanabe and Kano 2011; Crepel 1982). The early phase of CF synapse elimination starts at around P7 just after the functional differentiation. Unlike the late phase of CF synapse elimination, the early phase is not

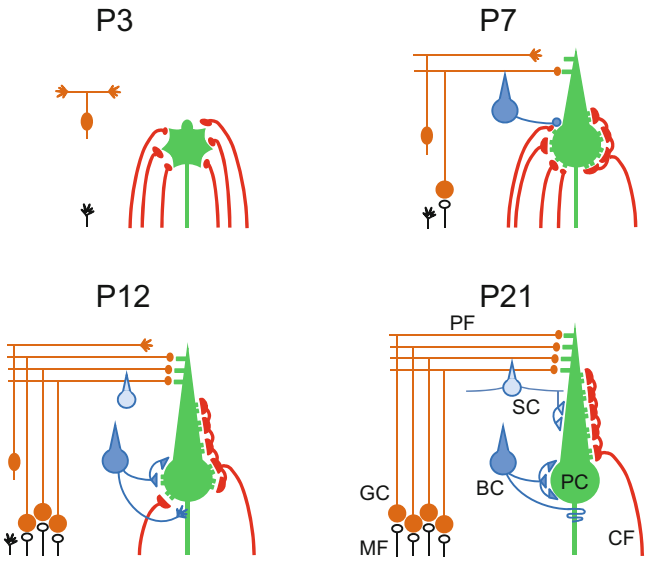

Fig. 18.1 Synaptogenesis and synapse elimination around PCs during postnatal development. *PC* Purkinje cell, *CF* climbing fiber, *PF* parallel fiber, *BC* basket cell, *SC* stellate cell, *GC* granule cell, *MF* mossy fiber. Note that PF synapses onto BCs and SCs are not illustrated for simplicity

dependent on proper generation of GCs and PF-PC synapses. Several lines of evidence suggest that the neuronal activity is crucial for this event (Hashimoto and Kano 2013; Watanabe and Kano 2011).

The late-phase of CF synapse elimination starts at around P12 (Hashimoto and Kano 2013; Watanabe and Kano 2011; Crepel 1982). This process is critically dependent on proper formation of excitatory PF synapses and inhibitory BC synapses on PCs. In mice deficient in the type 1 metabotropic glutamate receptor (mGluR1) or any of its downstream signaling molecules (Gαq, PLCβ4, PKCγ), the late-phase of CF elimination is severely impaired. A recent study has revealed that postsynaptic Sema7A, a GPI linked subtype of Semaphorin, and its receptors (ItgB1 and PlxnC1) on CFs are involved in the cascade downstream of mGluR1 (Uesaka et al. 2014). Moreover, a neurotrophin receptor, TrkB, is also involved in CF synapse elimination that starts at around P10–P12.

18.2 Synaptogenesis of PF to Immature PCs

During the prenatal period, GC precursors migrate to the cerebellar surface and form the external granular layer. After birth, they descend in the molecular and PC layers and form the internal granular layer (Altman and Bayer 1997; Rubenstein and Rakic 2013). In the premigratory zone, postmitotic spindle-shaped GCs extend

future PFs to both directions in the transverse plane parallel to the cortical surface (Fig. 18.1). Then, GCs start to migrate along Bergmann fibers in the molecular layer by extending downward fibers. As a consequence, T-shaped axons of GCs are constructed. At P7, the PF to PC synaptogenesis has already started on the immature PC dendrites, but the density of PF-PC synapses is low. Concomitant with active outgrowth of PC dendrites and migration of GCs, the density of PF-PC synapses is explosively increased thereafter. Formation and maturation of PFs in the molecular layer proceeds in an "inside-out" manner.

Mutant mice deficient in GluD2 or Cbln1 display similar defects in the PF synapse formation (Mishina et al. 2012; Yuzaki 2011). GluD2 is a member of ionotropic glutamate receptors but does not function as glutamate-gated ion channel. GluD2 is expressed predominantly in PCs. Cbln1 belongs to the C1q/tumor necrosis factor superfamily, and is highly expressed in cerebellar GCs. In GluD2 or Cbln1 knockout mice, the density of PF synapses is reduced to about a half of that in wild-type mice. Moreover, the number of free spines and mismatching of pre- and postsynaptic specialization at PF synapses are conspicuous. Recent studies have revealed that the molecular complex formed by GluD2 on PC dendritic spines, Cbln1 released from GCs and neurexin on PF terminals acts as a bidirectional synaptic organizer that stabilizes PF-PC synapses.

18.3 Synaptogenesis of SCs and BCs to PCs

BCs innervate the soma and construct the pinceau formation around the axon initial segment (AIS) of PCs in the mature cerebellum (Fig. 18.1). On the other hand, SCs mainly form synapses on PC dendrites (Fig. 18.1). The BC starts to form synapses on the PC soma at the end of the first postnatal week (Ango et al. 2004). Around P9, most of the perisomatic synapses are from CFs, but thereafter until P20, BC axons take over somatic synaptic sites with progress of somatic CF synapse elimination. From around P9, BC axons reach the AIS of PCs and begin to form the pinceau. Targeting of basket cell axons to the AIS is mediated by several molecules including membrane-associated adaptor protein ankyrin-G and one of its binding partner, neurofascin 186 (NF186) (Ango et al. 2004; Huang et al. 2007; Williams et al. 2010). NF186 exhibits subcellular concentration gradient highest at the AIS (Ango et al. 2004). Ankyrin-G is expressed exclusively at AISs in PCs. Ankyrin-G deficient mice show a defect in distribution of NF186 and abnormal widespread coverage of AISs with BC axons instead of the focal ensheathment of AIS in wild-type mice (Ango et al. 2004; Huang et al. 2007; Williams et al. 2010).

SC precursors migrate into the molecular layer a few days after the migration of BC precursors, which continue until around P14. Between P12 and P16, SCs extend neuritis in horizontal orientation (Ango et al. 2008). Then, at P16 to P18, SC axons send ascending and descending collaterals strictly associating with the radial process of Bergmann glia, which are further elaborated with appearance of plexus of finer branches up to P40. In Close Homologue of L1 (CHL1) knockout mice, SC

axons exhibit abnormal trajectories and orientation, and aberrant innervations of PC dendrites (Williams et al. 2010; Ango et al. 2008), whereas the formation of the pinceau by BC axons is normal. Importantly, SC specific abnormalities are also observed in Bergmann glia-specific CHL1-deleted mice. These lines of evidence demonstrate that CHL1 expressed in the Bergmann glia works as guiding scaffolds to organize SC axon arbors and synapse formation (Williams et al. 2010; Ango et al. 2008).

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