博士論文 (要約)

Impaired climbing fiber synapse elimination in the developing mouse cerebellum by Purkinje cell-specific knockdown of Fndc3b

（マウス発達期小脳における **Fndc3b** のプルキンエ細胞特異的ノックダウンによる登上線維シナプス刈り込み障害）

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In the embryonic development, neural circuits are assembled through events including neuronal differentiation, cell migration, axonal growth, target recognition and synaptogenesis. During synaptogenesis, initially many redundant synapses are formed. Subsequently, the neuronal connections need to be refined to form precise synaptic circuits for their proper function. Part of the initially formed synapses are then strengthened and maintained while others are eliminated through a competitive process termed “synapse elimination”, followed by axonal pruning. This synaptic refinement occurs in many regions of the developing nervous system. In the peripheral nervous system (PNS), synapse elimination in the neuromuscular junction (NMJ) and the autonomic ganglia has been reported. In the NMJ, each muscle fiber at the immature stage is initially innervated by several motor neurons, but after maturation each muscle fiber is innervated by a single motor axon. In the central nervous system (CNS), two systems in which synapse elimination occurs are notably well known, the visual cortex and the cerebellum. To understand the molecular basis of developmental synapse elimination, I focus on the climbing fiber to Purkinje cell synapse in the cerebellum, which provides a reliable model to study this process in the CNS.

In the adult mouse cerebellum, most Purkinje cells are innervated by only one climbing fiber. However, during the early development multiple climbing fibers make contact with each Purkinje cell on the soma. Each climbing fiber innervates a given Purkinje cell with similar synaptic strength. The climbing fiber to Purkinje cell synapse elimination occurs in four different stages: (1) the functional differentiation, (2) the climbing fiber translocation, (3) the early phase and (4) the late phase of synapse elimination. First, during the functional differentiation (from postnatal day 3 (P3) to P7) relative synaptic strengths among multiple climbing fibers innervating each Purkinje cell diverge. A single climbing fiber is selectively

strengthened to become a “winner” compared to the other climbing fibers that remain weak or to be “losers”. During this phase, the amplitude of excitatory postsynaptic current (EPSC) elicited by stimulating the winner climbing fiber selectively increases and the probability of multi-vesicular release becomes higher selectively for the winner climbing fiber. This biased strengthening of a single climbing fiber has been demonstrated to be dependent on P/Q-type voltage-dependent calcium channel (VDCC) in Purkinje cells. Then, the winner climbing fiber starts to translocate along growing Purkinje cell dendrites (from P9) while the other climbing fibers remain around the soma. In parallel with climbing fiber translocation, climbing fiber synapses are eliminated in two distinct phases. The early phase of climbing fiber synapse elimination starts after the biased strengthening of the “winner” climbing fiber and lasts from P8 to around P11. Neuronal activity via Ca^{2+} influx into the postsynaptic Purkinje cell through P/Q-type VDCC is crucial for the early phase of climbing fiber synapse elimination. Moreover, insulin-like growth factor-I (IGF-I) has been reported to act as a trophic support for the maintenance of climbing fibers from P8 to P12. The late phase of synapse elimination, taking place from around P12 to P17, is dependent on the proper formation of parallel fibers to Purkinje cell synapses. The type 1 metabotropic glutamate receptor (mGluR1) and the subsequent signaling cascade in Purkinje cells involving Gαq, phospholipase Cβ4, protein kinase Cγ (PKCγ) are crucial for the late phase of climbing fiber synapse elimination. This mGluR1 to PKCγ cascade is thought to be driven at parallel fiber to Purkinje cell synapses by neural activity along mossy fiber-granule cell-parallel fiber pathway. On the other hand, parallel fiber to Purkinje cell synapses restrict climbing fiber innervation territory to Purkinje cell proximal dendrites by heterosynaptic competition. Glutamate receptor $\delta 2$ (GluD2) and Cbln1 stabilize parallel fiber to Purkinje cell synapses and therefore

are important for confining climbing fiber innervation to Purkinje cell proximal dendrites. Besides excitatory parallel fiber inputs, GABAergic inhibition from basket cells to Purkinje cells facilitates removal of somatic climbing fiber synapses from P10. Climbing fiber mono-innervation to Purkinje cell is attained by the end of the third postnatal week (P18~).

Although mechanisms of developmental climbing fiber synapse elimination are being elucidated, molecules and pathways underlying interactions between postsynaptic Purkinje cells and presynaptic climbing fiber terminals are not well understood. For synaptic interactions, membrane-associated molecules could be assumed to be involved. Using microRNA (miRNA)-mediated knockdown (KD) of candidate molecules in Purkinje cells at embryonic stage and subsequent electrophysiological analysis in cerebellar slices from young adult mice, my laboratory previously performed screening of membrane-associated molecules expressed in developmental Purkinje cells. Fibronectin type III domain-containing protein 3b (Fndc3b) is one of such molecules that are potentially involved in climbing fiber synapse elimination. Fndc3b, also known as fad104 (factor for adipocyte differentiation-104), is a transmembrane protein found at the endoplasmic reticulum and the Golgi network membranes, depending on the cell type. Although its function in adipogenesis, osteogenesis, cell proliferation, adhesion, spreading, migration and lung maturation are known, there has been no report of its function at a neuronal or circuit level in the CNS. Here, I knocked down Fndc3b specifically in Purkinje cells during postnatal development by a miRNA targeted against Fndc3b, and examined climbing fiber synapse elimination by electrophysiological analyses. I found that climbing fiber synapse elimination was impaired in Purkinje cells with Fndc3b-KD during the early phase from P8. The amplitude of EPSCs elicited by the winner climbing fiber was significantly larger from P12 in Purkinje cells with Fndc3b-KD than in

control Purkinje cells. Fluorescent *in situ* hybridization revealed that the expression levels of Fndc3b becomes the highest at the early developmental stage. Immunohistochemical analysis demonstrated that elimination of climbing fiber terminals from the soma was impaired in Purkinje cells with Fndc3b-KD, as well as the translocation of the winner climbing fiber along the dendrites of Fndc3b-KD Purkinje cells. Fndc3b-KD in Purkinje cells did not affect the distribution of parallel fiber synapses, their function, distribution of inhibitory synapses or morphology of Purkinje cells and inhibitory neurons. These results suggest that Fndc3b promotes elimination of redundant climbing fibers from the Purkinje cell soma during the early phase of climbing fiber synapse elimination, negatively regulates climbing fiber synaptic strength and facilitates translocation of the strongest climbing fiber along Purkinje cell dendrites.