Neuroscience Research 83 (2014) 64–68

Contents lists available at ScienceDirect

Neuroscience Research

journal homepage: www.elsevier.com/locate/neures

Update Article

The role of Cbln1 on Purkinje cell synapse formation

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ARTICLE INFO

Article history:
Received 7 January 2014
Received in revised form 27 January 2014
Accepted 29 January 2014
Available online 7 March 2014

Keywords:
Cerebellum
Purkinje cell
Synapse formation
Cbln1

ABSTRACT

Cbln1 is a glycoprotein which belongs to the C1q family. In the cerebellum, Cbln1 is produced and secreted from granule cells and works as a strong synapse organizer between Purkinje cells and parallel fibers, the axons of the granule cells. In this update article, we will describe the molecular mechanisms by which Cbln1 induces synapse formation and will review our findings on the axonal structural changes which occur specifically during this process. We will also describe our recent finding that Cbln1 has a suppressive role in inhibitory synapse formation between Purkinje cells and molecular layer interneurons. Our results have revealed that Cbln1 plays an essential role to establish parallel fiber–Purkinje cell synapses and to regulate balance between excitatory and inhibitory input on Purkinje cells.

1. Introduction

The brain circuitry consists of millions of neurons of diverse types that communicate with each other through synapses. During development, functional circuitry is established through multiple steps (Goda and Davis, 2003). First, each neuron has to find its proper partner to establish synaptic connections, which is orchestrated by the "synapse organizers" (Fox and Umemori, 2006; Yuzaki, 2011). Synapse organizers include transmembrane proteins such as neurexin (NRX)/neuroligin (Chih et al., 2005), leucine-rich repeat transmembrane neuronal proteins (Linhoff et al., 2009), synaptic cell-adhesion molecule (Biederer et al., 2002), cadherin family proteins (Takeichi, 2007) and EphB/ephrinB (Kayser et al., 2006), and secretory proteins such as Wnt-7a (Hall et al., 2000), fibroblast growth factors (Hall et al., 2000), and neuronal pentraxins (Xu et al., 2003). Most of these proteins are expressed specifically on presynaptic or postsynaptic sites and their interaction mediates cell type-specific intercellular interactions which determine whether excitatory or inhibitory synapses are formed. As the number of synapses increases, neuronal activity has to be controlled to maintain balance and prevent cells from being damaged by too much excitation. This requires maintaining an appropriate ratio of excitatory and inhibitory synapses (Lu et al., 2009; Levinson and El-Husseini, 2005). Finally, immature connections need to be structurally and functionally refined by neuronal activity to establish a mature circuitry (Sanes and Lichtman, 1999; Kano and Hashimoto, 2009; Katz and Shatz, 1996). Studying the mechanisms by which each of these steps takes place is critical to understanding the development of the brain.

The cerebellar cortex consists of relatively simple circuits, which makes it an ideal system to study how neuronal connections are formed (Eccles, 1967; De Zeeuw et al., 2011). The only cell type which sends output from the cerebellar cortex is the Purkinje cell (Fig. 1A). Purkinje cells expand their vast dendritic arbors in the cerebellar molecular layer, where they receive excitatory input from the parallel fibers (PFs: granule cells' axons) and the climbing fibers (axons from inferior olive neurons), and inhibitory input from the molecular layer interneurons (MLIs). Excitatory synapses formed between the Purkinje cells and PFs comprise the highest number of synapses in the brain. Each Purkinje cell forms 100,000–200,000 synapses with the PFs in rodents (Harvey and Napper, 1991); proper function of these synapses is essential for motor coordination and learning (Ito, 2002).

Cbln1 is a synapse organizer that is indispensable for the formation and maintenance of PF–Purkinje cell synapses *in vivo* (Hirai et al., 2005; Ito-Ishida et al., 2008). In this review, we will describe multiple aspects of Cbln1's role on Purkinje cells' synapse development. We will describe how Cbln1 mediates structural and functional maturation of PF terminals. We will also give an update on our recent findings of Cbln1's role on MLI–Purkinje cell synapse function. These findings suggest that Cbln1 plays an important role

Abbreviations: PF, parallel fiber; MLI, molecular layer interneuron; GluD2, glutamate receptor delta 2; Nrx, neurexin; SFK, Src-family kinase; GABA, γ-aminobutyric acid.

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http://dx.doi.org/10.1016/j.neures.2014.01.009
0168-0102/

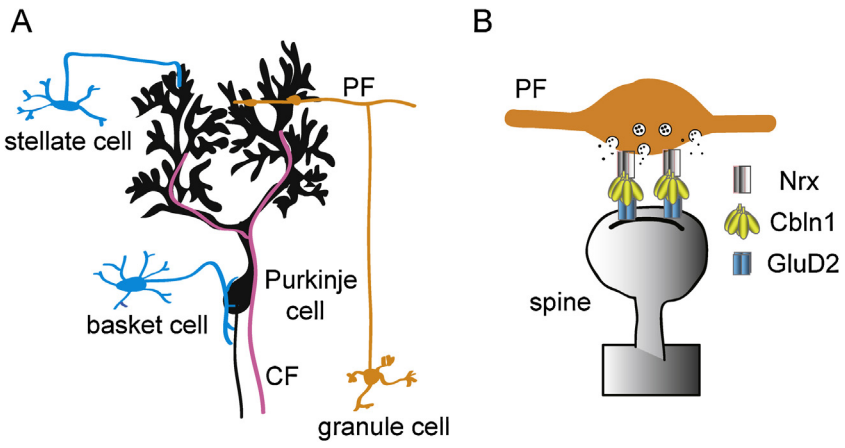

Fig. 1. Synapses on Purkinje cells and Cbln1. (A) Synaptic inputs onto the Purkinje cells. Purkinje cells receive excitatory synapses from PFs and climbing fibers, and inhibitory synapses from two kinds of interneurons, stellate cells and basket cells. (B) Cbln1 forms a tripartite protein complex with Nrx and GluD2. The complex works to hold the gap between PF terminals and Purkinje cells' spines.

in orchestrating excitatory and inhibitory synapse formation in Purkinje cells.

2. Cbln1 induces PF–Purkinje cell synapse formation *in vivo*

Cbln1 is a secretory glycoprotein produced in the cerebellar granule cells (Hirai et al., 2005). It belongs to the C1q family along with other proteins such as C1q, which is part of the complement cascade, and adiponectin (Yuzaki, 2011). Cbln1 was first identified as a precursor of cerebellin, which was found biochemically as a polypeptide specifically enriched in the synaptosomal fraction of the mouse cerebellum (Urade et al., 1991). Studies of mice genetically lacking Cbln1 (*cbln1*-null mice) have revealed a crucial role for full-length Cbln1 in cerebellar function (Hirai et al., 2005), as *cbln1*-null mice show severe ataxia due to a significant reduction of PF–Purkinje cell synapses. We have further demonstrated this importance by rescue experiments using recombinant Cbln1 (Ito-Ishida et al., 2008). When recombinant Cbln1 was injected into the subarachnoidal supracerebellar space of *cbln1*-null mice, normal densities of PF–Purkinje cell synapses were restored within 2 days, together with remarkable improvement of motor dysfunction. However, the effect did not last long. As the recombinant Cbln1 was degraded, the established synapses were lost and ataxia recurred 1 week after the injection, revealing that Cbln1 is also required for the maintenance of the synapses. Because many other synapse organizers identified *in vitro* are not essential for the formation of excitatory synapses *in vivo* (Dudanova et al., 2007; Missler et al., 2003; Varoqueaux et al., 2006), Cbln1 may be considered as a uniquely strong synapse organizer (Ito-Ishida et al., 2008; Hirai et al., 2005).

Recent studies identified postsynaptic and presynaptic receptors for Cbln1 as glutamate receptor delta 2 (GluD2) and Nrx (Fig. 1B) (Uemura et al., 2010; Matsuda et al., 2010; Matsuda and Yuzaki, 2011). GluD2 is a transmembrane protein specifically expressed in the Purkinje cells' spines. Although it was classified as an ionotropic glutamate receptor based on its amino acid sequence, it did not show any glutamate-induced channel activity and its ligand has been a mystery (Kakegawa et al., 2007). When the phenotype of *cbln1*-null mice was characterized, it was strikingly similar to that of mice lacking GluD2 (*glud2*-null mice). They both showed severe ataxia due to the specific loss of PF–Purkinje cell synapses and had abnormal Purkinje cell spine structures such as "naked spines", with no presynaptic endings, and "mismatched spines", which had long PSDs that did not match the length of active zones (Kashiwabuchi et al., 1995; Hirai et al., 2005; Kurihara et al., 1997). These findings suggested that Cbln1 and GluD2 share a common signaling pathway. Indeed, it was found that Cbln1 directly interacts with the N-terminal extracellular domain of GluD2, and that GluD2 is indispensable for Cbln1-induced synapse formation (Matsuda et al., 2010; Uemura et al., 2010). Cbln1 and GluD2, together with presynaptic Nrx, were shown to form a tripartite complex that functions to maintain the connection between PF terminals and Purkinje cells' spines (Matsuda and Yuzaki, 2011; Uemura et al., 2010). The Nrx–Cbln1–GluD2 tripartite complex works ideally to promote bidirectional differentiation of the presynaptic and postsynaptic components. When Cbln1 binds to GluD2, it promotes clustering of GluD2 in the spine, while GluD2's C-terminus intracellular domain recruits several scaffold proteins which may promote maturation of the postsynaptic sites (Uemura et al., 2004; Matsuda et al., 2010). At the same time, binding of Cbln1 to Nrx promotes clustering of Nrx at the presynaptic site, which promotes accumulation of active zone proteins and synaptic vesicles (Uemura et al., 2010; Matsuda and Yuzaki, 2011). Because Cbln1 is a hexamer and GluD2 is a tetramer, the large size of the in vivo Nrx–Cbln1–GluD2 complex may further promote accumulation of the synaptic components through a positive-feedback mechanism (Lee et al., 2012; Matsuda and Yuzaki, 2011).

3. Cbln1 induces dynamic structural changes in PFs during synapse formation

Synapse formation occurs in multiple steps. First, axonal terminals and dendrites make contacts. Second, pre- and postsynaptic structures are induced. Lastly, maturation takes place and nascent synapses become functional (Goda and Davis, 2003). This step-by-step process has been difficult to clarify because synapse formation occurs randomly and gradually. Most previous studies have focused on how dendritic spines are formed in hippocampal and cortical excitatory neurons and have led to the following two models that describe the process (Yuste and Bonhoeffer, 2004). In the "filopodial model", dendritic filopodia capture axonal terminals and induce presynaptic differentiation (Okabe et al., 2001; Knott et al., 2006; Ziv and Smith, 1996). The interaction between the dendritic filopodia and the axons further promotes maturation of mushroom-shaped spines. In the "Millier/Peters model", axonal terminals play a key role in initiating synapse formation and converting preexisting shaft synapses to spine synapses (Harris, 1999; Yuste and Bonhoeffer, 2004; Miller and Peters, 1981). In these models, interaction between axons and dendrites is essential for spine formation. In contrast, it has been known that Purkinje cells'