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OVERVIEW

Dendrite Formation of Cerebellar Purkinje Cells

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Abstract During postnatal cerebellar development, Purkinje cells form the most elaborate dendritic trees among neurons in the brain, which have been of great interest to many investigators. This article overviews various examples of cellular and molecular mechanisms of formation of Purkinje cell dendrites as well as the methodological aspects of investigating those mechanisms.

Keywords Purkinje cell · Dendrite · Cerebellum · Development · Slice culture · Transfection

Introduction

The cerebellum is a "little brain" in terms of its volume. However, the surface area of the cerebellar cortex is not as "little" as expected from the volume because the cerebellar cortex is folded in a complex form. If flattened in a plane, the area of the cerebellar cortex of humans is 1,128 cm^2, whereas that of the neocortex is 1,900 cm^2 [1]. As expected from such a large size, the cerebellum has crucial functions. It plays important roles in coordinated movement and motor learning [2]. In addition, recent investigations are revealing that it is also involved in higher cognitive functions and mental health [1, 3].

Histologically, the cerebellar cortex is three-layered (molecular, Purkinje cell and internal granular layers) and contains five major types of neurons (Purkinje, granule, basket, stellate and Golgi cells), which form relatively simple and well characterized neuronal circuitry [2, 4]. Purkinje cells are the most outstanding neurons in the cerebellar cortex due to their large somata and extensive dendritic trees (Fig. 1c). Purkinje described Purkinje cells in 1837 and they were the first neurons to be discovered. Later, introduction of various staining procedures such as Golgi techniques unveiled the elaborate dendritic trees of Purkinje cells [5]. Most Purkinje cells have a single primary dendrite, whereas a few have two or more primary dendrites. Primary dendrites extend toward the molecular layer (ML) and branch extensively to form secondary and tertiary dendrites. A number of synapses are made between spines on tertiary dendrites and parallel fibers, axons from granule cells, which are the most numerous neurons in the central nervous system (CNS). In addition, Purkinje cells are innervated by climbing fibers (axons from inferior olive neurons) and cortical inhibitory interneurons (basket and stellate cells). Purkinje cell dendrites develop in a planar form and the plane is oriented perpendicular to the long axis of the cerebellum.

Long-term depression (LTD) of synaptic transmission at parallel fiber–Purkinje cell synapses can be induced upon conjunctive stimulation of parallel and climbing fibers [6]. This synaptic plasticity is thought to provide a cellular basis for motor learning involving the cerebellum. Thus, Purkinje cells and their dendrites are key elements in cerebellar functions.

In this article, I will first review dendrite formation of Purkinje cells and then go on to the methodological aspects of investigating the dendrite formation of Purkinje cells. Next, various examples of cellular and molecular mechanisms of formation of Purkinje cell dendrites are described.

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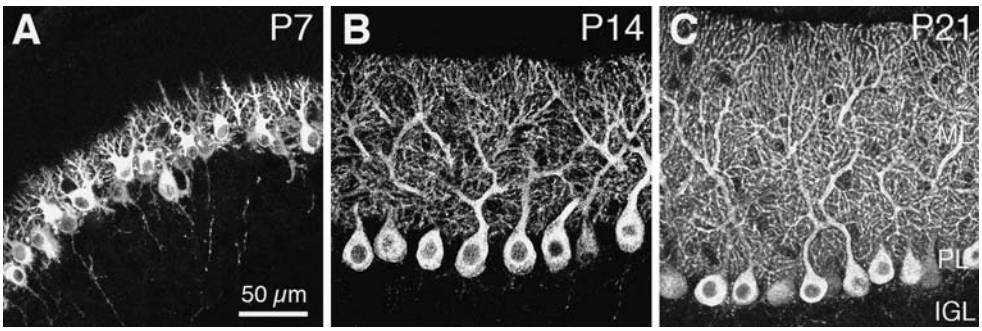

Fig. 1 Postnatal development of the cerebellar cortex. Immunohistochemistry against inositol 1,4,5-trisphosphate receptor (IP_3R) of cerebellar slices of mice on postnatal day (*P*) 7 (**a**), 14 (**b**) and 21 (**c**)

Development of the Cerebellar Cortex and Purkinje Cell Dendrites

The layered structure and the circuitry of the cerebellar cortex are formed postnatally in many vertebrates including mice, rats, monkey and humans [4]. Granule cells actively proliferate in the external granular layer (EGL) during postnatal 2 and 3 weeks in mice and rats, respectively. During this period, they successively become postmitotic and migrate from the EGL to the internal granular layer (IGL). Migration is completed at 2 and 3 weeks after birth in mice and rats, respectively.

During the same period as proliferation, migration and differentiation of granule cells, dendrites of Purkinje cells are formed. Purkinje cell dendrites extend, branch and form synapses with parallel and climbing fibers and interneurons during postnatal 3 weeks (Fig. 1).

One of the characteristic features in the morphogenesis of Purkinje cell dendrites is extension and retraction of primary dendrites. In contrast to mature Purkinje cells, immature Purkinje cells have several primary dendrites in the first postnatal week in mice and rats (Fig. 1a). During the second postnatal week, most Purkinje cells lose all of their primary dendrites except one, which extends toward the pial surface and differentiates into the mature morphology (Fig. 1b). Thus, the morphology of Purkinje cell dendrites changes dramatically during postnatal cerebellar development [7–9].

Methodological Aspects of Investigating Dendrite Formation of Purkinje Cells

Organotypic Slice Culture

Organotypic slice culture preserves the in vivo cytoarchitecture of the cerebellum for a long time in vitro. Thus, this technique has great advantages for investigating cerebellar development, including proliferation and migration of granule cells, dendritogenesis and synaptogenesis of Purkinje cells [10]. Gähwiler [11] established and successfully used the roller tube method for organotypic slice culture of the CNS. However, this technique includes some difficult procedures.

Another simple method for organotypic slice culture of the CNS was reported firstly by Yamamoto et al. [12, 13], who co-cultured the lateral geniculate nucleus and visual cortex by this method. Stoppini et al. [14], who mainly cultured the hippocampus, described this technique in detail and contributed to the spread of this technique. This method is called the "interface" culture technique because slices are mounted on a porous membrane settled at the interface between the air and culture medium. In some cases, it was called the "static" culture technique in contrast to the roller tube technique [15]. This feature enables efficient supply of oxygen and nutrients to slices. Although this method is basically similar to several types of classically used organ culture methods [16], it is simplified and adapted to the culture of the CNS.

A detailed description of culture of cerebellar slices by the "interface" culture technique was firstly reported by Tanaka et al. [17] (Fig. 2). As the membrane, Tanaka et al. [17] simply used a membrane floated at the air/medium interface (Fig. 2a), whereas many other investigators used the "cell culture insert" [18–20]. In the slices cultured by this method, the overall structure of cerebellar slices and the cytoarchitecture of the cerebellar cortex are well preserved (Fig. 2b). The serial process of granule cell development including proliferation, migration and extension of parallel fibers is reproduced over 6 days in vitro (Fig. 2c–e). Purkinje cells also survive well, are arranged in a row at the PL as in vivo, extend arborized dendritic trees toward the pial surface, and form synapses with parallel fibers (Fig. 2f, g). It should be noted that some reorganization of the events of cerebellar development occurs during culture. For example, in parasagittal slices, parallel fibers were observed to

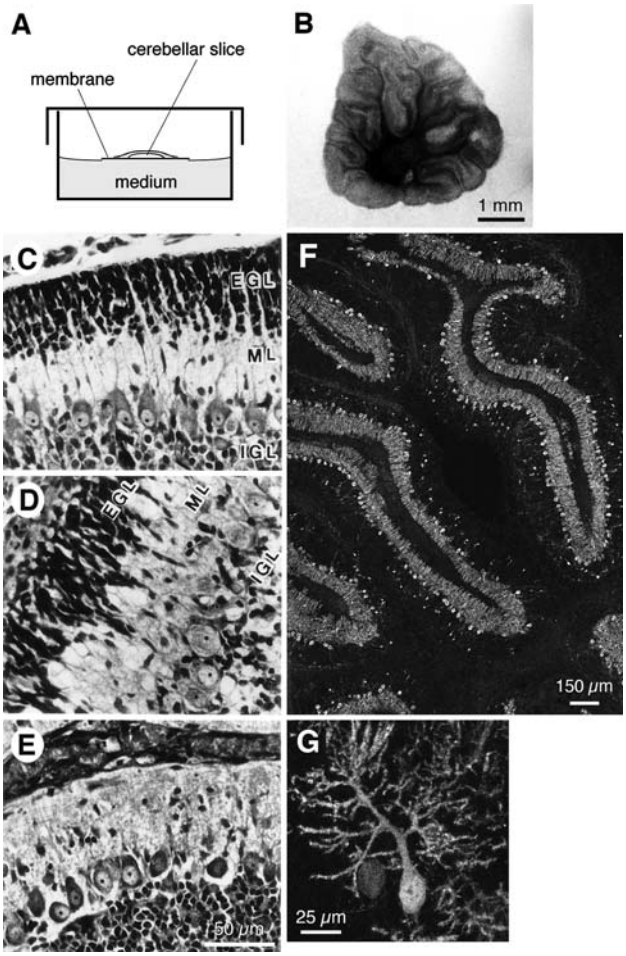

Fig. 2 Organotypic slice culture of the cerebellum. **a** Schema of the "interface" culture method. **b** Overview of a cerebellar slice derived from a P9 rat and cultured for 6 days. **c–e** Toluidine blue-stained sections of P9 rat cerebellum (**c**) and cerebellar slices derived from P9 rats and cultured for 3 (**d**) and 6 (**e**) days. **f**, **g** Low (**f**)- and high (**g**)-power views of immunohistochemistry against IP_3R of a cerebellar slice derived from a P9 rat and cultured for 6 days. **b–e**, **g** were reproduced from Tanaka et al. [9, 17, 132], with permissions from Elsevier Ltd. and Society for Neuroscience

elongate in the plane of the slice (sagittal plane) [17]. This orientation of parallel fiber elongation is perpendicular to that in vivo. Until now, this method has been widely used to investigate cerebellar development, including dendritogenesis of Purkinje cells (e.g., [9, 21–25]).

Time-Lapse or Repeated Observation of Living Cells

The morphology of Purkinje cell dendrites changes dramatically during postnatal cerebellar development, as described in the preceding section. However, such a dynamic process is poorly understood because it is extremely difficult to track the morphological changes of Purkinje cells in vivo. To elucidate the dynamic aspects of the morphogenesis of Purkinje cell dendrites, several investigators have performed time-lapse or repeated observation of living Purkinje cells in slice or cell cultures.

Fenili and De Boni [26] expressed red fluorescent protein (RFP) in Purkinje cells in slice cultures by gene gun biolistics and tracked dendritic differentiation of Purkinje cells over several days by repeated observation of the same cells. Lordkipanidze and Dunaevsky [27] used a similar method and analyzed morphological changes of Purkinje cell dendrites between 2 and 3 days in vitro to show that the extension and retraction of distal parts of Purkinje cell dendrites occurred concomitantly in the same cell.

Tanaka et al. [28] used glutamate decarboxylase 67-green fluorescent protein (GAD67-GFP) knock-in mice, in which GABAergic neurons, including cerebellar Purkinje cells, express GFP [29, 30]. Cerebellar cell cultures derived from these mice allowed observation of the morphogenesis of Purkinje cell dendrites during a prolonged culture period of up to 25 days in vitro (Fig. 3). Using this method, it was shown that retraction of some of the primary dendrites occurs during morphogenesis of Purkinje cells. Together with the morphological changes of Purkinje cell dendrites in vivo and the findings revealed by long-term tracking of identified living cells over a several-day or several-week period in vitro, it was suggested that the final morphology of Purkinje cells is achieved not only through extension, but also through retraction of their dendrites.

In addition to the observation of shafts of Purkinje cell dendrites as described above, analyses of spine morphology over a relatively short-term period have been performed in GFP-expressing [31] or fluorescent dye-injected [32] Purkinje cells in slice preparations.

Viral Vectors

Expression of foreign genes in Purkinje cells in vivo is useful to investigate molecular mechanisms of dendrite formation of Purkinje cells. Recent advances in viral vectors have markedly contributed to selective and efficient gene transfer to Purkinje cells in vivo [33]. Adenoviral vectors, the most commonly used vectors, preferentially transduce Bergmann glia and the transduction efficiency in Purkinje cells is very low, although Purkinje cells in culture could be transduced efficiently using these vectors [34]. Another virus that was used in earlier studies of gene transfer to Purkinje cells is herpes simplex virus 1 (HSV-1). It was shown that the defective HSV-1 amplicon vectors efficiently transduce Purkinje cells by injection of the vectors into the inferior olivary nucleus, suggesting these vectors are delivered by retrograde axonal transport [35]. Purkinje cells in culture could also be transduced efficiently by these vectors [36].

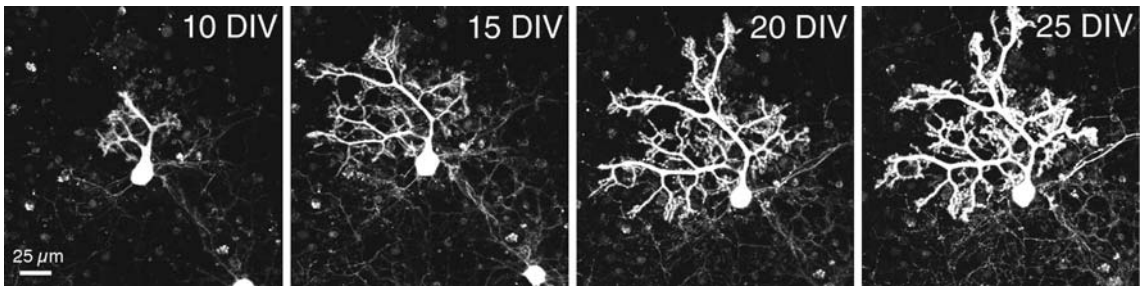

Fig. 3 Long-term observation of dendritic morphogenesis of living GFP-expressing Purkinje cells in dissociated cell cultures. The same Purkinje cell in a dissociated cell culture derived from neonatal GAD67-GFP knock-in mice was repeatedly observed at 10, 15, 20 and 25 days in vitro (*DIV*)

In contrast to these two viruses, adeno-associated virus (AAV) and the vesicular stomatitis virus glycoprotein (VSV-G)-pseudotyped lentivirus are advantageous for selective expression in Purkinje cells due to the viral tropism for Purkinje cells [33]. One major limitation of AAV is the insert capacity. The length of inserts that AAV vectors carry should be less than 4 kb [33]. Among at least ten serotypes of AAV that have been used, AAV1 [37], AAV2 [38], AAV5 [39] and AAV8 [40] have been shown to be efficient in transduction of Purkinje cells.

Lentivirus is one of the retroviruses which have a single-stranded RNA genome. The lentiviridae family includes human immunodeficiency virus (HIV)-1, HIV-2, feline immunodeficiency virus (FIV) and simian immunodeficiency virus. Like AAV vectors, lentiviral vectors have strict limitations in terms of insert capacity, but their capacity is almost twice (8 kb) as large as that of AAV vectors [33]. Using FIV-derived vectors, Alisky et al. [39] showed efficient transduction of Purkinje cells, as well as stellate and Golgi cells. Torashima et al. [41] used pseudotyped HIV-1-derived lentiviral vectors in which the native glycoprotein envelope of HIV-derived lentiviral vectors is replaced with that of vesicular stomatitis virus (VSV-G) to gain broader tropism than that of the wild-type virus. Using these vectors driven by the murine stem cell virus promoter, they showed Purkinje-cell-preferential and efficient transduction. In contrast, non-selective transduction in cerebellar neurons and glial cells was reported using HIV-1-derived lentiviral vectors driven by the human cytomegalovirus promoter [42]. The tropism of lentiviral vectors for Purkinje cells appears to be significantly affected by unidentified factors [33].

L7/pcp-2 Promoter

The L7/pcp2 promoter drives gene expression specifically in cerebellar Purkinje cells and retinal rod bipolar neurons [43]. This promoter has been used to express foreign genes specifically in Purkinje cells in transgenic mice [43] as well as in viral infection [34]. This technique can be applied not only to the investigation of transgene functions in Purkinje cells, but also to the visualization [44] and purification [45] of Purkinje cells. Unfortunately, however, the activity of this promoter is relatively low.

Single-Cell Electroporation

In addition to viral infection, gene gun biolistics [26, 31, 46–48] and bulk electroporation [23, 47] have been used for transfection of foreign genes into Purkinje cells in slice cultures. However, the transfection efficiency of these methods in Purkinje cells is low [23, 47, 48]. Lipofection, another widely used transfection method, appears to be worse in terms of transfection efficiency in Purkinje cells than gene gun biolistics and bulk electroporation [47]. Furthermore, it is impossible to introduce transgenes into single cells by all of these transfection methods.

Single-cell electroporation is a recently developed method to introduce polar and charged molecules such as dyes, drugs, peptides, proteins and nucleic acids [49]. In contrast to traditional bulk electroporation using large plane electrodes, single-cell electroporation uses electrolyte-filled capillaries [50], micropipettes (patch-clamp electrodes) [51–53] or chip structures [54–56] to transfer the molecules of interest into single cells. By this method, it is possible to transfer expression plasmids [51–53, 56] or small interfering RNA (siRNA) [56, 57] into microscopically identified cells.

Tanaka et al. [58] recently applied this technique to introduce siRNA into Purkinje cells in cerebellar cell cultures. By introducing siRNA against GFP in GFP-expressing Purkinje cells, the levels of GFP fluorescence were reduced at least up to 4 days after electroporation. Thus, single-cell electroporation of siRNA can be a simple but effective tool for silencing gene expression specifically in Purkinje cells without using a Purkinje-cell-specific promoter. Unfortunately, it appears to be impossible at present to express foreign genes in Purkinje cells by single-cell electroporation of expression plasmids.

Cellular and Molecular Mechanisms of Formation of Purkinje Cell Dendrites

To date, many studies have revealed aspects of mechanisms of Purkinje cell dendrite formation at cellular and molecular levels. These studies are reviewed in this section.

Granule Cells

Many studies have demonstrated that granule cells play important roles in the formation of Purkinje cell dendrites. Observations of agranular cerebella of X-irradiated and mutant animals showed that the presence and differentiation of granule cells are necessary for the normal development of Purkinje cell dendrites (e.g., [59–62]). Coculture experiments using dissociated Purkinje and granule cells clearly indicated that the granule–Purkinje cell interaction plays a crucial role in the branching and thickening of Purkinje cell dendrites [63, 64]. It has been suggested that granule cells exert trophic effects on Purkinje cells by providing neurotrophic substances and electrical activity, as described below.

Electrical Activity

Neuronal activity is widely known to affect dendritic differentiation [65–67]. Using dissociated cell cultures, Schilling et al. [68] showed that after dendritic elongation of Purkinje cells during the first week of culture, electrical activity emerges and dendrites stop growing and start branching. If electrical activity is inhibited by chronic tetrodotoxin or high magnesium treatment, dendrites continue to elongate, as if they are still immature. At the time that branching begins, intracellular calcium levels become sensitive to tetrodotoxin, suggesting that calcium may be involved in dendrite growth.

Glutamate

Glutamate is a neurotransmitter at the synapses between granule cells and Purkinje cells. Cohen-Cory et al. [69] showed that application of glutamate together with nerve growth factor (NGF) promoted survival, cell size and neurite elaboration of Purkinje cells in cell cultures. These effects required simultaneous exposure to glutamate and NGF and were not evoked by exposure to glutamate or NGF alone.

Hirai and Launey [64] showed that blockade of both α-amino-3-hydroxy-5-methyl-4-isoxazolepropinonic acid (AMPA)/kainate receptor and metabotropic glutamate receptor (mGluR) in cell cultures caused a significant decrease in the number of branch points and the diameter of Purkinje cell dendrites without apparently affecting dendrite extension and spine formation. The same treatment also reduced the number of primary dendrites [28]. Although blockade of *N*-methyl-D-aspartic acid (NMDA) receptor caused a strong reduction in differentiation of Purkinje cell dendrites, the effect of NMDA was shown to be indirect through the promotion of granule cell survival by the activation of NMDA receptors expressed on granule cells, leading to an increase in granule–Purkinje cell interaction [64]. Catania et al. [70] showed that blockade of mGluR1 or mGluR5 reduced differentiation of Purkinje cell dendrites both in cell cultures and in vivo.

There is a controversial report by Adcock et al. [71] showing that blockade of glutamate receptors had only minor effects on Purkinje cell dendrites in slice cultures. They suspected that this discrepancy may be caused by differences between dissociated cell cultures and slice cultures.

GDNF

Glial cell line-derived neurotrophic factor (GDNF) is a member of the type β transforming growth factor superfamily. Mount et al. [72] showed that GDNF increased survival and dendritic differentiation of Purkinje cells in dissociated cell cultures. GDNF did not alter the glial cell number or the number and morphology of the overall neuronal population, suggesting that GDNF acts specifically on Purkinje cells in the cerebellum.

BDNF

Purkinje and granule cells express both brain-derived neurotrophic factor (BDNF) and the BDNF receptor TrkB. Schwartz et al. [73] showed stunted growth of Purkinje cell dendrites in BDNF-knockout mice. They also observed greater death of granule cells in these mice. Thus, the effect of BDNF may be indirect through the promotion of granule cell survival by the activation of BDNF receptors expressed on granule cells [64]. It was also revealed that the granule–Purkinje cell interaction is maintained via BDNF, of which expression is promoted by inositol 1,4,5-trisphosphate receptor (IP_3R) signaling in granule and/or Purkinje cells [74, 75].

Progesterone and Estradiol

Over the past decade, it has been shown that the brain itself is capable of synthesizing steroids de novo from cholesterol, the so-called "neurosteroids" [76, 77]. Recently, Purkinje cells have been identified as a major site for neurosteroid synthesis in vertebrates [78]. This is the first demonstration of de novo neuronal neurosteroidogenesis in

the brain. In mammals, Purkinje cells actively synthesize progesterone and estradiol de novo from cholesterol during neonatal life. Both progesterone [22] and estradiol [79] promote dendritic growth, spinogenesis and synaptogenesis via each cognate nuclear receptor in the developing Purkinje cell. In knockout mice deficient in cytochrome P450 aromatase, a key enzyme in estradiol synthesis, reduced dendritic growth, spinogenesis and synaptogenesis were observed in Purkinje cells [80]. These actions of progesterone and estradiol may be mediated by neurotrophic factors such as BDNF [80].

Thyroid Hormone

Perinatal thyroid deficiency leads to a striking reduction in the growth and branching of Purkinje cell dendritic arborization [81]. Kimura-Kuroda et al. [82] and Heuer and Mason [83] showed that addition of triiodothyronine (T3) or thyroxine (T4) to cerebellar cell cultures caused a dramatic increase in dendritic branching of Purkinje cells. Using knockout mice deficient in thyroid hormone receptor $\alpha 1$ (TR$\alpha 1$) or β (TRβ), Heuer and Mason [83] further showed that T3 acts on Purkinje cells directly through TR$\alpha 1$ expressed on the Purkinje cell and not on the granule cell, and that TRβ isoform is not involved. Although BDNF and neurotrophin-3 was implicated in collaboration with T3 during cerebellar development, they showed that T3-promoted Purkinje cell differentiation is not mediated via these neurotrophins.

CRF and Urocortin

Corticotropin-releasing factor (CRF) is a neuromodulatory peptide that augments the effects of glutamate [84]. CRF is selectively localized in climbing and mossy fibers in the cerebellum [85]. Another member of the CRF peptide family, urocortin, is localized in Purkinje cells, and climbing and parallel fibers [86]. Both CRF and urocortin act via G-protein-coupled receptors, namely CRF receptor 1 (CRF-R1) and CRF receptor 2 (CRF-R2) with urocortin having a higher binding affinity for CRF-R2 [87]. In Purkinje cells, CRF-R1 is localized on the dendrites, whereas CRF-R2 is restricted to the somata, suggesting different roles of these two peptides at different locations within the Purkinje cell [88–90].

Using slice cultures, Swinny et al. [91] showed that intermittent exposure (12 h per day for 10 days in vitro) of CRF or urocortin induced significantly more dendritic outgrowth and elongation of Purkinje cells compared with untreated cells. CRF also promoted dendritic branching. The effects of urocortin appear to predominate in the initial outgrowth, whereas those of CRF appear to predominate in later dendritic differentiation such as elongation and branching. Of interest, constant exposure to CRF or urocortin significantly inhibited dendritic outgrowth. The trophic effects of CRF and urocortin are mediated by the protein kinase A and mitogen-activating protein (MAP) kinase pathways.

$\alpha 2\delta$-2 Voltage-Dependent Calcium Channel Accessory Subunit

Cacna2d2 is the gene that encodes the $\alpha 2\delta$-2 voltage-dependent calcium channel accessory subunit. $\alpha 2\delta$-2 mRNA is strongly expressed in cerebellar Purkinje cells. *Ducky* is a mouse mutant with a mutation in the *Cacna2d2* gene [92], which results in the introduction of a premature stop codon and the expression of a truncated protein [93]. *Ducky* mutant mice show spike-wave seizures and cerebellar ataxia. Brodbeck et al. [93] showed that dendritic trees of Purkinje cells in these mice have an immature and grossly abnormal morphology, including multiple primary dendrites and a reduction in the size of dendritic trees. They further showed that whereas the wild-type $\alpha 2\delta$-2 subunit increased the peak current density of the voltage-dependent calcium channel when co-expressed in vitro, co-expression with the truncated mutant $\alpha 2\delta$-2 protein reduced current density, suggesting that this abnormality may contribute to the *ducky* phenotype.

Pleiotrophin–PTPζ and GLAST

PTPζ is a receptor-type protein tyrosine phosphatase that is expressed predominantly in the CNS and synthesized as a chondroitin sulfate proteoglycan [94–96]. The extracellular region of PTPζ, which is generated by alternative splicing, is secreted as a soluble chondroitin sulfate proteoglycan, phosphacan. Pleiotrophin, a heparin-binding growth factor, binds to the chondroitin sulfate portion of PTPζ with high affinity and functions as a ligand of PTPζ. In the developing cerebellum, PTPζ is expressed by Purkinje cells and Bergmann glia [9, 97–99], whereas pleiotrophin is expressed by Bergmann glia [9, 100, 101].

Tanaka et al. [9] showed that pleiotrophin–PTPζ signaling is involved in the morphogenesis of Purkinje cell dendrites using cerebellar slice cultures. Aberrant morphology of Purkinje cell dendrites such as multiple and disoriented primary dendrites was induced in slice cultures in which pleiotrophin–PTPζ signaling was disturbed. Furthermore, it was revealed that pleiotrophin–PTPζ signaling regulates distribution of a glial glutamate transporter GLAST on Bergmann glial processes, which plays an important role in the morphogenesis of Purkinje cell dendrites. The morphological abnormality of multiple primary dendrites is similar to that observed in *ducky* mutant mice described in the

preceding subsection [93], suggesting that pleiotrophin–PTPζ signaling may be related to voltage-dependent calcium channels activated after glutamate stimulation.

One of the interesting questions concerning the role of PTPζ is whether some of the primary dendrites really retract while the others in the same cell extend and elongate during the morphogenesis of Purkinje cell dendrites. Tanaka et al. [28] used long-term observation of living Purkinje cells in cell cultures to directly show that retraction of some of the primary dendrites occurs during the morphogenesis of Purkinje cell dendrites, as described in the preceding section. Thus, pleiotrophin–PTPζ signaling regulates such a dynamic process of the morphogenesis of Purkinje cell dendrites. The extension and retraction of primary dendrites was revealed to involve neuronal activity and calcium/calmodulin-dependent protein kinase II (CaMKII).

Homer/Vesl

Homer/Vesl is a family of scaffolding proteins that couple diverse target molecules in postsynaptic density (PSD) [102–104]. Tanaka et al. [23] showed that Homer 1c, a long isoform with functional EVH1 and coiled-coil domains, but not short isoforms missing these features control properties of calcium release from intracellular stores and affect dendritic morphology of Purkinje cells.

While Homer 1–3 were reported to be expressed by Purkinje cells in the cerebellum [105, 106], a recent study showed that Homer 3 is the predominant isoform in Purkinje cells [107]. Mizutani et al. [108] revealed that Homer 3 is phosphorylated by CaMKII in Purkinje cells, and the phosphorylation reduces the affinity for mGluR1α, a target molecule of Homer.

CaMKII

CaMKII, a serine/threonine protein kinase abundantly expressed in neurons [109, 110], is a key intracellular molecule involved in dendritic differentiation [111–114]. Neuronal activity regulates the kinase activity of CaMKII, which mediates calcium-dependent dendritic growth. Of the four isoforms of CaMKII (α, β, γ and δ), the α isoform is predominant in the forebrain, while the β isoform is predominant in the cerebellum [115, 116]. In the cerebellum, CaMKIIα is expressed selectively in Purkinje cells, whereas CaMKIIβ is expressed in Purkinje and granule cells [117–119].

In cerebellar cell cultures, Tanaka et al. [28] and Ohkawa et al. [120] showed that treatment with a CaMKII inhibitor reduced the number of primary dendrites and the total dendritic length of Purkinje cells. Interestingly, the effect on primary dendrites is temporally specific between 5 and 15 days in vitro, a culture period when the extension and retraction of primary dendrites occur actively [28]. Blockade of AMPA/kainate-type glutamate receptors also reduced the number of primary dendrites over the same culture period [28]. These findings suggest that neuronal activity and downstream CaMKII signaling are involved in the morphogenesis of Purkinje cell dendrites.

PKCγ

Protein kinase C (PKC), another family of serine/threonine protein kinases, is a key molecule in signal transduction for the expression of LTD at the parallel fiber–Purkinje cell synapse [6]. Using cerebellar slice cultures, Metzger and Kapfhammer [21] showed that treatment with a PKC agonist reduced dendritic differentiation of Purkinje cells, whereas treatment with a PKC inhibitor increased it. Furthermore, Schrenk et al. [121] showed that Purkinje cell dendritic trees were enlarged and had an increased number of branching points in PKCγ-knockout mice compared to wild-type mice, indicating a role for the PKCγ isoform as a negative regulator of dendritic growth and branching.

RORα

Retinoid-related orphan receptor α (RORα) is a nuclear receptor that is required for survival [122, 123] and dendritic differentiation [124–126] of Purkinje cells. The gene encoding this protein is responsible for the *staggerer* mutation [127].

In the early stage of dendritogenesis, Purkinje cells change their morphology from a fusiform and bipolar shape to a stellate shape by retraction of primitive dendrites and extension of perisomatic protrusions [5, 8].

Boukhtouche et al. [24] showed that lentiviral-mediated RORα1 overexpression in fusiform Purkinje cells accelerates the transition of their morphology to the stellate shape in slice cultures of the embryonic or neonatal cerebellum. In addition, it was also demonstrated that RORα-deficient Purkinje cells in *staggerer* mutant mice remain in the embryonic fusiform stage in slice cultures, whereas RORα overexpression restores normal dendritogenesis. In contrast, RORα does not seem to have a major role in the late stage of dendritogenesis because RORα1 overexpression did not influence dendritic differentiation of Purkinje cells in slice cultures prepared from 7-day-old mice.

Stathmin Family

Stathmin is a candidate molecule that regulates microtubule dynamics in response to calcium signaling [128, 129],

which is essential for neuronal activity-dependent dendritic formation [66, 67]. Stathmin destabilizes microtubules in two distinct ways, by sequestering α- and β-tubulin heterodimers and by promoting microtubule catastrophe [128, 129]. These activities of stathmin are suppressed by phosphorylation by CaMKII etc. Ohkawa et al. [120] showed that stathmin is downregulated at both the expression and activity levels during cerebellar development. Furthermore, overexpression of stathmin limited the dendritic growth of Purkinje cells in cell cultures. Phosphorylation of stathmin at Ser16 was mediated by neuronal activity, voltage-dependent calcium channels, mGluR1 and CaMKII, suggesting that calcium elevation activates CaMKII, which in turn phosphorylates stathmin to stabilize dendritic microtubules. However, knockdown of endogenous stathmin also reduced dendritic growth of Purkinje cells. These findings suggest that proper regulation of stathmin activity is necessary for dendritic differentiation of Purkinje cells. Ohkawa et al. [120] also showed that overexpression of SCG10, a membrane-anchored member of the stathmin family, limited the dendritic growth of Purkinje cells as did stathmin, although SCG10 with a mutation at the predicted phosphorylation site by CaMKII maintained this effect. Thus, SCG10 may modulate dendritic differentiation independently of CaMKII.

SCLIP is another membrane-anchored member of the stathmin family [130]. Unlike stathmin and SCG10, SCLIP is specifically and strongly expressed in Purkinje cells and accumulates in their dendrites during cerebellar development [131]. Using lentiviral-mediated RNAi in slice cultures of the embryonic or neonatal cerebellum, Poulain et al. [25] showed that knockdown of SCLIP in Purkinje cells at the early stage of dendritogenesis promotes retraction of primitive dendrites and then prevents extension of new perisomatic protrusions, resulting in a round morphology. It also prevented elongation and branching of Purkinje cells at the late stage of dendritogenesis. Conversely, overexpression of SCLIP promoted dendritic differentiation. Thus, SCLIP appears to control the early and late stages of Purkinje cell dendritogenesis.

Future Directions

Cerebellar Purkinje cells are one of the most outstanding neurons due to their elaborate dendritic trees, which have been of great interest to many investigators. To date, many molecules have been revealed to influence the formation of Purkinje cell dendrites, as described in this article. While we could suppose complicated interplays of these molecules, the essential mechanisms regulating the formation of these characteristic dendrites is not fully understood. Further elucidation is needed to clarify the molecular mechanisms of dendrite formation of Purkinje cells especially regarding the oriented extension, overwhelmingly extensive branching, and morphogenesis through extension and retraction.

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