Review

Molecular and cellular mechanisms of developmental synapse elimination in the cerebellum: Involvement of autism spectrum disorder-related genes

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Abstract: Neural circuits are initially created with excessive synapse formation until around birth and undergo massive reorganization until they mature. During postnatal development, necessary synapses strengthen and remain, whereas unnecessary ones are weakened and eventually eliminated. These events, collectively called "synapse elimination" or "synapse pruning", are thought to be fundamental for creating functionally mature neural circuits in adult animals. In the cerebellum of neonatal rodents, Purkinje cells (PCs) receive synaptic inputs from multiple climbing fibers (CFs). Then, inputs from a single CF are strengthened and those from the other CFs are eliminated, and most PCs become innervated by single CFs by the end of the third postnatal week. These events are regarded as a representative model of synapse elimination. This review examines the molecular and cellular mechanisms of CF synapse elimination in the developing cerebellum and argues how autism spectrum disorder (ASD)-related genes are involved in CF synapse development. We introduce recent studies to update our knowledge, incorporate new data into the known scheme, and discuss the remaining issues and future directions.

Keywords: cerebellum, climbing fiber, Purkinje cell, development, synapse elimination, autism spectrum disorder

Introduction

The nervous system undergoes extensive synapse formation around birth, and the density of synapses in neonatal animals is much higher than that of mature animals. During synapse formation, presynaptic axons initially target and innervate a larger number of postsynaptic neurons resulting in an excess number of synaptic inputs on each neuron. During postnatal development, necessary synapses are strengthened and retained, and unnecessary synapses are weakened and eventually eliminated. Consequently, neural circuits are refined by eliminating redundant axonal projections and presynaptic axons come to innervate a limited number of postsynaptic neurons. These events, col-

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Non-standard abbreviation list: Aldoc: aldolase C; AMPAR: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; ASD: autism spectrum disorder; AUTS2: autism susceptibility candidate 2; BC: basket cell; BDNF: brain-derived neurotrophic factor; C1ql1: C1q-like molecule 1; CamK: Ca^{2+}/calmodulin-dependent protein kinase; Cbln1: cerebellin 1; CF: climbing fiber; cKO: conditional knockout; EPSC: excitatory postsynaptic current; FMRP: fragile X mental retardation protein; GABA: γ-aminobutyric acid; GC: granule cell; GLAST: glutamate transporter L-glutamate/L-aspartate transporter; GluD2: δ2 glutamate receptor; Grn: progranulin; Htr3a: serotonin 3A receptor; IGF1: insulin-like growth factor 1; Itgb1: integrin β1; KO: knockout; LTD: long-term depression; mGluR1: metabotropic glutamate receptor type 1; mIPSC: miniature inhibitory postsynaptic current; MLI: molecular layer interneuron; NLGN: neuroligin; NMDA: N-methyl-D-aspartate; NMDAR: N-methyl-D-aspartate receptor; NRXN: neurexin; patDp: paternally inherited 15q11–13 duplication; PC: Purkinje cell; PF: parallel fiber; PKC: protein kinase C; PLC: phospholipase C; Plxn: plexin; P/Q-VDCC: P/Q-type voltage-dependent calcium channel; PTPδ: protein tyrosine phosphatase δ; RORα: retinoid-related orphan receptor α; Sema: semaphorin; SFARI: Simons Foundation Autism Research Initiative; Sort1: sortilin 1; TrkB: tropomyosin receptor kinase B; Tsc1: tuberous sclerosis complex 1.

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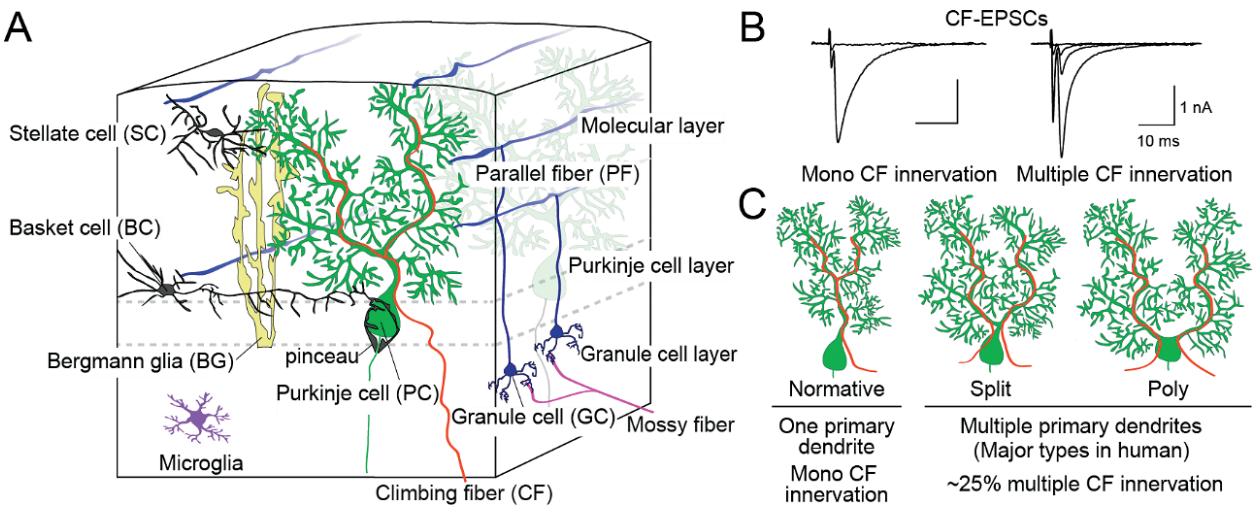

Fig. 1. Basic neural circuits in the cerebellar cortex and the morphological diversity in PC dendritic arbors related to CF innervation patterns. (A) Diagram of the major cell types and neural circuits constituting the adult mouse cerebellar cortex. (B) Representative traces of CF-mediated excitatory postsynaptic currents (CF-EPSCs) from two PCs. The PC with a single CF-EPSC step (left) and that with multiple steps (right) are judged to be mono-innervated and multiply-innervated by CFs, respectively. (C) Schematics depict a relationship between the morphology of PC dendritic arbors and CF innervation patterns. PCs are classified into three groups: (1) with one primary dendrite (Normative), (2) two multibranched dendrites bifurcated proximal to the soma (Split), and (3) multiple dendrites emerging directly from the soma (Poly).[16)]

lectively called "synapse elimination" or "synapse pruning", are essential for creating functionally mature neural circuits.[1)–5)] Synapse elimination occurs in various regions of the peripheral and central nervous systems, including the neuromuscular junction, autonomic ganglia, the visual and somatosensory thalamus, the cerebral cortex, and the cerebellum. Among them, the molecular and cellular mechanisms of postnatal changes in climbing fiber (CF) to Purkinje cell (PC) synapses in the cerebellum have been a representative experimental model. This review aims to provide a compact overview of the current understanding of CF synapse elimination in the developing cerebellum. As there are several comprehensive reviews on this issue,[6)–9)] we introduce recent studies to update our knowledge, incorporate new data into the known scheme of CF to PC synapse formation and elimination, and briefly discuss the remaining questions. Furthermore, we will mention that many genes related to autism spectrum disorder (ASD) are involved in CF to PC synapse formation and elimination, given that abnormal synapse pruning has been implicated in ASD[10)] and cerebellar defects or dysfunctions are suggested to be related to ASD.[11)]

Major cell types and neural circuits in the cerebellar cortex

PCs are GABAergic inhibitory neurons that send the sole outputs from the cerebellar cortex to the deep cerebellar nuclei and the vestibular nuclei.[12),13)] In adult animals, PCs receive two distinct types of glutamatergic excitatory synaptic inputs, parallel fibers (PFs) and CFs (Fig. 1A). PFs are bifurcated axons of granule cells (GCs) forming synapses on the PC distal dendrites. CFs are axon branches of neurons in the inferior olive innervating the PC proximal dendrites. In addition, PCs receive GABAergic inhibitory synaptic inputs from basket cells (BCs) and stellate cells in the molecular layer, collectively called molecular layer interneurons (MLIs). BCs are located in the deeper molecular layer near the PC layer and form inhibitory synapses around the somata and pinceau structures around the axon initial segments of PCs. Stellate cells are located in the middle of the molecular layer and form inhibitory synapses on the PC dendrites. While astrocytes, oligodendrocytes, and microglia are present, the cerebellum contains a special type of astrocyte, called Bergmann glia, extending processes from the PC layer to the cerebellar surface through the molecular layer. Neural circuits in the

cerebellum are shaped and refined during postnatal development through interactions of different types of neurons, synapses, and glial cells. Notably, the proper development of PF to PC synapses, MLI to PC synapses, and Bergmann glia is required for the elimination of redundant CF synapses, and thus the establishment of appropriate CF to PC wiring.

In adult rodents, each PC is typically innervated by a single CF on the proximal dendrites, while each PC receives more than 100,000 PF synaptic inputs on the distal dendrites.[14] The number of CFs innervating a PC can be quantitively estimated electrophysiologically by recording excitatory postsynaptic currents (EPSCs) elicited by stimulating CFs from a PC using whole-cell patch-clamp recording in acute cerebellar slices (Fig. 1B).[15] This method enables the investigator to count the number of discrete steps of CF-mediated EPSCs elicited in the recorded PC. To search all CFs innervating the PC, the site of CF stimulation is systematically moved, and the stimulus intensity is gradually increased at each stimulation site. The number of discrete CF-EPSCs thus obtained reflects the number of CFs innervating the recorded PC.

A recent study by Busch and Hansel (2023) revealed that PCs with multiple primary dendrites constitute the majority (∼95%) in the human cerebellum and about 50% in adult mice (Fig. 1C).[16] They found that ∼25% of multibranched PCs receive synaptic connections from more than two CFs, whereas almost all PCs with one primary dendrite have single CF innervation in adult mice. Consequently, about 15% of all PCs have multiple CF innervation, the value of which is consistent with data recorded from randomly chosen PCs in wild-type control mice, as reported in many previous studies. Multi-branched PCs are relatively more prevalent in posterior lobules of human and murine cerebellar vermis. However, the prevalence is not dependent on zebrin II/aldolase C (Aldoc), which is predominantly expressed in posterior lobules of the vermis.[17] Our study by Rai *et al.* (2021) found a tendency for a higher degree of multiple CF innervation in Aldoc$^+$ PCs than Aldoc$^-$ PCs at around P25.[18] These studies implicate that diversity in the morphology and biochemistry of PCs influences CF to PC synapse development.

Mechanisms of formation and elimination of CF-PC synapses in the developing cerebellum

In the newborn rodent cerebellum, CFs with many branches project to a broader area of the PC layer and innervate a larger number of PCs at postnatal day 3 (P3) than at later developmental stages (Fig. 2), as demonstrated by tracing of a single inferior olivary neuron and electron microscopic analysis.[19),20] During CF synapse formation around birth in mice, each PC receives synaptic inputs from more than five CFs on the soma, an average of around seven CFs based on electrophysiological data,[21] with nearly equal strengths. From around P3 to P7, synaptic inputs from a single CF selectively become larger in each PC, leading to the selection of a single "winner" CF and the other "loser" CFs in each PC (termed "functional differentiation").[22] These functional changes correspond to the morphological remodeling of CF terminal arbors from loose "creeper type" into dense "nest-type",[19] which include collaterals of CFs projecting to adjacent PCs.[23] After P9, the strongest "winner" CF expands its synaptic territory over the PC proximal dendrites, forming hundreds of synaptic contacts (termed "dendritic translocation").[23] Concurrently, from around P7 to around P17, synapses from both the winner and loser CFs remaining on the PC soma are eliminated. The elimination of somatic CF synapses consists of two distinct phases: the 'early phase' from around P7 to around P12,[24] and the 'late phase' from around P12 to around P17.[21] The formation of PF to PC synapses does not influence the early phase, but normal PF-PC synapse formation is a prerequisite for the late phase of CF elimination.

Molecular mechanisms of CF synapse formation on PCs have been relatively uncharacterized compared with those of CF-PC synapse elimination. Several studies have shown synapse organizers play important roles in CF-PC synapse formation (Fig. 3A). Roles of the presynaptic organizer neurexins (NRXNs: *Nrxn1/2/3*) and their postsynaptic binding partners neuroligins (NLGNs: *Nlgn1/2/3*) in CF-PC synapse formation have been analyzed (Fig. 3A, 4).[25),26] Chen *et al.* (2017) showed that sparse knockout (KO) of all NRXNs in some CFs caused retraction of the NRXN-deficient CFs from the cerebellar cortex. The NRXN-deficient CFs may not actively innervate PCs or may be eliminated as loser CFs through competition with other NRXN-expressing CFs. In contrast, the global KO of all NRXNs from almost all CFs caused a reduction in CF innervation territories over PC dendrites and decreased the CF-EPSC amplitude in young adulthood. As the postsynaptic binding partners for NRXNs, the roles of NL-

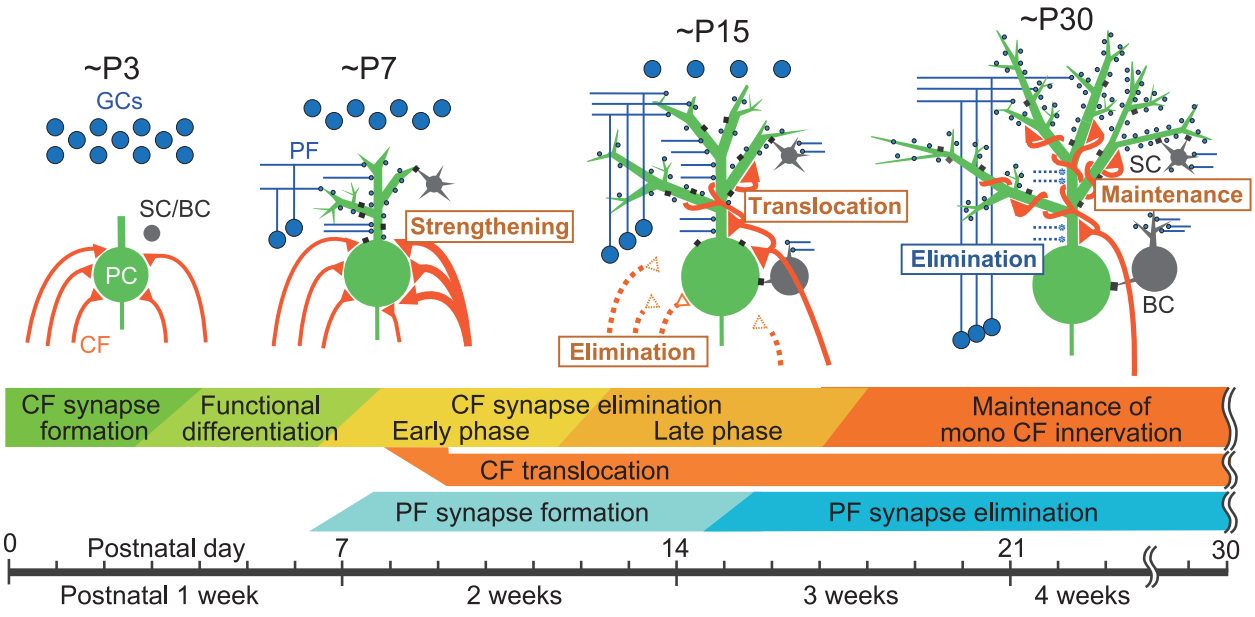

Fig. 2. Alterations in CF and PF synaptic wirings on PCs during postnatal development of the mouse cerebellum. (Upper) Schematics showing developmental alterations in CF and PF synaptic wiring to PCs at ~P3, ~P7, ~P15, and ~P30 in mice. (Lower) Major events related to postnatal development of CF-to-PC and PF-to-PC synapses from birth to ~P30 (from Kano and Watanabe, 2019).[7)]

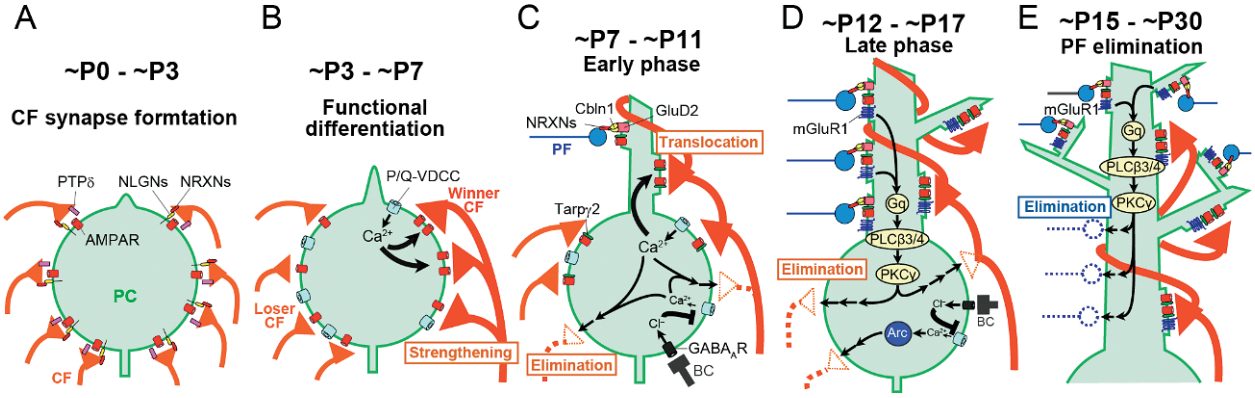

Fig. 3. Molecular and cellular mechanisms underlying the formation and remodeling of CF and PF synaptic wiring to PCs during postnatal development. (A) CF synapse formation onto neonatal PCs by ~P3 is facilitated by the action of PTPδ on presynaptic CFs and NRXN-NLGN interaction. (B) From ~P3 to ~P7, the strengthening of a single "winner" CF input (functional differentiation) is triggered and promoted by Ca^{2+} influx into PCs through P/Q-VDCC. (C) From ~P7 to ~P11, the translocation of the winner CF from the soma to the PC dendrite is promoted by Ca^{2+} influx through P/Q-VDCC. At the same time, the Ca^{2+} influx triggers and facilitates the elimination of CF synapses from the soma (early phase of CF elimination). GABAergic inhibition from BCs modulates this process by inhibiting Ca^{2+} influx into the PC soma and facilitates the elimination of somatic CF synapses from ~P10. In parallel, massive PF synapse formation occurs, which is promoted by NRXN-Cbln1-GluD2 interaction. (D) Between ~P12 and ~P17, mGluRs are activated at PF-PC synapses and drive Gαq-PLCβ3/4-PKCγ signaling in PCs to fuel the elimination of CF synapses from the soma (late phase of CF synapse elimination). At the same time, Arc is upregulated by Ca^{2+} influx through P/Q VDCCs and promotes late-phase CF synapse elimination. (E) From ~P15 to ~P30, mGluR1-PKCγ signaling in PCs drives PF synapse elimination from the proximal portions of PC dendrites, segregating PC dendrites into the CF innervation zone and PF innervation zone (modified from Kano and Watanabe, 2019).[7)]

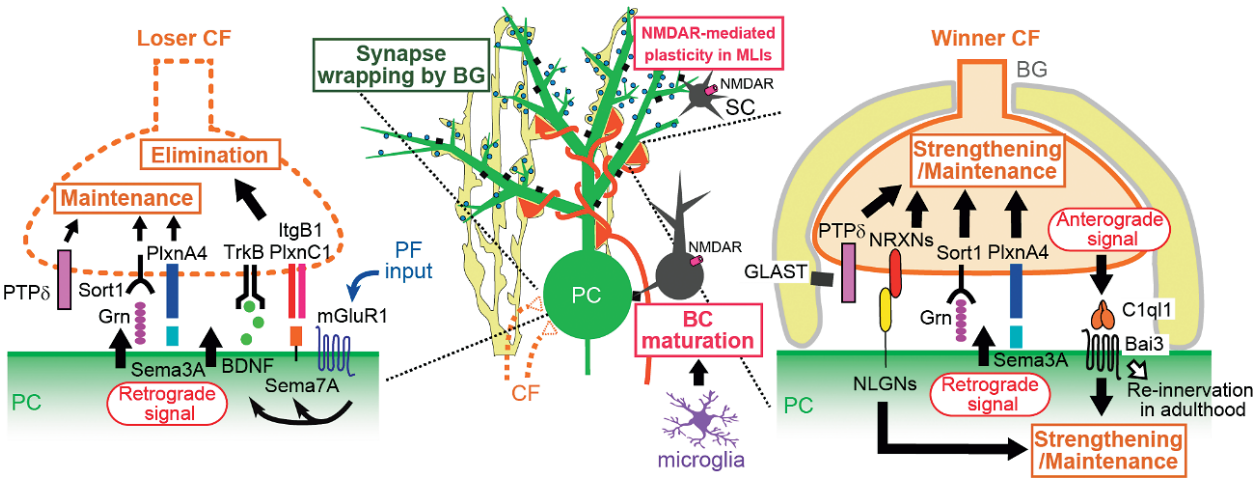

Fig. 4. Trans-synaptic signaling and cellular interactions underlying the elimination of loser CFs (left) and strengthening/maintenance of a winner CF (right) (modified from Kano and Watanabe, 2019).[7)]

GNs in PCs have been investigated.[26)] Zhang *et al.* (2015) showed that PC-specific triple NLGN KO mice showed reduced CF innervation territories over PC dendrites and smaller CF-EPSC amplitudes without altering the mono CF innervation in young adulthood.[26)] Thus, the presynaptic NRXNs in CFs and the postsynaptic NLGNs in PCs contribute to the strengthening of CF-PC synaptic connections and function during postnatal development and in young adulthood (Fig. 4). Recently, Okuno *et al.* (2023) reported that protein tyrosine phosphatase δ (PTPδ/*Ptprd*) acts as a presynaptic organizer for CF synapse formation and maintenance (Fig. 3A, 4).[27)] During development, PTPδ KO mice showed a reduction in CF puncta size, CF-EPSC amplitude, and CF translocation accompanied by accelerated CF synapse elimination in both Aldoc$^-$ and Aldoc$^+$ PCs. However, a decrease in the CF terminal density on PCs during synapse formation before P5 and reductions in CF translocation and the CF-EPSC amplitude in adulthood were only seen in Aldoc$^-$ PCs. The underlying mechanisms for the distinct phenotypes between Aldoc$^-$ and Aldoc$^+$ PCs in PTPδ KO mice and the postsynaptic partner(s) of PTPδ in PCs remain unknown.

Mechanisms for CF synapse elimination have been studied extensively, and molecules related to the activities of PCs, CFs, and cerebellar neural circuits have been shown to be involved. Transgenic mice with chloride channel-overexpressing PCs exhibited reduced burst firings in PCs and persistent multiple CF innervation until adulthood.[28)] Conversely, harmaline administration, which induces synchronous activation of inferior olivary neurons and disturbs normal CF activity patterns, impaired CF synapse elimination from P9 to P12 in rats.[29)] These findings emphasize the necessity of normal activity levels and specific firing patterns in postsynaptic PCs and presynaptic CFs for effective synapse elimination. As the main player mediating the activity of PCs and playing crucial roles in CF synapse elimination, the P/Q-type voltage-dependent calcium channel (P/Q-VDCC/Cav2.1/*Cacna1a*) has been identified (Fig. 3B–D).[24),30)] In PC-specific P/Q-VDCC KO mice, functional differentiation to select a single winner CF that should occur by P7, the early phase of CF synapse elimination, and expansion of CF innervation over PC dendrites are severely impaired, leading to persistent multiple CF innervation into adulthood.[24)] P/Q-VDCCs are activated in PCs by depolarization in response to CF inputs. Although the specific molecules and mechanisms are largely unknown, the subsequent Ca^{2+} influx into PCs is thought to trigger intracellular signaling cascades essential for the sequential events of CF synapse development. Moreover, the immediate early gene *Arc/Arg3.1* (Arc), which is upregulated in PCs by Ca^{2+} influx through P/Q-VDCCs, is required for the late phase of CF synapse elimination.[31)] On the other hand, GABAergic inhibition of PCs from BCs has been shown to regulate CF synapse elimination from around P10 by controlling depolarization and the following Ca^{2+} influx into the PC soma.[32)] In mice with PC-specific KO of TARPγ2/Stargazin/*Cacng2*, an auxiliary

subunit essential for α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) trafficking and postsynaptic accumulation, the synaptic currents in response to CF stimulation are almost halved, resulting in reduced Arc expression in PCs and impairment of the late phase of CF synapse elimination.[33] Together, these results show that the P/Q-VDCC in PCs triggers a canonical signaling pathway for developmental CF synapse elimination including functional differentiation, CF translocation, and early-phase and late-phase CF synapse elimination (Fig. 3C, D).

The late phase of CF synapse elimination, starting around P12, requires the proper formation of PF-PC synapses[21] and is thought to depend on synaptic transmission from PFs. Key molecules involved in the late phase include the metabotropic glutamate receptor type 1 (mGluR1)[34),35] and its downstream signaling molecules Gαq,[36] phospholipase C β3 and β4 (PLCβ3, PLCβ4),[18),37] and protein kinase Cγ (PKCγ)[38] (Fig. 3D). Because mGluR1 has been shown to be activated by brief burst activation of PFs in cerebellar slices,[39)–41] the mGluR1 signaling cascades for the late phase of CF synapse elimination are considered to be activated at PF-PC synapses *in vivo*. Recently, Nakayama *et al.* (2024) demonstrated that using GC-specific TARPγ2 KO mice lacking mossy fibers to GC excitatory synaptic transmission, mGluR1 is activated at PF-PC synapses by the activity along the mossy fiber-GC-PF circuit and promotes the late phase of CF synapse elimination.[42] PLCβ3 and PLCβ4 are expressed in Aldoc$^+$ and Aldoc$^-$ PCs, respectively[43] and either PLCβ3 or PLCβ4 contributes to CF synapse elimination in PCs expressing the corresponding PLCβ isoform. Moreover, Semaphorin 7A (Sema7A)[44] and brain-derived neurotrophic factor (BDNF)[45] have been identified to promote the late phase of CF elimination downstream of mGluR1 (Fig. 4). Both act retrogradely from postsynaptic PCs to receptor molecules at presynaptic CFs. Sema7A engages integrin β1 (Itgb1) and Plexin C1 (PlxnC1),[44] and BDNF acts on its receptor tropomyosin receptor kinase B (TrkB)[45] to promote the elimination of surplus CF synapses from around P15.

Conversely to the molecules that facilitate the elimination of CF synapses, molecules that counteract CF synapse elimination and promote their strengthening and maintenance have also been identified. Semaphorin 3A (Sema3A)[44] and progranulin (Grn)[46] are thought to be secreted by PCs and act in a retrograde manner on their receptor molecules at presynaptic CFs (Fig. 4). Sema3A has been shown to act on Plexin A4 (PlxnA4) from around P8 to around P18, and Grn on Sortilin 1 (Sort1) from around P11 to around P16, to enhance and maintain CF-PC synapses irrespective of whether they are winner or loser CFs.

Another molecule that strengthens and maintains CF-PC synapses is C1q-like molecule 1 (C1ql1) (Fig. 4).[47] C1ql1 belongs to the complement C1q family and is secreted from presynaptic CFs in an activity-dependent manner, and C1ql1 anterogradely acts on postsynaptic PCs by binding to the cell adhesion G-protein-coupled receptor 3 (Bai3). In contrast to Sema3A and Grn that non-selectively reinforce winner and loser CFs, C1ql1-Bai3 signaling selectively strengthens and maintains the winner CF while promoting the elimination of loser CFs after P7.

Mechanisms of PF-PC synapse development and its effects on CF-PC synapses in the developing cerebellum

The molecular mechanisms of PF-PC synapse formation and its influence on CF innervation have also been characterized. In the early postnatal period, GCs continue to be generated in the external granular layer at the surface of the cerebellar cortex.[48] Around P7, GCs migrate from the external granular layer to the internal granular layer, extending their axons PFs, and forming excitatory synapses from PFs on the dendrites of PCs (Fig. 2). An essential molecule for PF-PC synapse formation is cerebellin 1 (Cbln1), a member of the complement C1q family secreted from PFs in an activity-dependent manner.[49] Cbln1 forms a tripartite complex with NRXNs located on PFs and the δ2 glutamate receptor (GluD2/GluRδ2/*Grid2*) expressed on PCs for stabilizing the PF-PC synapses,[50),51] (Fig. 3C). KO mice lacking either GluD2 or Cbln1 exhibit a significant reduction in the number of PF synapses and show abnormalities in the late phase of CF synapse elimination after P12.[49),52] In addition to the increase in weak CF-EPSCs with fast rise times, small CF-EPSCs with slow rise times appear in GluD2 KO mice.[52)–54] These atypical CF-EPSCs are characteristic of GluD2 or Cbln1 KO mice and reflect ectopic CF innervations of the PC distal dendrites, resulting in CF-EPSCs with slow rise times because of the long electrotonic length from the site of synapses and the recording site at the soma. Therefore, these molecules are crucial not

only for PF-PC synapse formation but also for CF synapse elimination by determining the territory of CF innervation and preventing ectopic CF synapse formation.

The maintenance of CF mono-innervation in adult animals is dependent on the integrity of PF-PC synapses. When GluD2 is ablated in adult PCs, ectopic multiple CF innervation of the PC distal dendrites appears with transverse branches that elongate in a mediolateral direction.[55] Therefore, GluD2 in PCs is essential for maintaining CF mono-innervation. Furthermore, a recent study by Aimi *et al.* (2023) found that when C1ql1 or Bai3 expression is increased in CFs or PCs, respectively, excess innervation by CFs of mature PCs with transverse branches occurs in an activity-dependent manner.[56] The authors showed that multiple CF innervation of PCs in GluD2 KO mice was not observed when endogenous Bai3 was deleted from PCs, and C1ql1 immuno-positive puncta were upregulated in the upper molecular layer of GluD2 KO mice. Hence, C1ql1-Bai3 signaling is necessary for ectopic CF synapse formation in mature PCs of GluD2 KO mice (Fig. 4).

A previous report suggested that the serotonin 3A receptor (*Htr3a*/5-HT_{3A} receptor) in cerebellar GCs may contribute indirectly to CF synapse elimination. *Htr3a* is transiently expressed in GCs during the first 3 weeks after birth.[57] *Htr3a* KO mice exhibit facilitated maturation of PC dendrites, abnormal physiological properties of PF-PC synapses, and delayed CF synapse elimination. However, multiple CF innervation in *Htr3a* KO mice did not persist and mono CF innervation was attained after P24.[58]

Besides CF synapse elimination, PF synapse elimination occurs from PC dendrites between P15 and P30 (Fig. 3E).[59] From P9 to P15, the winner CF undergoes translocation and simultaneous massive PF-PC synapse formation occurs to growing PC proximal dendrites resulting in a marked expansion of the dendritic portions with concurrent CF and PF innervation. Beginning around P15, a substantial elimination of PF synapses from the CF and PF mixed innervated zone occurs. By P30, a clear segregation of the CF and PF innervation territories on the PC dendritic arbors is established: a single dominant CF occupies the proximal dendrites, while a vast number of PFs innervate the distal dendrites of each PC. Importantly, this PF synapse elimination is deficient in mice lacking mGluR1 or PKCγ, indicating that mGluR1-to-PKCγ signaling is essential for the segregation of CF and PF territories on PC dendrites by eliminating PF synapses from the proximal dendrites besides the removal of excess CF synapses from the soma and establishment of mono CF innervation.

Roles of molecular layer interneurons and glial cells in CF-PC synapse elimination

The strength of inhibitory synaptic inputs to PCs during postnatal development significantly contributes to CF synapse elimination.[32] In mice with heterozygous KO of the γ-aminobutyric acid (GABA)-synthesizing enzyme GAD67, inhibitory synaptic inputs to the cell bodies of PCs are weaker than in wild-type mice transiently from P7 to P15, leading to impaired CF synapse elimination after P10. In these GAD67 heterozygous KO mice, reduced inhibitory synaptic inputs from BCs result in larger Ca^{2+} transients in the cell bodies of PCs in response to the activation of weak CFs compared with wild-type mice (Fig. 3C–D). This may drive putative Ca^{2+}-dependent synapse reinforcement mechanisms and permit the retention of weak CFs that should normally be eliminated.

Nakayama *et al.* (2024) recently reported that the activation of N-methyl-D-aspartate (NMDA) receptors (NMDARs) in MLIs was required for the late phase of CF synapse elimination after P14.[42] They found that CF synapse elimination was normal in mice with GC-specific KO of GluN1/*Grin1*, the essential subunit of NMDARs. In contrast, the late phase of CF elimination was impaired in mice with GluN1 deletion in PCs and MLIs (Fig. 4). Because functional NMDARs are absent in PCs during the late phase of CF synapse elimination, NMDARs on MLIs were considered to be involved. These data are consistent with previous studies showing that NMDARs in the cerebellum are required for CF elimination, particularly during the third postnatal week.[60),61] However, NMDA receptors at mossy fiber to GC synapses were previously thought to be responsible for CF synapse elimination, so Nakayama *et al.* (2024) have updated our knowledge about how NMDARs contribute to shaping neural circuits in the cerebellum. Thus, neural activity along the mossy fiber-GC-PF circuit is crucial for the late phase of CF elimination by activating mGluR1 in PCs directly at PF-PC synapses and by enhancing inhibition from MLIs to PCs indirectly through activating NMDARs in MLIs at PF-MLIs synapses (see the cerebellar neural circuit diagram in Fig. 1A).

Microglia and astrocytes play crucial roles in synapse reorganization by actively removing redundant synapses in the developing visual thalamus, hippocampus, and cerebral cortex.[62),63)] In contrast, the roles of microglia in the developing cerebellum are different from those in the above brain regions.[64] In the cerebellum of microglia-depleted mice, the elimination of redundant CF synapses after P10 was impaired and GABAergic inhibitory inputs to PCs from MLIs were weakened.[64] Specifically, the amplitude and the frequency of miniature inhibitory postsynaptic currents (mIPSCs) in PCs were reduced at P10 to P12, and the immunohistochemical signal intensity of vesicular GABA transporter in the cerebellar cortex was decreased at P11.[64)] However, microglia did not exhibit phagocytosis of redundant CF synapses in wild-type mice. Importantly, the impairment of CF synapse elimination was restored by daily intraperitoneal administration of diazepam, a $GABA_A$ receptor sensitizer, from P9 to P12.[64)] Hence, this study indicates that microglia promote CF synapse elimination not by directly phagocytosing redundant CF synapses but by promoting the maturation of inhibitory synapses onto PCs (Fig. 4). Although detailed mechanisms by which microglia promote the maturation of MLIs are unclear, results from previous studies collectively suggest that BDNF-TrkB signaling may be involved. BDNF is reported to be produced and released from microglia,[65)] the amplitude and frequency of mIPSCs in PCs are reduced in TrkB KO mice,[66)] and the number of GAD65-positive boutons is reduced in cerebellum-specific TrkB KO mice[67)] Previous studies also suggested that cytokines derived from microglia may contribute to enhancing $GABA_A$ receptor functions in cells other than those in the cerebellum. Interleukin-1β enhances plasma membrane insertion of $GABA_A$ receptors in cultured neurons and oocytes,[68)] and *in vivo* nano-injection of tumor necrosis factor α induces $GABA_A$ receptor trafficking to synaptic membranes in spinal cord neurons.[69)] In the developing somatosensory cortex, it has been reported that microglial contact induces excitatory synapse formation on pyramidal neurons[70)] as well as axo-axonic synaptogenesis derived from a chandelier cell, a GABAergic interneuron subtype, via microglial $GABA_{B1}$ receptors.[71)] Further studies are needed to determine whether such contact-dependent or $GABA_B$ receptor-mediated mechanisms by cerebellar microglia underlie the maturation of inhibitory synapses on PCs.

Bergmann glia, a special type of astrocyte in the cerebellar cortex, express the glutamate transporter L-glutamate/L-aspartate transporter (GLAST/EAAT1/*Slc1a3*) abundantly in their processes that span the molecular layer.[72),73)] Bergmann glial processes tightly enwrap CF and PF synapses.[74)] In GLAST KO mice, Bergmann glial processes were retracted from PC dendrites and synapses, and therefore they were exposed to the extracellular milieus.[75),76)] Analogous to GluD2 KO mice, ectopic CF synapses were formed on proximal and distal PC dendrites, causing multiple CF innervation in almost all PCs.[75),76)] Activation of CFs with ectopic synapses on PC distal dendrites generates CF-EPSCs with slow rise times in GLAST KO mice.[75),76)] On the other hand, a previous study showed that ectopic expression of AMPAR GluA2 subunits in Bergmann glia rendered the originally Ca^{2+}-permeable AMPAR Ca^{2+}-impermeable, causing retraction of their processes and multiple CF innervation of PCs.[77)] Therefore, tight enwrapping of CF synapses, PF synapses, and PC dendrites by Bergmann glial processes and uptake of released glutamate by GLAST to Bergmann glia are essential for establishing CF mono-innervation of PCs (Fig. 4).

ASD-related genes are involved in CF-PC synapse elimination in the developing cerebellum

Abnormality in synapse elimination during development has been implicated in the pathophysiology of neurodevelopmental and psychiatric disorders including ASD and schizophrenia.[10)] On the other hand, accumulating evidence suggests that cerebellar dysfunctions are related to the disorders in ASD patients and animal models.[11),78)–80)] For example, mice with PC-specific conditional KO (cKO) of an ASD-related gene, *Tuberous sclerosis complex 1* (*Tsc1*), had reduced number and excitability of PCs in adulthood and exhibited ASD-like behavioral abnormalities including impaired sociality and reduced flexibilities.[78)] Furthermore, adult mice with PC-specific cKO of *Shank2*,[81)] *Pten*,[82)] or *Mecp2*,[83)] which are well-known ASD-related genes, have been reported to exhibit ASD-like phenotypes. However, there have been no reports that link developmental CF synapse elimination in the cerebellum and ASD-like phenotypes. To get insight into this issue, we summarize how the deletion or mutation of ASD-related genes alters or impairs CF synapse elimination in the developing

Table 1. Alterations in CF to PC synaptic wiring and transmission in mice with deletion or mutation of an ASD-related gene

Gene	Mouse	Developmental CF synapse elimination	CF innervation in the adult	CF transmission in the adult	References
***Cacna1a* (P/Q-VDCC)**	PC-selective P/Q-VDCC cKO (GluD2-Cre)	Impaired (P7–P18)	Multiple CF innervation (P19–P31)	Normal	Hashimoto *et al.*, 2011[24]
***Cacng2* (TARPγ2)**	PC-selective TARPγ2 cKO (GluD2-Cre)	Impaired (P15–P17)	Multiple CF innervation (P21–P43)	CF-EPSC amplitude↓	Kawata *et al.*, 2014[33]
	GC-selective TARPγ2 cKO (GluD2-Cre)	Impaired (P16–P18)	Multiple CF innervation (P60–P80)	Normal	Nakayama *et al.*, 2024[42]
***Grid2* (GluD2)**	Global *Grid2* KO	Impaired (>P10)	Multiple CF innervation (P24–P64), Branched CFs	Appearance of slow and small CF-EPSCs	Hashimoto *et al.*, 2001[52]
***Grin1* (GluN1)**	MLI-selective GluN1 cKO (GluD2-Cre)	Impaired (P15–P18)	Multiple CF innervation (P30–P50)	Normal	Nakayama *et al.*, 2024[42]
Htr3a	Global *Htr3a* KO	Delayed (P7–P24)	Normal (P68–P72)	Normal	Oostland *et al.*, 2013[58]
Igf1	IGF1 application into the cerebellum from P8	Impaired (P13–P15)	Multiple CF innervation (P24–P36)	CF-EPSC amplitude↑ (weaker CFs)	Kakizawa *et al.*, 2003[89]
	Anti-IGF antibody application into the cerebellum from P8	Accelerated (P13–P15)	Mono CF innervation↑ (P24–P36)	CF-EPSC amplitude↓	Kakizawa *et al.*, 2003[89]
Nrxn1/2	CF-selective NRXN1/2/3 cKO (Adeno associated virus-Cre)	ND	Normal (P24)	CF-EPSC amplitude↓	Chen *et al.*, 2017[25]
Nlgn1/2/3	PC-selective NLGN1/2/3 cKO (L7/Pcp2-Cre)	ND	Normal (P21–P25, ~P63)	CF-EPSC amplitude↓	Zhang *et al.*, 2015[26]
***Ntrk2* (TrkB)**	CF-selective KD of TrkB /PC-selective BDNF cKO (GluD2-Cre)	Impaired (P16–P19)	Multiple CF innervation (P21–P59)	Normal	Choo *et al.*, 2017[45]
PlxnA4	CF-selective KD of *PlxnA4*/PC-selective KD of *Sema3A*	Accelerated (P8–P18)	Normal (P21–P30)	CF-EPSC amplitude↓	Uesaka *et al.*, 2014[44]
Myo5a	Global *Myo5a* KO	Delayed (P11–P15)	Normal (2 months-old)	CF-EPSC amplitude↓	Takagishi *et al.*, 2007[84]
CamKIIa	Global *CamKIIa* KO	Delayed (P21–P28)	Normal (P90–P100)	Normal	Hansel *et al.*, 2006[85]
CamKIV	Global *CamKIV* KO	Delayed/impaired (P14–P23)	ND	CF-EPSC amplitude↓	Ribar *et al.*, 2000[86]
Rora	Global *Rora* KO (*staggerer* mutant)	ND	Multiple CF innervation (3 weeks-old)	Normal	Iizuka *et al.*, 2016[87]
	PC-selective *Rora* cKO (L7/Pcp2-Cre)	Impaired (>P16)	Multiple CF innervation (1 month-old)	Normal	Chen *et al.*, 2013[88]
***Ptprd* (PTPδ)**	Global *Ptprd* KO, CF-selective KD of PTPδ	Accelerated (P7–P12 in Aldoc$^-$, P13–P15 in Aldoc$^+$ PCs)	Normal (P19–P29)	CF-EPSC amplitude↓ (Aldoc$^-$ PCs)	Okuno *et al.*, 2023[27]
Fmr1	Global *Fmr1* KO	Accelerated (P21–P48)	Mono CF innervation↑ (P21–P48)	Normal	Koekkoek *et al.*, 2005[91]
Auts2	PC-selective KD of AUTS2	ND	Multiple CF innervation (P21–P30)	Normal	Yamashiro *et al.*, 2020[94]
***Nlgn3*-R451C**	Global mutant of NLGN3-R451C	Delayed (P10–P15)	Normal (P21–P35)	CF-EPSC amplitude↑ (weaker CFs)	Lai *et al.*, 2021[97]
15q11-13 duplication	Global mutant of paternal 15q11-13 duplication (patDp/+)	Impaired (P10–P12)	Multiple CF innervation (P63–P70)	CF-EPSC amplitude↑	Piochon *et al.*, 2014[98] Simmons *et al.*, 2022[100]

cerebellum based on published studies (Table 1).

We first argue that a substantial portion of genes encoding the molecules involved in CF synapse elimination discussed so far are listed in the Simons Foundation Autism Research Initiative (SFARI) database (https://www.sfari.org/resource/sfari-gene/) as ASD-related genes. These genes include: *Cacna1a* (encoding P/Q-VDCC), *Cacng2* (TARPγ2), *Grid2* (GluD2), *Grin1* (GluN1), *Htr3a*, *Nrxn1/2*, *Nlgn1/2/3*, *Ntrk2* (TrkB), and *PlxnA4*. Moreover, mice with deletion of *Myo5a* (myosin Va),[84]

CamkIIa (Ca^{2+}/calmodulin-dependent protein kinase II α),[85] *CamKIV* (Ca^{2+}/calmodulin-dependent protein kinase IV),[86] or *Rora* (retinoid-related orphan receptor α [RORα]),[87),88] the genes listed in the SFARI database, are reported to exhibit impairment of CF synapse elimination. Administration of antibodies against insulin-like growth factor 1 (IGF1), the gene for which (*Igf1*) is listed in the SFARI database, is reported to accelerate CF synapse elimination.[89] In addition, mutations of *Ptprd* (encoding PTPδ), although not listed in the SFARI database, are associated with ASD and various types of neurological disorders such as restless legs syndrome, obsessive-compulsive disorder, attention deficit and hyperactivity disorder, and schizophrenia.[90] However, because comprehensive analyses of ASD-like behavioral abnormalities have not been performed on mice deficient in the above-listed genes, how impaired or altered CF synapse elimination contributes to ASD-like behaviors remains largely unknown.

Next, we show four examples in which ASD-model mice with ASD-related behavioral abnormalities have impairment or alteration in CF synapse elimination. The first example is mice with PC-specific deletion of *Fmr1* (encoding fragile X mental retardation protein [FMRP]).[91] PC-specific *Fmr1* KO mice were reported to exhibit immature elongated spines and enhanced long-term depression (LTD) in PCs and accelerated CF synapse elimination when examined between P21 and P48.[91] Moreover, a recent study showed that adult PC-specific *Fmr1* KO mice displayed reduced social behaviors, sensory hypersensitivity, and reduced PC excitability.[92] Introduction of *Fmr1* into PCs of global *Fmr1* KO mice ameliorated the impaired social behaviors and cerebellar dysfunctions.[92] These results showed that *Fmr1* in PCs is important for regulating ASD-like behaviors. However, it remains unclear whether the abnormal synaptic wiring in PCs including accelerated CF synapse elimination contributes to ASD-related behavioral abnormalities.

The second example is mice with cerebellum- and midbrain-specific deletion of *autism susceptibility candidate 2* (*Auts2*), which acts as a transcriptional activator and is specifically expressed in PCs and Golgi cells during postnatal development.[93] AUTS2 cKO mice had a smaller and deformed cerebellum containing immature-shaped PCs with reduced expression of P/Q-VDCC essential for CF synapse elimination.[94] AUTS2 cKO mice and mice with PC-specific AUTS2 knockdown (KD) exhibited impairment of CF synapse elimination, reduced dendritic translocation of CFs, increased PF synaptic density, and enhanced PF-PC synaptic transmission.[94] These data showed that AUTS2 is required for the maturation of PCs and proper formation of CF and PF synaptic wiring patterns of PCs presumably partly through the regulation of P/Q-VDCC expression. AUTS2 cKO mice exhibited deficits in motor learning and vocal communication.

The third example is mice with an arginine to cysteine substitution of amino acid residue 451 (R451C) in *Nlgn3*, which has been identified in patients with ASD. NLGN3-R451C mutant mice, one of the best-studied mouse models of ASD, exhibit impaired social interactions and enhanced repetitive behaviors.[95),96] Lai *et al.* (2021) reported that the expression of NLGN3 protein in the cerebellum of NLGN3-R451C mutant mice was reduced to about 10% of the level of wild-type mice.[97] CF synapse elimination was impaired between P10 and P15 in NLGN3-R451C mutant mice, but most PCs became mono-innervated after P16 similar to wild-type mice.[97] However, selective strengthening of inputs from a single CF relative to those from the other CFs in each PC was impaired from P16, and this impairment persisted into the juvenile stage. Furthermore, the inhibition to excitation balance of synaptic inputs to PCs was elevated, and calcium transients in the PC soma induced by the strongest and weaker CF inputs were both reduced in NLGN3-R451C mutant mice. These findings indicate that NLGN3-R451C mutation significantly impacts synapse development and refinement in cerebellar circuits.[97]

The fourth example is the copy-number variation ASD mouse model of the human paternally inherited 15q11–13 duplication (patDp/+)[98),99] that exhibits reduced social interaction, few ultrasonic vocalizations, behavioral inflexibility, and motor coordination and learning deficits.[98),99] The patDp/+ mice exhibited impaired LTD at PF-PC synapses, impaired CF synapse elimination from P10, and persistent multiple CF innervation into adulthood.[98] A follow-up study by Simmons *et al.* (2022) reported that spontaneous and sensory-evoked CF-induced calcium transients and CF-EPSCs were enhanced, possibly resulting from enhanced NRXN1 expression in patDp/+ mice. The results of these studies implicate that excessive CF-PC inputs with sensory over-responsivity and aber-

rant PF-PC synaptic plasticity may be associated with dysfunction in certain ASD model mice.[100]

As summarized in Table 1, the studies mentioned above suggest that many ASD-associated genes are involved in CF synapse elimination during postnatal development. Although the effects of deletion or mutation of ASD-associated genes on CF synapse elimination are complex, they can be roughly categorized into the following three types. Deletion or mutation of a gene causes, (1) impairment of CF synapse elimination during postnatal development and persistent multiple CF innervation into the mature stage, (2) acceleration of CF synapse elimination during postnatal development, or (3) a transient impairment of CF synapse elimination during postnatal development but normal CF mono-innervation in the mature stage. In addition to these three types of effects, deletion or mutation of certain genes induces altered CF-PC synaptic transmission in adulthood. Although changes in CF to PC synaptic wiring and transmission may lead to cerebellar dysfunctions, they can arise from various causes including the reduction in the number of PCs, alterations of PC excitability, and changes in PF to PC synaptic wiring, transmission, and plasticity. Notably, recent studies on mouse models of ASD suggest that decreased PC activity in the right Crus I is associated with the emergence of ASD-like behavioral abnormalities.[80),101] It remains to be determined through future research whether the abnormalities in the development and function of CF-PC synapses mentioned above are related to ASD-like behavioral abnormalities.

Remaining issues and future directions

Several important issues regarding CF synapse elimination remain to be clarified. First, it is largely unknown about the molecular mechanisms downstream of P/Q-VDCCs in PCs, which mediate the selection of a single "winner" CF, translocation of the winner CF along the PC dendrites, and the early and late phases of CF elimination. In particular, it is important to investigate how calcium elevation in PCs strengthens a single CF and eliminates the other weaker CFs at the same time. It is hypothesized that inputs to the stronger synapse generate some "punishment signal" and eliminate the weaker synapse, whereas some local "protection signal" is generated at the stronger synapse and protects it from the punishment signal.[2] In mitral cells of the developing olfactory bulb, the elimination of dendritic branches is mediated by RhoA generated at synapses on the dendritic branch that will survive, indicating that RhoA functions as a "punishment signal".[102] In contrast, the nature of the hypothetical 'punishment signal' and 'protection signal' involved in CF synapse elimination in the developing cerebellum remains unknown.

Second, it is unknown how redundant CF synapses are detached and eliminated from PCs during postnatal development. Microglia and astrocytes have been shown to phagocytose redundant synapses in the developing retinogeniculate synapses and the cerebral cortex. In the neuromuscular synapses, Schwann cells phagocytose retracting motor axons.[103] In marked contrast, microglia contribute to CF synapse elimination not by phagocytosing redundant CF synapses but by promoting the maturation of inhibitory synaptic inputs to PCs.[64] A previous study showed that lysosomal activity was increased in the deeper half of the molecular layer of the cerebellum during the second postnatal week, suggesting increased phagocytosis during CF synapse elimination.[104] Further studies are needed to clarify whether and how phagocytosis of redundant CFs occurs during CF synapse elimination.

Third, molecular mechanisms underlying the activity-dependent maintenance of CF-PC synapses and heterosynaptic competition between CFs and PFs for PC dendrites are largely unknown. Blockade of PC activity or CF-PC synaptic transmission in mature cerebellum *in vivo* causes retraction of CF innervation and expansion of PF synaptic territories on PC dendritic arbors.[105),106] These results indicate that CF-PC synaptic activity is a prerequisite for maintaining CF-PC synapses and determining CF and PF innervation territories over PC dendritic arbors. Therefore, investigation of the molecular mechanisms for the activity-dependent maintenance of CF-PC synapses will lead to the elucidation of those for heterosynaptic competition between CFs and PFs.

Fourth, recent studies have disclosed that cerebellar neural circuits are more diverse than previously assumed. For example, the patterns of PC dendritic arbors,[16] the degree of persistent multiple CF innervation,[18] and the level of PC activity *in vivo*[107] are not identical between Aldoc$^+$ and Aldoc$^-$ PCs. Moreover, transcriptional profiling using single nucleus RNA sequences in adult mouse cerebellum revealed a great diversity of PCs in the posterior lobules expressing Aldoc.[108] Therefore, future studies should consider the possibility

that patterns of CF-PC synaptic wiring may vary depending on cerebellar lobules.

Finally, future research should further elucidate the molecular and cellular mechanisms of postnatal development of CF-PC synaptic wiring and function. This will deepen our understanding of how the mechanisms are related to not only motor control but also ASD-associated behaviors such as social interaction and behavioral flexibility.

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References

1) Purves, D. and Lichtman, J.W. (1980) Elimination of synapses in the developing nervous system. Science **210**, 153–157.
2) Lichtman, J.W. and Colman, H. (2000) Synapse elimination and indelible memory. Neuron. **25**, 269–278.
3) Kano, M. and Hashimoto, K. (2009) Synapse elimination in the central nervous system. Curr. Opin. Neurobiol. **19**, 154–161.
4) Riccomagno, M.M. and Kolodkin, A.L. (2015) Sculpting neural circuits by axon and dendrite pruning. Annu. Rev. Cell Dev. Biol. **31**, 779–805.
5) Faust, T.E., Gunner, G. and Schafer, D.P. (2021) Mechanisms governing activity-dependent synaptic pruning in the developing mammalian CNS. Nat. Rev. Neurosci. **22**, 657–673.
6) Kano, M., Watanabe, T., Uesaka, N. and Watanabe, M. (2018) Multiple phases of climbing fiber synapse elimination in the developing cerebellum. Cerebellum **17**, 722–734.
7) Kano, M. and Watanabe, T. (2019) Developmental synapse remodeling in the cerebellum and visual thalamus. F1000Research **8**, 1191.
8) Watanabe, M. and Kano, M. (2011) Climbing fiber synapse elimination in cerebellar Purkinje cells. Euro. J. Neuroscience **34**, 1697–1710.
9) Hashimoto, K. and Kano, M. (2013) Synapse elimination in the developing cerebellum. Cell. Mol. Life Sci. **70**, 4667–4680.
10) Penzes, P., Cahill, M.E., Jones, K.A., Vanleeuwen, J.E. and Woolfrey, K.M. (2011) Dendritic spine pathology in neuropsychiatric disorders. Nat. Neurosci. **14**, 285–293.
11) Wang, S.S.H., Kloth, A.D. and Badura, A. (2014) The cerebellum, sensitive periods, and autism. Neuron. **83**, 518–532.
12) Eccles, J.C., Ito, M. and Szentágothai, J. (1967) The Cerebellum as a Neuronal Machine. Springer Science+Business Media, New York.
13) Ito, M. (1984) The Cerebellum and Neural Control. Raven Press, New York.
14) Napper, R.M.A. and Harvey, R.J. (1988) Number of parallel fiber synapses on an individual Purkinje cell in the cerebellum of the rat. J Comp Neurol. **274**, 168–177.
15) Kano, M., Hashimoto, K., Chen, C., Abeliovich, A., Aiba, A., Kurihara, H. *et al.* (1995) Impaired synapse elimination during cerebellar development in PKCγ mutant mice. Cell **83**, 1223–1231.
16) Busch, S.E. and Hansel, C. (2023) Climbing fiber multi-innervation of mouse Purkinje dendrites with arborization common to human. Science **381**, 420–427.
17) Sillitoe, R. V. and Hawkes, R. (2002) Whole-mount immunohistochemistry: A high-throughput screen for patterning defects in the mouse cerebellum. J. Histochem. Cytochem. **50**, 235–244.
18) Rai, Y., Watanabe, T., Matsuyama, K., Sakimura, K., Uesaka, N. and Kano, M. (2021) Phospholipase C β3 is required for climbing fiber synapse elimination in aldolase C-positive compartments of the developing mouse cerebellum. Neuroscience **462**, 36–43.
19) Sugihara, I. (2005) Microzonal projection and climbing fiber remodeling in single olivocerebellar axons of newborn rats at postnatal days 4–7. J. Comp. Neurol. **487**, 93–106.
20) Wilson, A.M., Schalek, R., Suissa-Peleg, A., Jones, T.R., Knowles-Barley, S., Pfister, H. *et al.* (2019) Developmental rewiring between cerebellar climbing fibers and Purkinje cells begins with positive feedback synapse addition. Cell Rep. **29**, 2849–2861.e6.
21) Hashimoto, K., Yoshida, T., Sakimura, K., Mishina, M., Watanabe, M. and Kano, M. (2009) Influence of parallel fiber-Purkinje cell synapse formation on postnatal development of climbing fiber-Purkinje cell synapses in the cerebellum. Neuroscience **162**, 601–611.
22) Hashimoto, K. and Kano, M. (2003) Functional differentiation of multiple climbing fiber inputs during synapse elimination in the developing cerebellum. Neuron **38**, 785–796.
23) Hashimoto, K., Ichikawa, R., Kitamura, K., Watanabe, M. and Kano, M. (2009) Translocation of a "winner" climbing fiber to the Purkinje cell dendrite and subsequent elimination of "losers" from the soma in developing cerebellum. Neuron. **63**, 106–118.
24) Hashimoto, K., Tsujita, M., Miyazaki, T., Kitamura, K., Yamazaki, M., Shin, H.S. *et al.* (2011) Postsynaptic P/Q-type Ca^{2+} channel in Purkinje cell mediates synaptic competition and elimination in developing cerebellum. Proc. Natl. Acad. Sci. U.S.A. **108**, 9987–9992.
25) Chen, L.Y., Jiang, M., Zhang, B., Gokce, O. and Südhof, T.C. (2017) Conditional deletion of all neurexins defines diversity of essential synaptic organizer functions for neurexins. Neuron **94**, 611–625.e4.
26) Zhang, B., Chen, L.Y., Liu, X., Maxeiner, S.,

Lee, S.J., Gokce, O. *et al.* (2015) Neuroligins sculpt cerebellar Purkinje cell circuits by differential control of distinct classes of synapses. Neuron. **87**, 781–796.
27) Okuno, Y., Sakoori, K., Matsuyama, K., Yamasaki, M., Watanabe, M., Hashimoto, K. *et al.* (2023) PTPδ is a presynaptic organizer for the formation and maintenance of climbing fiber to Purkinje cell synapses in the developing cerebellum. Front. Mol. Neurosci. **16**, 1206245.
28) Lorenzetto, E., Caselli, L., Feng, G., Yuan, W., Nerbonne, J.M., Sanes, J.R. *et al.* (2009) Genetic perturbation of postsynaptic activity regulates synapse elimination in developing cerebellum. Proc. Natl. Acad. Sci. U.S.A. **106**, 16475–16480.
29) Andjus, P.R., Zhu, L., Cesa, R., Carulli, D. and Strata, P.A. (2003) change in the pattern of activity affects the developmental regression of the Purkinje cell polyinnervation by climbing fibers in the rat cerebellum. Neuroscience. **121**, 563–572.
30) Miyazaki, T., Hashimoto, K., Shin, H.S., Kano, M. and Watanabe, M. (2004) P/Q-type Ca^{2+} channel α1A regulates synaptic competition on developing cerebellar Purkinje cells. J. Neurosci. **24**, 1734–1743.
31) Mikuni, T., Uesaka, N., Okuno, H., Hirai, H., Deisseroth, K., Bito, H. *et al.* (2013) Arc/Arg3.1 is a postsynaptic mediator of activity-dependent synapse elimination in the developing cerebellum. Neuron **78**, 1024–1035.
32) Nakayama, H., Miyazaki, T., Kitamura, K., Hashimoto, K., Yanagawa, Y., Obata, K. *et al.* (2012) GABAergic inhibition regulates developmental synapse elimination in the cerebellum. Neuron **74**, 384–396.
33) Kawata, S., Miyazaki, T., Yamazaki, M., Mikuni, T., Yamasaki, M., Hashimoto, K. *et al.* (2014) Global scaling down of excitatory postsynaptic responses in cerebellar Purkinje cells impairs developmental synapse elimination. Cell Rep. **8**, 1119–1129.
34) Kano, M., Hashimoto, K., Kurihara, H., Watanabe, M., Inoue, Y., Aiba, A. *et al.* (1997) Persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking mGluR1. Neuron **18**, 71–79.
35) Ichise, T., Kano, M., Hashimoto, K., Yanagihara, D., Nakao, K., Shigemoto, R. *et al.* (2000) mGluR1 in cerebellar Purkinje cells essential for long-term depression, synapse elimination, and motor coordination. Science **288**, 1832–1835.
36) Offermanns, S., Hashimoto, K., Watanabe, M., Sun, W., Kurihara, H., Thompson, R.F. *et al.* (1997) Impaired motor coordination and persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking Gαq. Proc. Natl. Acad. Sci. U.S.A. **94**, 14089–14094.
37) Kano, M., Hashimoto, K., Watanabe, M., Kurihara, H., Offermanns, S., Jiang, H. *et al.* (1998) Phospholipase Cβ4 is specifically involved in climbing fiber synapse elimination in the developing cerebellum. Proc. Natl. Acad. Sci. U.S.A. **95**, 15724–15729.
38) Chen, C., Kano, M., Abeliovich, A., Chen, L., Bao, S., Kim, J.J. *et al.* (1995) Impaired motor coordination correlates with persistent multiple climbing fiber innervation in PKCγ mutant mice. Cell **83**, 1233–1242.
39) Batchelor, A.M. and Garthwaite, J. (1997) Frequency detection and temporally dispersed synaptic signal association through a metabotropic glutamate receptor pathway. Nature **385**, 74–77.
40) Finch, E.A. and Augustine, G.J. (1998) Local calcium signalling by inositol-1,4,5-trisphosphate in Purkinje cell dendrites. Nature. **396**, 753–756.
41) Takechi, H., Eilers, J. and Konnerth, A. (1998) A new class of synaptic response involving calcium release in dendritic spines. Nature **396**, 757–760.
42) Nakayama, H., Miyazaki, T., Abe, M., Yamazaki, M., Kawamura, Y., Choo, M. *et al.* (2024) Direct and indirect pathways for heterosynaptic interaction underlying developmental synapse elimination in the mouse cerebellum. Commun. Biol. **7**, 806.
43) Sarna, J.R., Marzban, H., Watanabe, M. and Hawkes, R. (2006) Complementary stripes of phospholipase Cβ3 and Cβ4 expression by Purkinje cell subsets in the mouse cerebellum. J. Comp. Neurol. **496**, 303–313.
44) Uesaka, N., Uchigashima, M., Mikuni, T., Nakazawa, T., Nakao, H., Hirai, H. *et al.* (2014) Retrograde semaphorin signaling regulates synapse elimination in the developing mouse brain. Science **344**, 1020–1023.
45) Choo, M., Miyazaki, T., Yamazaki, M., Kawamura, M., Nakazawa, T., Zhang, J. *et al.* (2017) Retrograde BDNF to TrkB signaling promotes synapse elimination in the developing cerebellum. Nat Commun. **8**, 195.
46) Uesaka, N., Abe, M., Konno, K., Yamazaki, M., Sakoori, K., Watanabe, T. *et al.* (2018) Retrograde signaling from progranulin to sort1 counteracts synapse elimination in the developing cerebellum. Neuron. **97**, 796–805.e5.
47) Kakegawa, W., Mitakidis, N., Miura, E., Abe, M., Matsuda, K., Takeo, Y.H. *et al.* (2015) Anterograde C1ql1 signaling is required in order to determine and maintain a single-winner climbing fiber in the mouse cerebellum. Neuron **85**, 316–329.
48) Altman, J. and Anderson, W.J. (1972) Experimental reorganization of the cerebellar cortex. I. Morphological effects of elimination of all microneurons with prolonged x-irradiation started at birth. J. Comp. Neurol. **146**, 355–405.
49) Hirai, H., Pang, Z., Bao, D., Miyazaki, T., Li, L., Miura, E. *et al.* (2005) Cbln1 is essential for

synaptic integrity and plasticity in the cerebellum. Nat. Neurosci. **8**, 1534–1541.
50) Uemura, T., Lee, S.J., Yasumura, M., Takeuchi, T., Yoshida, T., Ra, M. *et al.* (2010) Trans-synaptic interaction of GluRδ2 and neurexin through Cbln1 mediates synapse formation in the cerebellum. Cell. **141**, 1068–1079.
51) Matsuda, K., Miura, E., Miyazaki, T., Kakegawa, W., Emi, K., Narumi, S. *et al.* (2010) Cbln1 is a ligand for an orphan glutamate receptor δ2, a bidirectional synapse organizer. Science **328**, 363–368.
52) Hashimoto, K., Ichikawa, R., Takechi, H., Inoue, Y., Aiba, A., Sakimura, K. *et al.* (2001) Roles of glutamate receptor δ2 subunit (GluRδ2) and metabotropic glutamate receptor subtype 1 (mGluR1) in climbing fiber synapse elimination during postnatal cerebellar development. J. Neurosci. **21**, 9701–9712.
53) Yamasaki, M., Miyazaki, T., Azechi, H., Abe, M., Natsume, R., Hagiwara, T. *et al.* (2011)Glutamate receptor δ2 is essential for input pathway-dependent regulation of synaptic AMPAR contents in cerebellar Purkinje cells. J. Neurosci. **31**, 3362–3374.
54) Ichikawa, R., Miyazaki, T., Kano, M., Hashikawa, T., Tatsumi, H., Sakimura, K. *et al.* (2002) Distal extension of climbing fiber territory and multiple innervation caused by aberrant wiring to adjacent spiny branchlets in cerebellar purkinje cells lacking glutamate receptor δ2. J. Neurosci. **22**, 8487–8503.
55) Miyazaki, T., Yamasaki, M., Takeuchi, T., Sakimura, K., Mishina, M. and Watanabe, M. (2010) Ablation of glutamate receptor glurδ2 in adult Purkinje cells causes multiple innervation of climbing fibers by inducing aberrant invasion to parallel fiber innervation territory. J. Neurosci. **30**, 15196–15209.
56) Aimi, T., Matsuda, K. and Yuzaki, M. (2023) C1ql1-Bai3 signaling is necessary for climbing fiber synapse formation in mature Purkinje cells in coordination with neuronal activity. Mol. Brain. **16**, 61.
57) Oostland, M., Sellmeijer, J. and Van Hooft, J.A. (2011) Transient expression of functional serotonin 5-HT 3 receptors by glutamatergic granule cells in the early postnatal mouse cerebellum. J. Physiol. **589**, 4837–4846.
58) Oostland, M., Buijink, M.R. and van Hooft, J.A. (2013) Serotonergic control of Purkinje cell maturation and climbing fibre elimination by 5-HT3 receptors in the juvenile mouse cerebellum. J. Physiol. **591**, 1793–1807.
59) Ichikawa, R., Hashimoto, K., Miyazaki, T., Uchigashima, M., Yamasaki, M., Aiba, A. *et al.* (2016) Territories of heterologous inputs onto Purkinje cell dendrites are segregated by mGluR1-dependent parallel fiber synapse elimination. Proc. Natl. Acad. Sci. U.S.A. **113**, 2282–2287.
60) Kakizawa, S., Yamasaki, M., Watanabe, M. and Kano, M. (2000) Critical period for activity-dependent synapse elimination in developing cerebellum. J. Neurosci. **20**, 4954–4961.
61) Rabacchi, S., Bailly, Y., Delhaye-Bouchaud, N. and Maraini, J. (1992) Involvement of the N-methyl D-aspartate (NMDA) receptor in synapse elimination during cerebellar development. Science **256**, 1823–1825.
62) Stephan, A.H., Barres, B.A. and Stevens, B. (2012) The complement system: An unexpected role in synaptic pruning during development and disease. Annu. Rev. Neurosci. **35**, 369–389.
63) Neniskyte, U. and Gross, C.T. (2017) Errant gardeners: Glial-cell-dependent synaptic pruning and neurodevelopmental disorders. Nat. Rev. Neurosci. **18**, 658–670.
64) Nakayama, H., Abe, M., Morimoto, C., Iida, T., Okabe, S., Sakimura, K. *et al.* (2018) Microglia permit climbing fiber elimination by promoting GABAergic inhibition in the developing cerebellum. Nat. Commun. **9**, 2830.
65) Parkhurst, C.N., Yang, G., Ninan, I., Savas, J.N., Yates, J.R., Lafaille, J.J. *et al.* (2013) Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell **155**, 1596–1609.
66) Bosman, L.W.J., Hartmann, J., Barski, J.J., Lepier, A., Noll-Hussong, M., Reichardt, L.F. *et al.* (2007) Requirement of TrkB for synapse elimination in developing cerebellar Purkinje cells. Brain Cell Biol. **35**, 87–101.
67) Rico, B., Xu, B. and Reichardt, L.F. (2002) TrkB receptor signaling is required for establishment of GABAergic synapses in the cerebellum. Nat. Neurosci. **5**, 225–233.
68) Serantes, R., Arnalich, F., Figueroa, M., Salinas, M., Andrés-Mateos, E., Codoceo, R. *et al.* (2006) Interleukin-1β enhances $GABA_A$ receptor cell-surface expression by a phosphatidylinositol 3-kinase/Akt pathway. J. Biol. Chem. **281**, 14632–14643.
69) Stück, E.D., Christensen, R.N., Huie, J.R., Tovar, C.A., Miller, B.A., Nout, Y.S. *et al.* (2012) Tumor necrosis factor alpha mediates $GABA_A$ receptor trafficking to the plasma membrane of spinal cord neurons *in vivo*. Neural Plast. **2012**, 261345.
70) Miyamoto, A., Wake, H., Ishikawa, A.W., Eto, K., Shibata, K., Murakoshi, H. *et al.* (2016) Microglia contact induces synapse formation in developing somatosensory cortex. Nat. Commun. **7**, 12540.
71) Gallo, N.B., Berisha, A. and Van Aelst, L. (2022) Microglia regulate chandelier cell axo-axonic synaptogenesis. Proc. Natl. Acad. Sci. U.S.A. **119**, e2114476119.
72) Rothstein, J.D., Martin, L., Levey, A.I., Dykes-Hoberg, M., Jin, L., Wu, D. *et al.* (1994) Localization of neuronal and glial glutamate transporters. Neuron **13**, 713–725.
73) Chaudhry, F.A., Lehre, K.P., van Lookeren Cam-

pagne, M., Ottersen, O.P., Danbolt, N.C. and Storm-Mathisen, J. (1995) Glutamate transporters in glial plasma membranes: Highly differentiated localizations revealed by quantitative ultrastructural immunocytochemistry. Neuron **15**, 711–720.

74) Yamada, K., Fukaya, M., Shibata, T., Kurihara, H., Tanaka, K., Inoue, Y. *et al.* (2000) Dynamic transformation of Bergmann glial fibers proceeds in correlation with dendritic outgrowth and synapse formation of cerebellar Purkinje cells. J. Comp. Neurol. **418**, 106–120.

75) Miyazaki, T., Yamasaki, M., Hashimoto, K., Kohda, K., Yuzaki, M., Shimamoto, K. *et al.* (2017) Glutamate transporter GLAST controls synaptic wrapping by Bergmann glia and ensures proper wiring of Purkinje cells. Proc. Natl. Acad. Sci. U.S.A. **114**, 7438–7443.

76) Watase, K., Hashimoto, K., Kano, M., Yamada, K., Watanabe, M., Inoue, Y. *et al.* (1998) Motor discoordination and increased susceptibility to cerebellar injury in GLAST mutant mice. European J. Neurosci. **10**, 976–988.

77) Iino, M., Goto, K., Kakegawa, W., Okado, H., Sudo, M., Ishiuchi, S. *et al.* (2001) Glia-synapse interaction through Ca^{2+}-permeable AMPA receptors in Bergmann glia. Science **292**, 926–929.

78) Tsai, P.T., Hull, C., Chu, Y., Greene-Colozzi, E., Sadowski, A.R., Leech, J.M. *et al.* (2012) Autistic-like behaviour and cerebellar dysfunction in Purkinje cell *Tsc1* mutant mice. Nature **488**, 647–651.

79) Amaral, D.G., Schumann, C.M. and Nordahl, C.W. (2008) Neuroanatomy of autism. Trends Neurosci. **31**, 137–145.

80) Stoodley, C.J., D'Mello, A.M., Ellegood, J., Jakkamsetti, V., Liu, P., Nebel, M.B. *et al.* (2017) Altered cerebellar connectivity in autism and cerebellar-mediated rescue of autism-related behaviors in mice. Nat. Neurosci. **20**, 1744–1751.

81) Peter, S., Ten Brinke, M.M., Stedehouder, J., Reinelt, C.M., Wu, B., Zhou, H. *et al.* (2016) Dysfunctional cerebellar Purkinje cells contribute to autism-like behaviour in Shank2-deficient mice. Nat. Commun. **7**, 12627.

82) Cupolillo, D., Hoxha, E., Faralli, A., De Luca, A., Rossi, F., Tempia, F. *et al.* (2016) Autistic-like traits and cerebellar dysfunction in Purkinje cell PTEN knock-out mice. Neuropsychopharmacology **41**, 1457–1466.

83) Xu, F.X., Wang, X.T., Cai, X.Y., Liu, J.Y., Guo, J.W., Yang, F. *et al.* (2023) Purkinje-cell-specific MeCP2 deficiency leads to motor deficits and autistic-like behavior due to aberrations in PTP1B-TrkB-SK signaling. Cell Rep. **42**, 113559.

84) Takagishi, Y., Hashimoto, K., Kayahara, T., Watanabe, M., Otsuka, H., Mizoguchi, A. *et al.* (2007) Diminished climbing fiber innervation of Purkinje cells in the cerebellum of myosin Va mutant mice and rats. Dev. Neurobiol. **67**, 909–923.

85) Hansel, C., de Jeu, M., Belmeguenai, A., Houtman, S.H., Buitendijk, G.H.H.S., Andreev, D. *et al.* (2006) αCaMKII is essential for cerebellar LTD and motor learning. Neuron **51**, 835–843.

86) Ribar, T.J., Rodriguiz, R.M., Khiroug, L., Wetsel, W.C., Augustine, G.J. and Means, A.R. (2000) Cerebellar defects in Ca^{2+}/calmodulin kinase IV-deficient mice. J. Neurosci. **20**, RC107.

87) Iizuka, A., Matsuzaki, Y., Konno, A. and Hirai, H. (2016) Plasticity of the developmentally arrested staggerer cerebellum in response to exogenous RORα. Brain Struct Funct. **221**, 2879–2889.

88) Chen, X.R., Heck, N., Lohof, A.M., Rochefort, C., Morel, M.P., Wehrlé, R. *et al.* (2013) Mature Purkinje cells require the retinoic acid-related orphan receptor-α (rorα) to maintain climbing fiber mono-innervation and other adult characteristics. J. Neurosci. **33**, 9546–9562.

89) Kakizawa, S., Yamada, K., Iino, M., Watanabe, M. and Kano, M. (2003) Effects of insulin-like growth factor I on climbing fibre synapse elimination during cerebellar development. Euro. J. Neurosci. **17**, 545–554.

90) Cornejo, F., Cortés, B.I., Findlay, G.M. and Cancino, G.I. (2021) LAR receptor tyrosine phosphatase family in healthy and diseased brain. Front. Cell Dev. Biol. **9**, 659951.

91) Koekkoek, S.K.E., Yamaguchi, K., Milojkovic, B.A., Dortland, B.R., Ruigrok, T.J.H., Maex, R. *et al.* (2005) Deletion of FMR1 in Purkinje cells enhances parallel fiber LTD., enlarges spines, and attenuates cerebellar eyelid conditioning in fragile X syndrome. Neuron **47**, 339–352.

92) Gibson, J.M., Vazquez, A.H., Yamashiro, K., Jakkamsetti, V., Ren, C., Lei, K. *et al.* (2023) Cerebellar contribution to autism-relevant behaviors in fragile X syndrome models. Cell Rep. **42**, 113533.

93) Bedogni, F., Hodge, R.D., Nelson, B.R., Frederick, E.A., Shiba, N., Daza, R.A. *et al.* (2010) *Autism susceptibility candidate 2 (Auts2)* encodes a nuclear protein expressed in developing brain regions implicated in autism neuropathology. Gene Expr. Patterns **10**, 9.

94) Yamashiro, K., Hori, K., Lai, E.S.K., Aoki, R., Shimaoka, K., Arimura, N. *et al.* (2020) AUTS2 governs cerebellar development, Purkinje cell maturation, motor function and social communication. iScience **23**, 101820.

95) Tabuchi, K., Blundell, J., Etherton, M.R., Hammer, R.E., Liu, X., Powell, C.M. *et al.* (2007) A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science **318**, 71–76.

96) Rothwell, P.E., Fuccillo, M.V., Maxeiner, S., Hayton, S.J., Gokce, O., Lim, B.K. *et al.* (2014)

Autism-associated neuroligin-3 mutations commonly impair striatal circuits to boost repetitive behaviors. Cell **158**, 198–212.
97) Lai, E.S.K., Nakayama, H., Miyazaki, T., Nakazawa, T., Tabuchi, K., Hashimoto, K. *et al.* (2021) An autism-associated neuroligin-3 mutation affects developmental synapse elimination in the cerebellum. Front. Neural Circuits. **15**, 676891.
98) Piochon, C., Kloth, A.D., Grasselli, G., Titley, H.K., Nakayama, H., Hashimoto, K. *et al.* (2014) Cerebellar plasticity and motor learning deficits in a copy-number variation mouse model of autism. Nat. Commun. **5**, 5586.
99) Nakatani, J., Tamada, K., Hatanaka, F., Ise, S., Ohta, H., Inoue, K. *et al.* (2009) Abnormal behavior in a chromosome-engineered mouse model for human 15q11-13 duplication seen in autism. Cell **137**, 1235–1246.
100) Simmons, D.H., Busch, S.E., Titley, H.K., Grasselli, G., Shih, J., Du, X. *et al.* (2022) Sensory over-responsivity and aberrant plasticity in cerebellar cortex in a mouse model of syndromic autism. Biol. Psychiatry Global Open Sci. **2**, 450–459.
101) Kelly, E., Meng, F., Fujita, H., Morgado, F., Kazemi, Y., Rice, L.C. *et al.* (2020) Regulation of autism-relevant behaviors by cerebellar–prefrontal cortical circuits. Nat. Neurosci. **23**, 1102–1110.
102) Fujimoto, S., Leiwe, M.N., Aihara, S., Sakaguchi, R., Muroyama, Y., Kobayakawa, R. *et al.* (2023) Activity-dependent local protection and lateral inhibition control synaptic competition in developing mitral cells in mice. Dev. Cell. **58**, 1221–1236.e7.
103) Smith, I.W., Mikesh, M., Lee, Y.i. and Thompson, W.J. (2013) Terminal Schwann cells participate in the competition underlying neuromuscular synapse elimination. J. Neurosci. **33**, 17724–17736.
104) Song, J.W., Misgeld, T., Kang, H., Knecht, S., Lu, J., Cao, Y. *et al.* (2008) Lysosomal activity associated with developmental axon pruning. J. Neurosci. **28**, 8993–9001.
105) Bravin, M., Morando, L., Vercelli, A., Rossi, F. and Strata, P. (1999) Control of spine formation by electrical activity in the adult rat cerebellum. Proc. Natl. Acad. Sci. U.S.A. **96**, 1704–1709.
106) Kakizawa, S., Miyazaki, T., Yanagihara, D., Iino, M., Watanabe, M. and Kano, M. (2005) Maintenance of presynaptic function by AMPA receptor-mediated excitatory postsynaptic activity in adult brain. Proc. Natl. Acad. Sci. U.S.A. **102**, 19180–19185.
107) Zhou, H., Lin, Z., Voges, K., Ju, C., Gao, Z., Bosman, L.W.J. *et al.* (2014) Cerebellar modules operate at different frequencies. eLife **3**, e02536.
108) Kozareva, V., Martin, C., Osorno, T., Rudolph, S., Guo, C., Vanderburg, C. *et al.* (2021) A transcriptomic atlas of mouse cerebellar cortex comprehensively defines cell types. Nature. **598**, 214–219.

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Profile

Masanobu Kano was born at Shizuoka, Japan, in 1957. He received his medical degree from Tokyo Medical and Dental University in 1982. He completed his PhD at The University of Tokyo, Graduate School of Medicine in 1986, where he identified the glutamate receptor subtype involved in cerebellar long-term depression. He became a research associate at Jichi Medical School (Tochigi, Japan) in 1986. Then he joined the Max-Planck Institute for Biophysical Chemistry (Göttingen, Germany) in 1990, where he discovered long-term potentiation of inhibitory synapses. He returned to Jichi Medical School in 1992 and started examining synapse elimination in the developing cerebellum, a representative model of establishing functional neural circuits during postnatal development, which he continues to investigate to date. He moved to the RIKEN Institute (Wako, Japan) in 1995, and became a Professor of Physiology at Kanazawa University School of Medicine (Kanazawa, Japan) in 1998. In Kanazawa, his group discovered that endogenous cannabinoids function as retrograde messengers at synapses. He moved to Osaka University, Graduate School of Medicine in 2005 and then became a Professor of Neurophysiology at The University of Tokyo, Graduate School of Medicine in 2007. He retired from The University of Tokyo in 2023 and moved to Teikyo University. He has made significant contributions to elucidating the basic principles of neural circuit development, establishing new concepts of modification of synaptic transmission, and pioneering new fields of research on synaptic plasticity.