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Collapsin Response Mediator Proteins Regulate Neuronal Development and Plasticity by Switching Their Phosphorylation Status

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Abstract Collapsin response mediator protein (CRMP) was originally identified as a molecule involved in semaphorin3A signaling. CRMPs are now known to consist of five homologous cytosolic proteins, CRMP1–5. All of them are phosphorylated and highly expressed in the developing and adult nervous system. In vitro experiments have clearly demonstrated that CRMPs play important roles in neuronal development and maturation through the regulation of their phosphorylation. Several recent knockout mice studies have revealed in vivo roles of CRMPs in neuronal migration, neuronal network formation, synapse formation, synaptic plasticity, and neuronal diseases. Dynamic spatiotemporal regulation of phosphorylation status of CRMPs is involved in many aspects of neuronal development.

Keywords Collapsin response mediator protein · Semaphorin · Reelin · BDNF · Phosphorylation

Introduction

The organization of the nervous system relies on the intricate morphological complexity of neurons. The development of a functional neural circuitry involves several discrete steps. Newborn neurons must migrate to their proper locations, then extend axons and dendrites into proper target regions, and form synapses with appropriate partners. Neuronal migration, neurite extension, and synapse formation are thus essential processes by which neurons acquire their polarity and characteristic functional morphology. Impairment during these steps is thought to be involved in the onset of several neuronal diseases, underscoring the importance of understanding the detailed mechanisms of these processes [1, 2]. These processes particularly rely on specific and coordinated dynamics and organization of the actin and microtubule cytoskeletons. Microtubules are now considered to be essential regulators of neuronal morphogenesis. They not only provide the support for active transport of the membranes, organelles, and macromolecules required for development, but also actively participate in controlling shape changes through their dynamicity and restructuring. Numerous families of proteins regulate microtubules and their dynamics. Some molecules act directly on microtubules to stabilize them and promote their assembly, depolymerization, or fragmentation, whereas others indirectly regulate their dynamicity by controlling tubulin availability [3].

The collapsin response mediator protein (CRMP; the family is also known as Unc-33-like proteins (Ulip), dihydropyrimidinase-related proteins (DRP), TUC (TOAD/Ulip/DRP), and dihydropyrimidinase-like proteins) was identified as a molecule involved in the signaling of semaphorin3A (Sema3A), a repulsive axonal guidance molecule [4]. CRMPs are now known to consist of five homologous cytosolic proteins, CRMP1–5. All family members are highly expressed in the developing and adult nervous system [5–11]. CRMPs share homology with UNC-33 in the nematode *Caenorhabditis elegans*. Mutations in the *unc-33* gene lead to severe uncoordinated movements and abnormalities in the guidance and outgrowth of the axons of many neurons and a superabundance of microtubules in neuronal processes [12]. In cultured neurons, CRMPs have been shown to be involved in axon specification, elongation, and navigation, suggesting that CRMPs play multifunctional roles in neuronal development (Table 1).

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Table 1 The phenotypes observed in *crmp* knockout mice and functions of CRMPs demonstrated by loss-of-function and/or gain-of-function experiments in primary cultured neurons

CRMPs	Phenotypes in knockout mice	Functions in cultured neurons
CRMP1	Decrease in granule cell proliferation and apoptosis [20]	Sema3A-induced axonal guidance effect [18]
	Retarded neuronal migration [25]	Axon formation/extension induced by NT3 [72]
	Disorientation of apical dendrites [23]	Death of spinal cord neurons [102]
	Impaired dendritic spine density [23]	
	Impaired LTP and spatial memory [22]	
CRMP2	Severely abnormal dendritic patterning [27] (This phenotype is CRMP2S522A knock-in mutant mice in *crmp1*$^{-/-}$ background)	Axonal guidance effect by chemorepellent [4, 18, 40, 70, 103–106] (e.g., semaphorins, ephrinA5, myelin-derived inhibitors)
		Axon specification, elongation and branching [17, 71, 107–109]
		NT3-induced axon outgrowth [19]
		Negative effect on axon extension induced by NGF [72]
		Accelerates axon regeneration of nerve-injured motor neurons [110]
		Neurotransmitter release [64, 65]
		Resistance to glutamate toxicity through NR2B trafficking [111, 112]
CRMP3	Impaired dendritic spine maturation [21]	Neuronal cell death [113–115]
	Impaired LTP [21]	
	Decrease of prepulse inhibition [95]	
CRMP4	Increased proximal bifurcation phenotype in the CA1 hippocampus [26]	Axon elongation and branching [53, 69]
		Inhibition of axon regeneration by myelin-derived inhibitors [116, 117]
		Axonal degeneration and cell death [115, 118]
		Sema3A-induced extension and branching of dendrites [26]
CRMP5	Atrophy of Purkinje cells [24]	Filopodia and growth cone development [119]
	Impaired LTD [24]	Abrogation of the neurite outgrowth promotional activity of CRMP2 [77]

Structurally, CRMPs are homologous to the dihydropyrimidinase enzyme (DPYS) responsible for uracil and thymine catabolism. CRMPs themselves have no known enzymatic activity but interact with their binding partners to affect their function [13–15]. The C-terminal tail regions of CRMPs have no detectable sequence homology to other proteins; they have a high pI and a high content of serine residues and they are predicted to be unfolded in secondary structure predictions [15]. These characteristics closely resemble those of tau/MAP microtubule binding proteins [15]. Extensive post translational modifications such as phosphorylation, deamidation, oxidation, isoaspartyl conversion, and *O*-glycosylation of CRMPs have been detected [16], and these modifications may regulate the interactions with their binding partners [15]. The best characterized modification is phosphorylation, mainly at the C-terminal tail (Table 2). For example, CRMPs bind to tubulin heterodimer, and upon phosphorylation of CRMPs by Rho/ROCK kinase (Rho kinase), cyclin-dependent kinase-5 (Cdk5), and glycogen synthase kinase-3β (GSK-3β), the binding affinity of CRMPs to tubulin is lowered [17–19]. The regulation of CRMPs by these kinases is thought to play important roles in neuronal development and maturation.

Although the functions of CRMPs in axon development have been well described using in vitro experimental systems, recent studies on CRMP knockout mice are revealing their in vivo roles in neuronal development and functions [20–27] (Table 1). In this review, we discuss the in vivo roles of CRMPs especially focusing on the regulation of their phosphorylation in response to various external stimuli.

Neuronal Migration

A curious feature of brain development is that, although neurons are generated from precursor cells that line the

Table 2 The known phosphorylation sites of CRMPs for different kinases and their functional implications

CRMPs	Residue(s)	Kinase(s)	Molecular function	Physiological function
CRMP1	Tyr504 [43]	Fyn [43]	Unknown	Unknown
	Thr509, Ser 522 [53]	Cdk5 [53]	Lowers binding affinity to tubulin [120]	Sema3A-induced dendritic spine development [23]
	Thr509, Thr514, Ser518[a] [53]	GSK-3β [53]	Unknown	Unknown
	Tyr residue(s) [25, 40]	Fyn, Fes [25, 40]	Unknown	Reelin-induced neuronal migration [25]
CRMP2	Tyr32 [40]	Fyn, Fes [40]	Unknown	Sema3A-induced axonal guidance effect [40]
	Tyr479 [44]	Yes [44]	Unknown	CXCL12-induced T lymphocyte migration [44]
	Thr509, Thr514, Ser518 [18, 19, 53, 121]	GSK-3β [18, 19, 53, 121]	Lowers binding affinity to tubulin, KLC, and Slip1 [19, 122]	Regulation of cell polarity and semaphorin-induced axonal guidance effect [18, 19, 104]
	Ser522 [18, 53, 54]	Cdk5 [18, 53, 54]	Lowers binding affinity to tubulin and Numb, facilitation of phosphorylation by GSK-3β [18, 53, 71]	Regulation of cell polarity, Sema3A-induced axonal guidance effect, Numb-mediated endocytosis, and regulation of dendritic field organization [18, 27, 53, 54, 71]
	Thr555 [70, 103, 112]	Rho kinase [70], CaMKII [112]	Lowers binding affinity to tubulin and Numb [70]	ephrin-A5 and myelin-derived inhibitor-induced axonal guidance effect and axonal resistance to glutamate toxicity [70, 105, 112]
	Ser/Thr residue(s) [123]	PKA [123]	Unknown	Related to behavioral sensitization to cocaine [123]
CRMP3	Tyr reside(s) [40]	Fyn [40]	Unknown	Unknown
CRMP4	Thr509, Thr514, Ser518 [53, 121]	GSK-3β [53, 121]	Lowers binding affinity to RhoA[b] [117]	Inhibition of the axon regeneration by the myelin-derived inhibitors and regulation of mitotic chromosomal alignment [117, 124]
	Ser522 [53]	DYRK2, Cdk5[c] [53]	Facilitation of phosphorylation by GSK-3β [53]	Unknown
	Tyr residue(s) [40]	Fyn [40]	Unknown	Unknown
CRMP5	Tyr residue(s) [24, 40]	TrkB, Fyn [24, 40]	Unknown	Synaptic plasticity in cerebellar Purkinje cells [24]
	Ser/Thr residue(s) [18]	Cdk5[d] [18]	Unknown	Unknown

KLC kinesin light chain, *CaMKII* calmodulin kinase II, *PKA* protein kinase A, *DYRK2* dual tyrosine-regulated kinase 2

[a] Only CRMP1 of human origin is phosphorylated at Thr509, Thr514, and Ser518 by GSK-3β [53]

[b] This molecular function of CRMP4 was reported using the long form of CRMP4, a splicing variant form (Thr622, Thr627, Ser631 of long form of CRMP4) [117]

[c] Cdk5 is not required for phosphorylation of CRMP4 at Ser522 in vivo [53]

[d] Phosphorylation site of CRMP5 was estimated at Thr514 but have not been identified experimentally [18]

walls of the ventricular system deep within the brain, newborn neurons often migrate long distances to reach their final destinations. This is particularly true in the process of cortical development [28]. The neocortex consists of six layers of neurons that have distinct morphological and functional identities. The development of these neuronal layers involves the migration of neurons to their final laminar positions [29, 30]. Two distinct modes of migration have been identified so far: radial and tangential migration. Radial migration, the most extensively studied form of neuronal movement, is the principal mode of migration in the developing cerebral cortex [31].

Novel functions of CRMPs discovered by phenotypic analysis of CRMP-deficient mice include regulation of cell migration during cortical development [25]. In the mouse cortex at embryonic day 16.5 (E16.5), *crmp1* mRNA is strongly expressed in the whole region of the cortical plate where migrating neurons are located [23]. In the upper and deep

layers of the *crmp1*$^{-/-}$ cerebral cortex, retardation in radial migration is observed by BrdU birthdating analysis, suggesting that CRMP1 regulates neuronal migration [25]. This retardation is probably not due to the lack of Sema3A-CRMP1 signaling during the period of cortical layer formation because the overall expression pattern of mRNA of *neuropilin 1* (*nrp1*), the Sema3A binding receptor, is different from that of *crmp1* in the cerebral cortex at E16.5 [23, 32]. These findings suggest that CRMP1 mediates signals for some molecules other than Sema3A during cortical layer formation.

Reelin, a secreted protein, plays a crucial role in radial migration [33]. Upon binding to ApoER2/VLDLR receptors, Reelin induces tyrosine phosphorylation of the cytoplasmic adaptor disabled-1 (Dab1) by Src-type tyrosine kinases [34–36]. Although how Reelin regulates neuronal migration remains unclear, several intracellular molecules, including Lis1 (PAFAH1b1), doublecortin, Nudel, MAP1B, MAP2, and the small GTPase Rac1, have been implicated as mediators of migration [30]. In radial migration in the cerebral cortex, tyrosine phosphorylation of Dab1 by a Reelin signaling cascade has been shown to be essential [35–37]. Biochemical analyses reveals a decreased tyrosine phosphorylation of Dab1 in the cerebral cortex of *reeler* mice lacking functional Reelin protein [36, 38, 39]. One of the tyrosine kinases involved in the phosphorylation of Dab1 is Fyn, a Src-type tyrosine kinase [34]. Because CRMP1 is a substrate of Fyn [25, 40], CRMP1 might also acts as a mediator of Reelin signaling through its phosphorylation by tyrosine kinases. Consistent with this hypothesis, the tyrosine phosphorylation level of CRMP1 is decreased in the *reeler* mouse cerebral cortex [25]. Furthermore, deficiency in *crmp1* gene in a *dab1* heterozygous background leads to the disruption of hippocampal lamination, a typical *reeler*-like phenotype. Thus, CRMP1 and Dab1 may synergistically regulate neuronal migration through Reelin signaling via their tyrosine phosphorylation (Fig. 1a) [25]. Reelin signaling negatively regulates Dab1 protein levels during embryonic development [36, 41]. Interestingly, the expression of CRMP1 is higher in *reeler* cerebral cortex compared with *reeler* heterozygote mice, suggesting that Reelin regulates protein levels of CRMP1 as well as Dab1 in the cerebral cortex. This further indicates that CRMP1 possesses a biochemical property similar to Dab1. An increased immunoreactivity of CRMP1 is observed in some neurons of the *reeler* cortical plate at E16.5, while that of Dab1 is observed in almost all neurons (Fig. 1b) [25]. This result suggests that CRMP1 may play a role in Reelin signaling in a subset of the cortical neurons, whereas Dab1 has a more ubiquitous role. Consistently, a retarded migration phenotype is observed in a subset of neurons in both the upper and deep layers of the *crmp1*$^{-/-}$ cerebral cortex, whereas overall inverted cerebral cortex is observed in *dab1*$^{-/-}$ (*yotari*) mice [42]. These findings suggest that CRMP1 may regulate migration of a heterogeneous subpopulation, unrelated to a specific cortical layer. The physiological significance of the subset of CRMP1-positive neurons remains unknown.

Recently, some tyrosine phosphorylation sites of CRMPs were identified [40, 43, 44]. The phosphorylation at these tyrosine residues appears to be differentially regulated, but the distinct roles of the phosphorylation of these residues are unknown. For example, CRMP2 is phosphorylated at Tyr-32 by Fyn, which is involved in the regulation of axonal guidance rather than neuronal migration (see "Neuronal network formation and synapse formation" section) [40]. Among other phosphorylation sites, Tyr-479 of CRMP2 is phosphorylated by Yes, a Src-type tyrosine kinase. The phosphorylation of CRMP2 at Tyr-479 regulates chemokine CXCL12-induced T lymphocyte migration [44]. Interestingly, the increase in phospho-Tyr-479 of CRMP2 upon CXCL12 stimulation is associated with the decrease in phospho-Thr-509/514 [44]. This complementary phosphorylation on tyrosine versus serine/threonine residues is also observed in Dab1 [45]. Phosphorylation of CRMPs at Tyr-479 might be involved in Reelin-induced radial migration because Tyr-479 is conserved in all CRMP family proteins. However, the CRMP1 phosphorylation site of Tyr-504 by Fyn is not conserved among CRMP2–5, giving us a clue about the specific function(s) of CRMP1 [43].

Neuronal Network Formation and Synapse Formation

After neurons migrate to their final destinations, they send axon and dendrites to their correct targets to create the neuronal network. In vivo observations of developing axonal projections revealed that axons extend to the vicinity of their appropriate target regions in a highly stereotyped and directed manner, making very few errors in navigation [2, 46]. This feature of neuronal development is thus defined as axonal guidance. Axonal growth cones are guided by extracellular cues called axonal guidance molecules, which initially include netrins, semaphorins, ephrins, and slits. These molecules can either attract or repel axons, dendrites, or neuronal cell bodies, depending on the type of membrane receptors that they interact with. In addition to directional guidance of migrating neurons and projecting axons, the axonal guidance molecules also have important roles in outgrowth, branching, pruning, regeneration of axons, dendritic branching, and synaptic plasticity [46]. Among these molecules, the semaphorins constitute a major family of repellent axonal guidance cues in the central as well as the peripheral nervous system [47, 48]. Sema3A repulses axons through the co-receptor protein NRP1 and plexinAs [49, 50], and its signal transduction mechanism is the best characterized within the class 3 semaphorin subfamily. Ras and

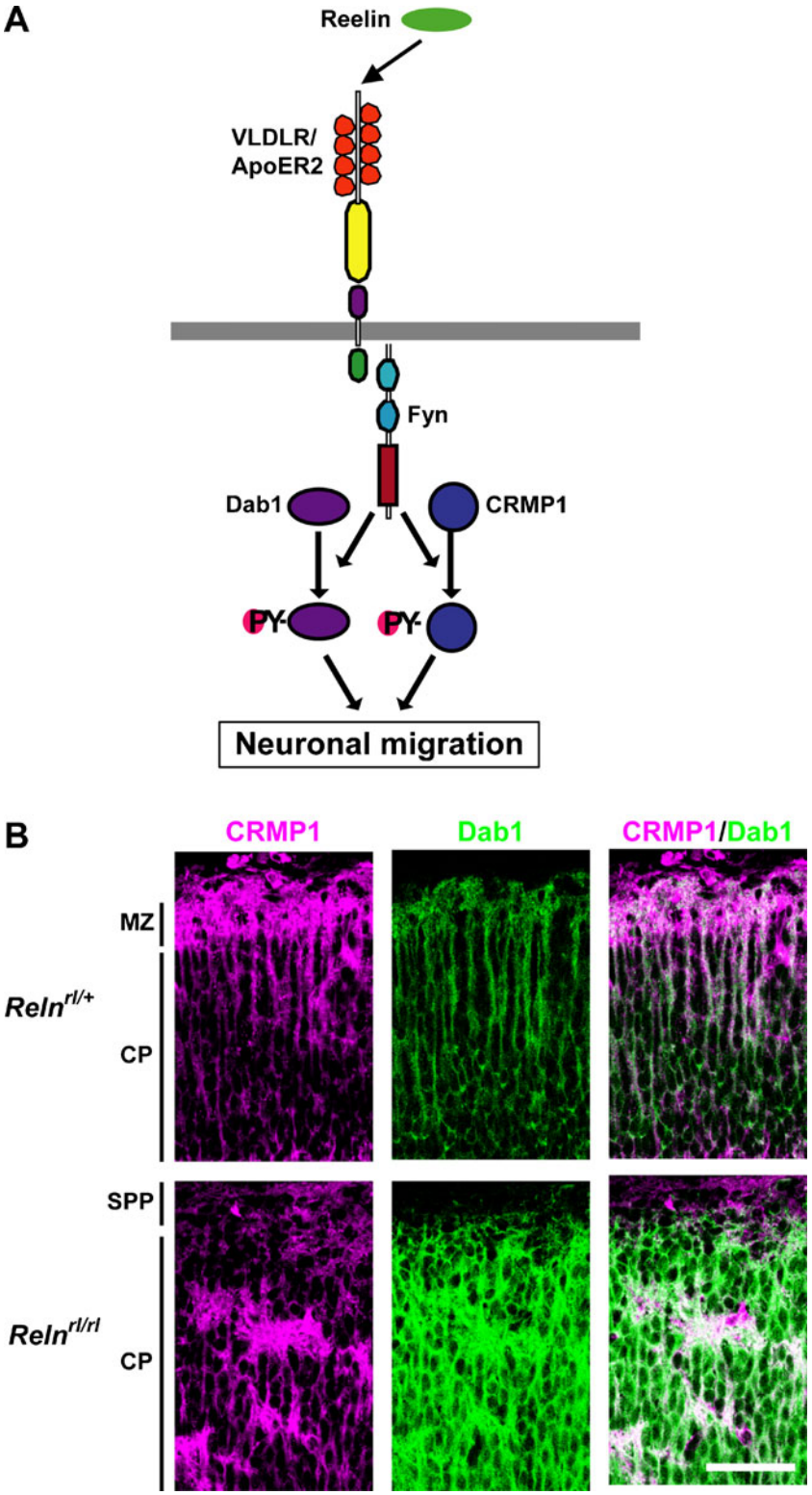

Fig. 1 CRMP1 mediates Reelin signaling in cortical neuronal migration. **a** In radial migration in the cerebral cortex, upon Reelin binding to ApoER2/VLDLR receptors, the cytoplasmic adaptor Dab1 and CRMP1 become phosphorylated on tyrosine residues by Fyn. The tyrosine-phosphorylated Dab1 and CRMP1 then synergistically regulate neuronal migration. **b** Immunohistochemistry with anti-CRMP1 (*magenta*) and anti-Dab1 (*green*) antibodies in E16.5 *reeler* heterozygote (*Reln*$^{rl/+}$) and *reeler* (*Reln*$^{rl/rl}$) coronal cortical sections. In *reeler* cortex, reduced Dab1 expression is observed at superplate, whereas its increased expression is observed at cortical plate [41]. Intense anti-CRMP1 immunoreactivity is observed at the marginal zone in reeler heterozygote cortex, whereas reduced immunoreactivity is observed at superplate in reeler cortex. Increased immunoreactivity of anti-CRMP1 at the cortical plate is also observed in *reeler* cortex. Cortical layers are shown on the *left*. *MZ* marginal zone, *CP* cortical plate, *SPP* superplate. Scale bar, 50 μm. Reproduced by permission from [25]

Rho family small GTPases, Fyn, Cdk5, and GSK-3β have shown to mediate growth cone collapse activity of Sema3A [51, 52].

Because CRMP2/chick CRMP-62 was identified as a signaling molecule of Sema3A, CRMPs are thought to play important roles in axonal guidance [4]. In vitro experiments shows that CRMP1 is phosphorylated at Thr-509 and Ser-522 by Cdk5 [53]. Cdk5 also phosphorylates CRMP2 at Ser-522 and CRMP2 is subsequently phosphorylated by GSK-3β at Thr-509, Thr-514, and Ser-518 [18, 54]. Missing

the phosphorylation steps by these kinases in CRMP1 and CRMP2 mutants or introducing short interfering RNA to CRMP1 and CRMP2 leads to the suppression of Sema3A-induced growth cone collapse in dorsal root ganglia (DRG) neurons [18, 23, 54]; therefore phosphorylation of both CRMP1 and CRMP2 are essential for Sema3A-regulated axonal guidance. Several axonal guidance defects have been reported in *sema3A*$^{-/-}$ mice [55–57]. However, similar axonal guidance defects were not observed in any single *crmp gene*-deficient animals (unpublished observation). In contrast, *crmp1*$^{-/-}$ mice exhibit dendritic guidance defects such as disorientation of apical dendrites in layer V cerebral cortex, similar to the phenotype seen in *sema3A*$^{-/-}$ mice [23, 52]. To address the role of phosphorylation of CRMP2, we recently generated CRMP2S522A knock-in mutant mice, in which CRMP2 cannot be phosphorylated by Cdk5. We have not detected any severe phenotype in CRMP2S522A knock-in mutant mice so far. However, in *crmp1*$^{-/-}$ mutant background, CRMP2S522A knock-in mice shows a severely abnormal dendritic patterning, which we defined as the "curling phenotype" [27]. Although the molecular mechanism(s) underlying the curling phenotype are unknown, this phenotype demonstrates the importance of phosphorylation of CRMP2 at S522 for proper dendritic guidance. Other types of CRMPs may also play a role in dendritic patterning. We recently generated *crmp4*$^{-/-}$ mutant mice. The *crmp4*$^{-/-}$ apical dendrites of the CA1 pyramidal neurons shows an increased proximal bifurcation phenotype [26]. This is also observed in *sema3A*$^{-/-}$ hippocampus [58], and Sema3A-induced extension and branching of the dendrites of hippocampal neurons is compromised in the *crmp4*$^{-/-}$ hippocampal neurons. These results suggest that CRMP4 negatively regulates apical dendrite bifurcation of CA1 pyramidal neurons in the mouse hippocampus and that this effect is at least partly dependent on Sema3A signaling [26].

Because Sema3A plays a role in dendritic spine maturation [32], it is possible that CRMP1 plays a role in dendritic development. The formation of synapses is a highly complex process that needs to be orchestrated with great temporal and spatial precision. Synapse formation is accompanied by accumulation of synaptic organelles and proteins at the sites where axons and dendrites contact each other. The initial contacts between filopodia of axonal or dendritic growth cones are subsequently transformed into functional synapses through cytoskeletal reorganization [59]. In cultured cortical neurons, Sema3A treatment increases the density of one of the postsynaptic proteins, postsynaptic density-95 (PSD-95) clusters, at actin-rich protrusions on dendritic processes [32]. In contrast, in *crmp1*$^{-/-}$ cortical neurons, Sema3A does not alter the density of clusters of PSD-95 [23]. Attenuation of the Sema3A effect is also seen in *cdk5*$^{-/-}$ neurons. Furthermore, the introduction of wild-type CRMP1 but not CRMP1 mutant (CRMP1-T509A/S522A), which cannot be phosphorylated by Cdk5, into *crmp1*$^{-/-}$ neurons rescues the defect in Sema3A responsiveness. These findings clearly demonstrate that phosphorylation of CRMP1 by Cdk5 plays a crucial role in mediating the effect of Sema3A on the clustering of PSD-95 [23]. Interestingly, in the absence of Sema3A, PSD-95 clusters are enlarged in *crmp1*$^{-/-}$ neurons [23]. This phenotype is similar to that obtained from *cdk5*$^{-/-}$ neurons [23, 60]. The initial explanation for the enlarged size of PSD-95 clusters in *cdk5*$^{-/-}$ neurons is that direct phosphorylation of PSD-95 by Cdk5 is required for the regulation of PSD-95 clustering [60]. However, because introduction of wild-type CRMP1 but not CRMP1-T509A/S522A rescues this phenotype, PSD-95 clustering is also regulated by phosphorylation of CRMP1 by Cdk5 [23]. On the other hand, the *crmp1*$^{-/-}$ layer V cortical neurons of adult brains examined by the Golgi-Cox impregnation method shows lower density of synaptic bouton-like structures on their dendritic processes. There is a genetic interaction between *crmp1* and *sema3A* genes influencing this phenotype [23]. This observation provides an apparent contrast to the finding that in *crmp1*$^{-/-}$ cultured cortical neurons the cluster size of PSD-95 is enlarged. However, the enlarged clusters of PSD-95 are not in close apposition with the presynaptic protein synapsin I (Fig. 2a). It is possible that the enlarged clusters seen in *crmp1*$^{-/-}$ neurons may reflect aberrant spine formation rather than normal synapse formation. During the cortical development, when pre- and/or postsynaptic structures are unstable, they might be eliminated in adult brain neurons of *crmp1*$^{-/-}$ mutant animals (Fig. 2b).

Other CRMP family proteins are also involved in spine morphogenesis and functions. A slight decrease in mushroom spines and a slight increase in finger spines are observed in *crmp3*$^{-/-}$ CA1 hippocampus, suggesting that CRMP3 is involved in dendritic spine maturation [21]. Unlike other CRMPs, CRMP3 does not have the consensus sequence of phosphorylation by Cdk5 near 522 [18], suggesting that CRMPs may regulate dendritic spine morphology via pathways other than Cdk5. Fyn is also involved in Sema3A-induced dendritic spine development [32], probably through phosphorylating CRMPs at several tyrosine residues [40, 43]. Among these phosphorylation sites, Tyr-32 of CRMP2 is phosphorylated by Fyn and Fes/Fps [40, 61], and this site is involved in Sema3A-induced growth cone collapse [40]. Whether this phosphorylation site is also essential for synapse formation and maturation remains to be determined.

Maintenance of Neuronal Circuit and Synaptic Plasticity

The stability of a neuronal network is maintained by a dynamic equilibrium between excitatory and inhibitory processes where synaptic mechanisms play an essential role

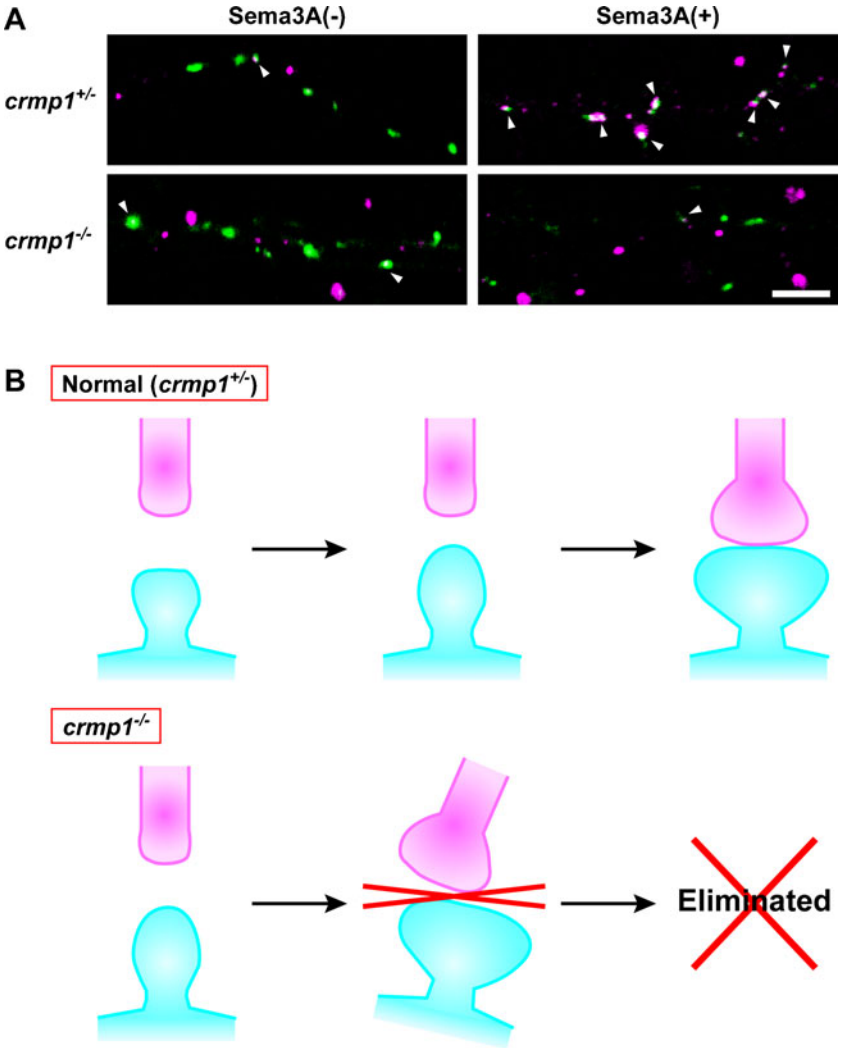

Fig. 2 CRMP1 is required for the stability of dendritic spines. **a** The colocalization of synapsin I and PSD-95 clusters corresponds to functional synapses [125]. Sema3A not only induces enlargement of PSD-95 clustering but also increases the double-positive clusters of synapsin I and PSD-95, suggesting that Sema3A induces spine maturation [32]. In the absence of Sema3A treatment, there are no differences in the double-positive clusters in *crmp1*$^{+/-}$ and *crmp1*$^{-/-}$ neurons. Sema3A enhances the double-positive clusters in *crmp1*$^{+/-}$ neurons. Sema3A does not alter the double-positive clusters in *crmp1*$^{-/-}$ neurons. These results show that Sema3A induces formation of mature spines in *crmp1*$^{+/-}$, but not in *crmp1*$^{-/-}$ neurons. These results also indicate that the enlarged clusters seen in *crmp1*$^{-/-}$ neurons may reflect aberrant rather than mature spine formation. *Arrowheads* indicate the double-positive clusters. Scale bar, 5 μm. Reproduced by permission from [23]. **b** In normal (*crmp1*$^{+/-}$) mice, local and stepwise assembly of pre- and postsynaptic components occur during the formation of mature spines [126–128]. The enlarged clusters in pre- and postsynaptic neurons of *crmp1*$^{-/-}$ mice are not in close apposition to each other. These enlarged clusters may be eliminated at the later stages of synapse development. Consistently, the adult *crmp1*$^{-/-}$ layer V cortical neurons show lower density of synaptic bouton-like structures [23]

[62]. The synapse formation is continuously changing in order to accommodate changes in activity and/or usage. This is defined as synaptic plasticity [63]. Being involved in synapse formation, CRMPs may also function in neural plasticity. In both *crmp1*$^{-/-}$ and *crmp3*$^{-/-}$ CA1 hippocampus, the long-term potentiation (LTP) is impaired [21, 22]. Additionally, in *crmp1*$^{-/-}$ mice, the spatial learning and memory are also impaired [22]. Because the paired pulse facilitation is normal in these mutant mice, the impairment of LTP is thought to be due to the impaired dendritic spine morphology [21, 22].

In contrast, some evidence suggests a link between CRMPs and presynaptic activity. CRMP2 directly binds to N-type voltage-gated Ca^{2+} channel (CaV2.2) at presynaptic sites [64–66]. In cultured hippocampal and DRG neurons, overexpression of CRMP2 increases surface expression levels of CaV2.2 at presynaptic sites and enhances both Ca^{2+} influx and neurotransmitter release. In contrast, the knockdown of CRMP2 decreases both Ca^{2+} influx and neurotransmitter release [64–66]. Furthermore, a peptide that uncouples CRMP2 and CaV2.2 suppresses inflammatory and neuropathic pain in vivo [67]. These findings

clearly demonstrate that CRMPs modulate presynaptic activity. Neurotransmitter release is tightly regulated through the synaptic vesicle cycle, which comprises Ca^{2+}-triggered exocytosis of synaptic vesicles, followed by retrieval and recycling of synaptic vesicles via clathrin-mediated endocytosis [68]. Interestingly, CRMP4 is partially colocalized with synaptic vesicle protein 2 and is thought to regulate exocytosis at the growth cone [69]. CRMP4 also interacts with intersectin, one of the molecules involved in endocytosis [69]. In addition, CRMP2 associates with Numb, and phosphorylation of CRMP2 regulates Numb-mediated endocytosis [70, 71]. These interactions between CRMPs and their interacting protein(s) might further explain the role(s) of CRMPs in presynaptic function.

Emerging evidence suggests that CRMPs have multiple roles in mediating extracellular signals other than Sema3A for maintenance of the neuronal circuit and synaptic plasticity [19, 72]. In the cerebellar Purkinje cells, CRMP5 plays a role in synaptic plasticity [24]. CRMP5 is expressed in cerebellar Purkinje cells at postnatal day 21 (P21) and P28 but not P14. In addition, the size of soma and diameter of primary dendrite of the *crmp5*$^{-/-}$ cerebellar Purkinje cells are decreased at P21 and P28 but not P14 [24]. Furthermore, the induction of long-term depression (LTD) of excitatory synaptic transmission between parallel fibers and Purkinje cells is deficient in cerebellar slices of *crmp5*$^{-/-}$ mice. Hence, CRMP5 is involved in the development, maintenance, and synaptic plasticity of Purkinje cells [24].

Brain-derived neurotrophic factor (BDNF) is highly expressed in the cerebellum, and both granule and Purkinje cells express the BDNF receptor tyrosine kinase receptor type 2 (TrkB). Exogenous BDNF acts directly on granule cells to promote survival and axonal elongation, and exogenous neurotrophins promotes dendritic arborization of cultured Purkinje cells [73]. Although the effect of BDNF in *crmp5*$^{-/-}$ Purkinje cell has not been investigated, BDNF-induced dendrite development is attenuated in cultured *crmp5*$^{-/-}$ hippocampal neurons and dendritic morphology in *crmp5*$^{-/-}$ hippocampus is impaired [24]. In addition, CRMP5 is tyrosine phosphorylated by TrkB. These findings suggest that the temporal regulation of CRMP5 expression plays essential roles in the regulation of dendritic morphology of Purkinje cells through BDNF–TrkB signaling [24]. In the postnatal stage, however, BDNF–TrkB signaling regulates dendritic spine density but not dendritic outgrowth [74]. This result raised the possibility that the loss of CRMP5 may impair BDNF-regulated dendritic spine density and thereby cause impairment of LTD. Because the formation of LTD is required for the activity of AMPA type and metabotropic glutamate receptors [75], the suppression of the activity of these receptors can lead to a decrease in the diameter of Purkinje cell dendrite [76]. The atrophied phenotype seen in the *crmp5*$^{-/-}$ Purkinje cell dendrites may therefore be explained by the secondary effects of impairment of LTD in the *crmp5*$^{-/-}$ cerebellum (Fig. 3). In addition to the possible involvement of CRMP5 in BDNF–TrkB signaling, CRMP5 may also be a possible modifier of tubulin [77], which may explain the abnormalities in dendritic development and impairment of LTD (Fig. 3).

Neuronal Diseases

In the central nervous system, neuronal degeneration in a specific area plays a key role in the onset of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and amyotrophic lateral sclerosis [78, 79]. Emerging evidence increasingly suggests that mental disorders such as schizophrenia represent a subtle disorder of brain development and plasticity [80]. CRMPs are thought to be involved in the onset and/or progression of these neuronal diseases. Both Cdk5 and GSK-3β are responsible for pathological tau phosphorylation in neurofibrillary tangles (NFTs), one marker of AD. Additionally, the antiphosphorylated CRMP2 antibody called 3F4 was raised against crude NFTs [81]. Because 3F4 antibody recognizes Cdk5 and GSK-3β phosphorylated CRMP2, phosphorylation of CRMP2 is considered to be one of the pathological features of AD [18, 82]. The phosphorylation of CRMP2 is increased

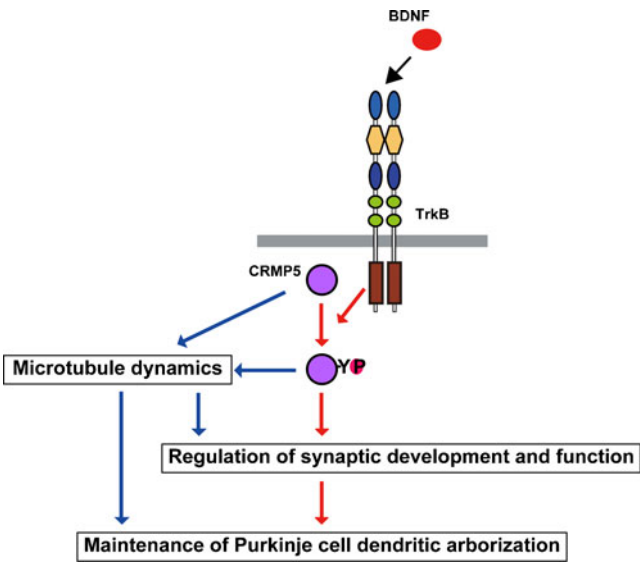

Fig. 3 Hypothetical pathway by which CRMP5 regulates the Purkinje cell morphology. Upon BDNF binding to TrkB, CRMP5 becomes phosphorylated on tyrosine residue(s) by TrkB. The tyrosine-phosphorylated CRMP5 may then regulate synaptic development and function, contributing to maintenance of Purkinje cell dendritic arborization (*red arrows*). Alternatively, CRMP5, a possible modifier of tubulin [77], may directly regulate synaptic plasticity and/or Purkinje cell morphology through microtubule dynamics via its phosphorylation mechanism (*blue arrows*)

in AD brain as well as in a transgenic mouse model of AD [83]. Importantly, increased phosphorylation of CRMP2 occurs prior to the onset of pathology in the mouse model [83]. As described above, CRMPs are involved in synaptic function, and memory impairment occurs prior to the manifestation of the pathology of AD [84]. Phosphorylation of CRMP2 therefore might be involved in the onset of preclinical stages of AD. If this is the case, the phosphorylation of CRMP2 can be a potential target for diagnosis and therapeutic treatment before the onset of AD. Increased phosphorylation of CRMP2 might be due to increased Cdk5 activity, which is induced by the cleavage of p35, an activator of Cdk5, to p25 [85–87]. It remains unclear whether Sema3A is involved in the pathology of AD, but the number of Sema3A-immunopositive neurons is elevated in the CA1 hippocampal region in AD [88]. Because Sema3A activates Cdk5 [52], it is possible that phosphorylation of CRMP2 is induced by enhanced signaling of Sema3A in AD brain. Further studies are required to understand how phosphorylated CRMP2 is increased in AD brain.

CRMP2 gene polymorphism and abnormal CRMP1 or CRMP2 protein levels have been found in schizophrenia [89–92], whereas CRMP2 gene polymorphism may not affect the risk of schizophrenia [93]. However, in *crmp3*$^{-/-}$ mice, prepulse inhibition of the acoustic startle response, a behavioral feature of schizophrenia [94], is drastically decreased [95]. Further behavioral studies using *crmp* mutants may reveal the exact roles of CRMPs in schizophrenia. Indeed, we recently found that *crmp1*$^{-/-}$ mice also show schizophrenia-like phenotype (unpublished observation). Taken together, CRMPs may be potential clinical targets of these neuronal diseases. Several small molecules that could manipulate CRMP function have been found [16].

Conclusions and Future Research

Through phenotypic analysis of *crmp* gene-deficient animals, the in vivo roles of CRMPs are beginning to be elucidated. Studies of *crmp* mutants enabled the discovery of novel roles of CRMPs, including neuronal migration, synapse formation, and synaptic plasticity. No axonal defects have been reported in *crmp* mutants so far, although the roles of CRMPs in axon development have been described in cultured neurons. In the early developmental stage when axon specification and elongation occur, all CRMPs are highly expressed in the nervous system [6, 8, 10]. Therefore, the single knockout of CRMPs may not be sufficient to cause axonal defects, which is probably due to the compensation by other CRMPs. Although CRMP proteins may form heterooligomeric complexes in vivo [96], the phenotype seen in *crmp* mutants does not completely overlap. This might reflect the different expression patterns of CRMPs [97]. In addition, our preliminary data indicate that subcellular localization of CRMPs in cultured neurons does not completely overlap. At the axonal growth cone, for example, CRMP2 was distributed in the central domain, whereas CRMP1 was distributed in both the central domain and filopodia. Interestingly, local inactivation of CRMP1 or CRMP2 at the axonal growth cone exhibited different phenotypes (unpublished observation). The complexity of different spatiotemporal expression profiles at the tissue level as well as the subcellular level may explain how CRMPs are widely involved in neuronal development and functions. Further studies are required using single mutant mice as well as multiple *crmp*-deleted mutants.

CRMPs are one of the major phosphoproteins in the developing and adult nervous system. A wide variety of CRMP functions may be regulated by multiple phosphorylation sites of these proteins. The phosphorylation of CRMPs might change their binding partner(s) and functions, depending on the residue(s) phosphorylated, which is analogous to Dab1. For example, Dab1 is phosphorylated by Fyn and Cdk5 [45]. The phosphorylation of Dab1 by Fyn regulates the interaction with Lis1, PI3 kinase, and the Crk family [98–100], whereas phosphorylation of Dab1 by Cdk5 regulates the interaction with CIN85 [101]. In conclusion, phosphorylation of CRMPs is implicated in neuronal development, maintenance, functions, and diseases. Further studies are required to elucidate the exact in vivo functions of CRMPs in the nervous system.

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