CRMP2: Functional Roles in Neural Development and Therapeutic Potential in Neurological Diseases

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Abstract

Cytoskeletal restructuring is essential for nearly all cellular processes in the developing brain. After cell fate determination, newborn cortical neurons must migrate to their final positions while establishing proper axon-dendrite polarity. Significant progress has recently been made towards understanding the cellular and molecular mechanisms underlying neuronal polarization in vivo. Collapsin response mediator protein 2 (CRMP2) has long been identified as a microtubule-binding protein that regulates neuronal polarity in vitro. Recent studies provide new insights into the roles of CRMP2 in neuronal migration and subsequent neuronal differentiation. Both the expression and activity of CRMP2 are tightly regulated during cortex development. CRMP2 is suggested to be important in the multipolar-bipolar transition in radial migration. The increasing number of known interaction partners indicates that CRMP2 has functions beyond cytoskeletal regulation, including axonal transport, vesicle trafficking, and neurotransmitter release. This review discusses the current knowledge about CRMP2 in the context of neuronal development and highlights a recent emerging theme regarding its potential therapeutic applications.

Keywords

neuronal polarization, microtubule, neuronal migration, cortex development, neurological disease

The mammalian cortex is organized into a six-layered laminated structure with functionally connected columns comprising both excitatory and inhibitory neurons. The proper development of such a complex architecture requires several precisely controlled processes including neuronal generation and migration, establishment of axon-dendrite polarity, formation of neural circuits, and maintenance of wired circuits. Disruptions to any of these steps can potentially lead to impaired neural functions and are associated with various neurological disorders in humans. Genetic studies have revealed that many neurodevelopmental diseases are caused by mutations in genes encoding cytoskeletal-related proteins, highlighting the importance of cytoskeletal restructuring and polarization during development of the nervous system (Gleeson 2000; Gleeson and Walsh 2000; Jaglin and Chelly 2009).

Cytoskeletal reorganization plays critical roles in nearly all cellular processes during neurodevelopment. For example, rapid cytoskeletal rearrangement is required for immature neurons to undergo changes in morphology, polarity, adhesion, and directional movement (Bielas and Gleeson 2004). Therefore, many signaling pathways implicated in neurodevelopment actively regulate cytoskeletal components to direct neuronal morphogenesis (Barnes and Polleux 2009). Continuing research efforts toward understanding the mechanisms of neuronal morphogenesis have led to major breakthroughs in the identification of cytoskeletal regulators. The collapsin response mediator protein (CRMP) family comprises five homologous cytosolic tubulin-binding proteins (CRMP1–5) that are multifunctional regulators in neurodevelopment. CRMPs share about 50% homology with the UNC-33 protein in the nematode *Caenorhabditis elegans*. Mutations in the *unc-33* gene cause severe uncoordinated movements and abnormal axonal guidance (Li and others 1992). CRMP proteins, also known as Unc-33-like phosphoprotein (Ulip) and dihydropyrimidinase-related protein (DRP) , are highly expressed in the mammalian nervous system (Charrier and others 2003). Recent knockout mouse studies revealed that CRMPs play

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important roles in various stages of neurodevelopment. CRMP1 and CRMP3 are involved in dendritic patterning and spine development in the cerebral cortex and hippocampus, respectively (Quach and others 2008; Yamashita and others 2007). Meanwhile, CRMP4 mediates dendritic bifurcation in the CA1 hippocampus, and CRMP5 is required for dendritic development and plasticity in the cerebellum (Niisato and others 2012; Yamashita and others 2011). Although CRMP2 was the first identified CRMP family member, there are no reports of studies using CRMP2 knockout mice. CRMP2 has long been identified as a critical regulator of axonal guidance and neuronal polarity in vitro. Its roles in cortex development have recently begun to be unraveled.

This review provides an overview of the functional roles of CRMP2 in neural development with a particular focus on recent discoveries in the establishment of axon-dendrite polarity during cortical migration. In addition, recent findings about the potential therapeutic applications of CRMP2 are reviewed. For additional details about the differential expression of CRMPs, functions of other CRMPs, and other possible roles of CRMPs in neurological diseases, the reader is referred to several outstanding reviews on these topics (Charrier and others 2003; Hensley and others 2011; Khanna and others 2012; Yamashita and Goshima 2012).

Role of CRMP2 in Neuronal Polarization

Much of the initial knowledge about neuronal polarization was obtained using dissociated neurons. Early pioneering work established how cultured dissociated hippocampal neurons develop polarity and allow a neuron to form structurally and functionally distinct subcellular compartments, that is, axons and dendrites (Barnes and Polleux 2009). The polarization of hippocampal pyramidal neurons is well characterized and has been classified into various stages of morphological changes. The key step in the establishment of neuronal polarity is the specification of a single neurite to become the axon and the differentiation of the remaining neurites into dendrites. This process is divided into the following four stages: 1) the formation of actin-based lamellipodia from hippocampal neurons, 2) the extension of multiple short neurites with no fixed polarity, 3) the breaking of symmetry and rapid extension of one of the neurites that will become the axon, and 4) the growth and differentiation of the remaining neurites into dendrites (Arimura and others 2004; Barnes and Polleux 2009). It is well established that the activation/inactivation of signaling components in a localized region of a single neurite is important for the establishment of polarity. Proper neuronal polarization requires the orchestrated restrictive localization of multiple signaling pathways. Studies reported by Arimura and Kaibuchi (2007) demonstrate that the spatial and temporal regulation of CRMP2 acts as a critical cytoskeletal regulator for establishing and maintaining neuronal polarity. CRMP2 is enriched in elongating axons, and its overexpression induces multiple axon-like neurites that express Tau1, an axonal marker and microtubule-associated protein (Yoshimura and others 2005). Indeed, CRMP2 binds the tubulin heterodimer and promotes microtubule assembly at the plus end. CRMP2 is also thought to stabilize microtubules during neuronal polarization, although the detailed mechanism remains unclear (Lin and others 2011). Subsequent studies revealed that CRMP2 has more diverse functions besides tubulin binding. CRMP2 also interacts with the Rac1-associated protein 1 (Sra-1)/WASP family verprolin-homologous protein 1 (WAVE1) complex to regulate actin filament stability at the growth cone (Kawano and others 2005). CRMP2 associates with Numb proteins in the central regions of axonal growth cones and regulates the clathrin-mediated endocytosis of the neuronal cell adhesion molecule, L1 (Nishimura and others 2003). Taken together, these findings indicate that CRMP2 serves as a multifunctional protein that promotes neuronal polarization by regulating microtubule assembly, actin filament reorganization, and the endocytosis of adhesion molecules. In addition to its major role as a cytoskeletal regulator, an increasing number of CRMP2 binding partners have been identified, suggesting that CRMP2 has other molecular functions such as vesicle trafficking and axonal transport (Khanna and others 2012). CRMP2 can be considered a scaffold adaptor protein that links the motor protein kinesin-1 to speci□c cargos such as tubulin heterodimers, the Sra-1/WAVE1 complex, and the receptor tyrosine kinase TrkB, thereby promoting anterograde axonal transport (Hensley and others 2011).

CRMP2 activity is precisely regulated by its phosphorylation status. Glycogen synthase kinase 3β (GSK3β) phosphorylates CRMP2 at the Thr509 and Thr514 sites after priming phosphorylation at the Ser522 site by cyclin-dependent kinase 5 (Cdk5) (Yoshimura and others 2005). This priming phosphorylation is required for subsequent GSK3β-mediated phosphorylation. CRMP2 phosphorylation by GSK3β reduces CRMP2's ability to interact with tubulin, thus inhibiting neurite extension (Yoshimura and others 2005). Nonphosphorylated CRMP2 is active and promotes tubulin assembly in the growing growth cone; this active form of CRMP2 is sufficient to determine axon specification. The overexpression of active CRMP2 disrupts polarity and induces multiple axon-like neurites (Yoshimura and others 2005). In cultured hippocampal neurons, phosphorylated CRMP2 is enriched in the distal part of the growing axons but not at the axonal growth cones (Yoshimura and others 2005). One intriguing question is whether inactive CRMP2 specifically regulates microtubule stabilization

and/or axonal growth. Further studies examining whether the phosphorylated CRMP2 protein plays any functional roles are warranted.

Several extracellular cues that regulate CRMP2 activity in different processes have been identified. These extracellular cues include ephrins, semaphorins, and neurotrophins. Activation of the neurotrophin receptor tyrosine kinase Trk increases CRMP2 activity by reducing GSK3β-dependent phosphorylation during axon specification (Yoshimura and others 2005). Brain-derived neurotrophic factor (BDNF) and neurotrophin-3, which are the endogenous ligands of the TrkB receptor, induce the local activation of phosphatidylinositol 3-kinase at the tip of one of the neurites; this leads to GSK3β phosphorylation at Ser9, thereby reducing CRMP2 phosphorylation at Thr514 and promoting axon outgrowth in vitro (Arimura and Kaibuchi 2007). We recently identified that the RhoGAP α2-chimaerin is required for this TrkB-mediated activation of CRMP2. In α2-chimaerin knockdown neurons, neurotrophin-induced Trk receptor autophosphorylation and CRMP2 phosphorylation at Thr514 are attenuated, highlighting the importance of α2-chimaerin in neurotrophin-mediated CRMP2 signaling (Ip and others 2012). Activated TrkB at the cell surface promotes axonal outgrowth by enhancing F-actin polymerization in distal neurite shafts and growth cones (Arimura and others 2009). CRMP2 also regulates the surface level of TrkB on the cell membrane by promoting anterograde axonal transport. CRMP2 forms a complex with the TrkB receptor, Slp1, and Rab27. In this case, CRMP2 functions as an adaptor protein and links kinesin-1 to the TrkB cargo. The complex transports the functional TrkB receptor to the distal end of the axon in a kinesin-1–dependent manner. This CRMP2-mediated axonal transport mechanism is important for BDNF-induced extracellular-signal-regulated kinase (ERK) phosphorylation and subsequent axonal outgrowth (Arimura and others 2009). Corroborating this notion, a recent study involving *C. elegans* proposes a causative relationship among CRMP (UNC-33), the motor kinesin Kif1A (UNC-104), and the diffusion barrier ankyrin (UNC-44); specifically, CRMP together with ankyrin is proposed to be required for the polarized sorting of kinesin to the axonal domain and thus for the establishment of axon-dendrite sorting (Maniar and others 2012).

CRMP2 is dynamically regulated by semaphorin 3A and ephrin A5 activation in the context of growth cone guidance. The growth cone acts as a sensor for guidance molecules and changes their morphology in response to attractive and repulsive guidance cues (Kalil and Dent 2005). The function of CRMP2 in growth cone guidance was first reported by Goshima and colleagues (1995) as a critical signal transducer downstream of the repulsive guidance cue semaphorin 3A. Semaphorin 3A signaling via its receptors neuropilin-1 and Plexin A (Plex1–3) activates GSK3β and phosphorylates CRMP2 at the Thr509 and Thr514 sites in semaphorin 3A–mediated growth cone collapse (Brown and others 2004). Moreover, CRMP2 activity is regulated by the RhoA-Rho kinase pathway because CRMP2 is a substrate of Rho kinases. Lysophosphatidic acid and ephrin A5 are known to activate RhoA GTPase, which in turn activates Rho kinases and phosphorylates CRMP2 at Thr555 (Arimura and others 2005). CRMP2 phosphorylation at Thr555 reduces the binding activity of CRMP2 to tubulin dimers and inhibits Numb-mediated endocytosis, causing increased contractibility and collapse of the growth cone. This pathway is involved in lysophosphatidic acid/ephrin A5–induced growth cone collapse independent of the GSK3β pathway (Arimura and others 2005; Brown and others 2004).

Roles of CRMP2 in Cortex Development In Vivo and Its Emerging Roles

Recent advances in techniques such as in utero gene transfer have enabled the manipulation of gene expression speci□cally in neural progenitor cells in the cerebral cortex (Saito and Nakatsuji 2001; Tabata and Nakajima 2001). This provides a new paradigm for examining neural development in vivo as well as probing the molecular mechanisms underlying the neurogenesis and integration of newborn neurons in the embryonic and early postnatal brain (Barnes and Polleux 2009). The mammalian cerebral cortex is characterized by six layers of highly laminated neurons generated through coordinated neuronal production and migration in the embryonic stage. During forebrain formation, the mammalian cerebral cortex arises from a sheet of proliferative cells in the dorsal telencephalon. Neural progenitors, also known as radial glia, proliferate in the ventricular zone. During neurogenesis, progenitor cells undergo asymmetric cell division to produce two daughter cells: one retains its stem cell fate, and the other adopts a neuronal fate. After cell cycle exit, postmitotic neurons leave the germinal ventricular zone and migrate towards their final resting positions in the cortex (Ayala and others 2007).

A notable multipolar stage of migrating neurons is observed in the lower intermediate zone and is characterized by the rapid extension and retraction of multiple short neurites (LoTurco and Bai 2006). Live imaging analysis of brain slices has revealed that this multipolar stage is transient and does not generate any fixed cell polarity (Fig. 1). These cells do not move directly towards the pial surface and frequently change their migration direction and rate (Barnes and others 2008). This highly dynamic stage represents a phase in which the neurons

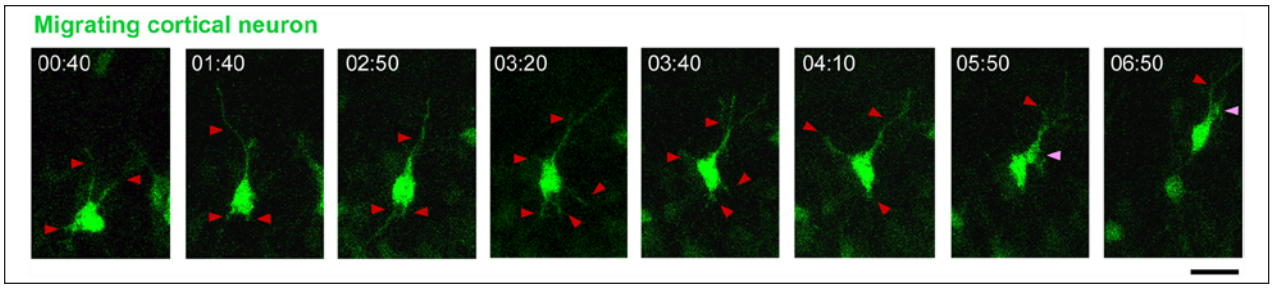

Figure 1. Multipolar-bipolar transition in migrating cortical neurons. Time lapse imaging of green fluorescent protein (GFP)-expressing migrating neurons in the intermediate zone. These multipolar neurons rapidly extend and retract processes until a single leading process is formed and the cell takes on a bipolar morphology. After obtaining a bipolar morphology, migrating neurons attach to the radial scaffold and move radially towards the pial surface. Recordings were carried out for 7 hours, and images were taken at the indicated time points. Neurites extended from cell bodies are indicated by red arrowheads. Pink arrowheads indicate the swelling of the leading process. Scale bar = 20 μm. Data adapted from Ip and others (2012).

actively explore the microenvironment for directional cues (Ayala and others 2007). Live imaging analysis further shows that microtubule stability controls extension/retraction processes and directional movement during the multipolar stage (LoTurco and Bai 2006). One neurite in each multipolar neuron is stabilized and specified as the leading process to initiate the neuron's transition into the bipolar morphology (Fig. 1). During radial translocation through the intermediate zone, the trailing process elongates rapidly and develops into an axon, while the cell body of the migrating neuron translocates towards the cortical plate (LoTurco and Bai 2006). The leading process is actively involved in the translocation of neurons and eventually differentiates into apical dendrites when the neurons reach their final destination in the cortical plate (Ayala and others 2007). Thus, neuronal polarization is a critical feature in neuronal migration and axon specification in the developing cortex.

Various signaling proteins that regulate cytoskeletal dynamics are implicated in neuronal polarization and migration in vivo. Serine/threonine kinases including liver kinase B1 (LKB1), Cdk5, and GSK3β are important regulators of the multipolar-bipolar transition and axonal outgrowth in migrating cortical neurons (Barnes and Polleux 2009). However, the molecular mechanisms underlying the actions of these kinases during neuronal polarization remain unclear. Our recent work provides molecular clues on how the kinases transduce extracellular cues to the cellular effectors in specific subcellular compartments. Immunohistochemical analysis shows that the CRMP2 protein is highly expressed in the cerebral cortex including the intermediate zone and the cortical plate (Fig. 2A). Importantly, CRMP2 silencing by in utero electroporation perturbs the radial migration of cortical neurons, causing the accumulation of neurons in the intermediate zone of the mouse neocortex (Fig. 2B). Morphological analysis shows that CRMP2-depleted

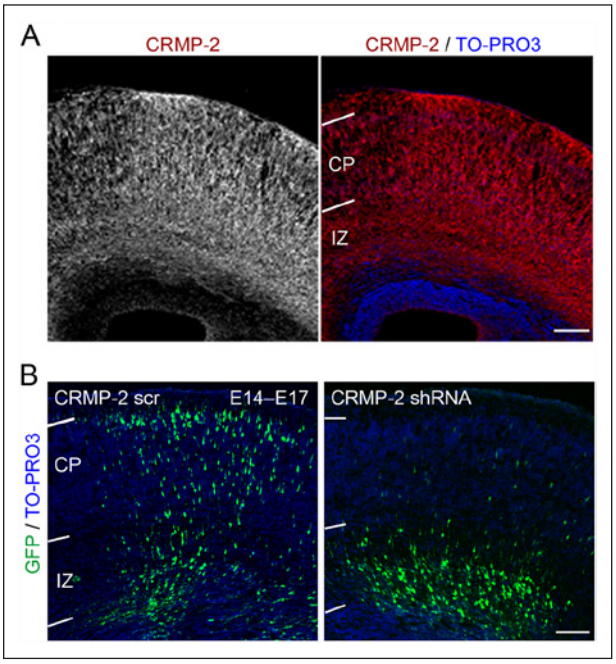

Figure 2. Functional roles of CRMP2 in cortex development. (A) CRMP2 is highly expressed in the intermediate zone and cortical plate of the cerebral cortex. Scale bar = 100 μm. (B) Silencing of CRMP2 by in utero electroporation impairs neuronal migration and arrests migrating neurons in the intermediate zone. Scale bar = 100 μm. Data adapted from Ip and others (2012).

neurons extend multiple minor processes or even exhibit a rounded morphology with minor processes. CRMP2-depleted neurons show inhibited formation of the leading process. Importantly, the precise regulation of CRMP2 activity in migrating neurons at the multipolar stage is sufficient for breaking cell symmetry and stabilizing one of the processes as the leading process. In this process, the RhoGAP α2-chimaerin regulates the activity and

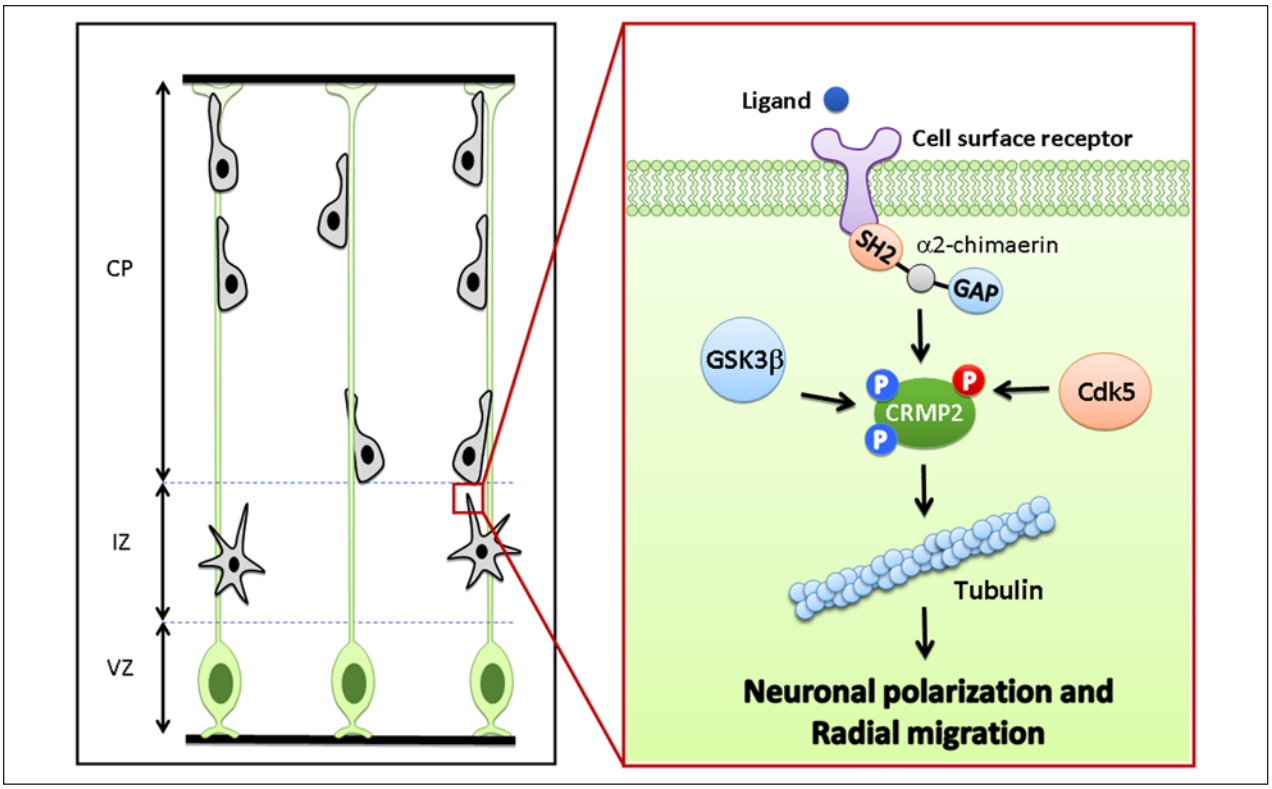

Figure 3. Tight regulation of CRMP2 activity during neuronal polarization and migration. CRMP2 is dynamically regulated by its phosphorylation status. GSK3β phosphorylates CRMP2 at the Thr509 and Thr514 sites after priming phosphorylation at the Ser522 site by Cdk5. The activity and localization of CRMP2 are regulated by its interaction with α2-chimaerin during the multipolar-bipolar transition in the intermediate zone (IZ) of the developing cortex. CP = cortical plate; VZ = ventricular zone.

localization of CRMP2 and directs the formation of a stabilized leading process (Ip and others 2012). Therefore, we propose that the precise activity and localization of CRMP2 are regulated by its interaction with α2-chimaerin and are required for the formation of a stabilized leading process and progression to the bipolar stage (Fig. 3). However, how CRMP2 regulates the multipolar-bipolar transition remains unclear. The endocytotic adaptor protein Numb is suggested to promote cell migration by directing integrin endocytosis to the leading edge of migrating cells (Nishimura and Kaibuchi 2007). It is plausible that CRMP2 promotes the specification of the leading process by regulating microtubule stability and Numb-mediated endocytosis of adhesion molecules.

CRMP2 has also been implicated in transduction of the signals from TrkB to regulate axon outgrowth in migrating cortical neurons during development of the cerebral cortex. A recent study shows that the phosphorylation of a scaffold protein Axin is critical for axon formation in the mouse cerebral cortex (Fang and others 2011). Axin knockdown in utero abolishes the formation and projection of axons. Importantly, Axin is phosphorylated by Cdk5; this phosphorylation facilitates the interaction between Axin and GSK3β, resulting in the inhibition of GSK3β activity and dephosphorylation of CRMP2. These findings shed light on the regulatory mechanism of axon formation in vivo via the Cdk5-dependent phosphorylation of Axin and regulation of CRMP2 activity (Fang and others 2011). Another recent study revealed that in addition to its phosphorylation status, CRMP2 can be regulated in vivo at the transcriptional level by extracellular cues. CRMP2 transcription is tightly regulated by bone morphogenetic protein (BMP) gradients during cortex development (Sun and others 2010). As a member of the transforming growth factor (TGF) superfamily, BMP suppresses CRMP2 expression in the neocortex via the transcription factor SMAD1. Elevated CRMP2 expression is associated with the decrease of SMADs in the intermediate zone and cortical plate. SMAD1 can bind to the CRMP2 promoter in cortical neurons, and its overexpression by in utero electroporation suppresses CRMP2 expression (Sun and others 2010). These findings collectively suggest that CRMP2 plays critical roles in the establishment of polarity in vivo and that its expression level is tightly regulated during cortex development.

The migration of the newly generated neurons to their final destination is followed by neuronal differentiation, which is characterized by dendritic outgrowth and synaptogenesis. Although the precise roles of CRMP2 remain to be elucidated, the protein is implicated in the dendritic patterning process. A CRMP2-S522A knockin mutant that lacks the Cdk5-dependent phosphorylation site was recently generated. With a CRMP1 mutant background, these knockin mice exhibit abnormal dendritic patterning, indicating the functional importance of Ser522 phosphorylation for dendritic guidance (Yamashita and others 2012). While the majority of published studies describe the functions of CRMP2 in cytoskeletal restructuring, CRMP2 was recently shown to interact with the N-type calcium channel (CaV2.2) at presynaptic terminals. CRMP2 overexpression in hippocampal neurons increases calcium channel current and neurotransmitter release, whereas CRMP2 knockdown abolishes these effects (Brittain and others 2009). Enhanced CRMP2-CaV2.2 interaction increases the quantity of CaV2.2 proteins at the membrane surface. This study demonstrates that CRMP2 plays an atypical role in the regulation of synaptic strength by modulating calcium influx into the presynaptic terminal (Brittain and others 2009).

Role of CRMP2 in Neurological Disorders and Its Therapeutic Potential

Given that CRMP2 is a multifunctional protein with diverse roles including tubulin assembly, protein trafficking, endocytosis, and channel modulation, it is not surprising that aberrant CRMP2 activity is implicated in various neurological disorders such as Alzheimer disease. The two major pathological hallmarks of Alzheimer disease are the presence of aggregated amyloid plaques comprising the amyloid β (Aβ) peptide and intracellular neurofibrillary tangles (NFTs) comprising the hyperphosphorylated tau protein (Finder 2010). Aβ42 oligomers and phosphorylated tau in the cerebral spinal fluid are considered reliable biomarkers of Alzheimer disease, even in the early phase of the disease (Blennow and Zetterberg 2013). This clinical application highlights the significance of aberrant phosphorylation in Alzheimer disease. Both Cdk5 and GSK3β are tau kinases; the phosphorylation of tau impairs its microtubule-binding affinity, thereby destabilizing microtubules (Cheung and Ip 2011). Intriguingly, CRMP2 is reported to be heavily phosphorylated in the brains of patients with Alzheimer disease and in genetic mouse models (Gu and others 2000; Soutar and others 2009; Yoshida and others 1998). Phosphorylated CRMP2 has been detected in NFTs and aggregated plaques in Alzheimer disease. Hyperphosphorylation of CRMP2 is also mediated by Cdk5 and GSK3β (Cheung and Ip 2011; Sutherland 2011). Moreover, excessive Cdk5 activity inhibits the phosphatase PP1 activity, thereby enhancing the phosphorylation of tau and CRMP2 (Cole and others 2008). Although CRMP2 shares several similar pathological features with tau, the important question is whether CRMP2 hyperphosphorylation plays a role in the disease pathogenesis or is secondary to other cellular defects induced by Alzheimer disease. Interestingly, CRMP2 hyperphosphorylation is suggested to manifest before the formation of plaques and tangles (Cole and others 2007). Therefore, this raises the intriguing possibility that CRMP2 hyperphosphorylation by Cdk5 and GSK3β is an early event in the progression of Alzheimer disease. One attractive hypothesis is that CRMP2 hyperphosphorylation reduces the quantity of functional CRMP2, leading to impaired microtubule dynamics, defects in axonal transport, and eventually neuronal cell death (Hensley and others 2011) (Fig. 4). Indeed, substantial evidence suggests that axonal transport plays an important role in the pathology of Alzheimer disease. Key proteins involved in Alzheimer disease, including amyloid precursor protein (APP), presenilins, and β-secretase (BACE), are transported into the axon. In particular, APP is transported along the axon by interacting with the kinesin light chain directly or through an adaptor protein, JNK-interacting protein 1 (JIP1). Furthermore, excess APP is known to impair axonal transport (Goldstein 2012). One possibility is that CRMP2 hyperphosphorylation impairs APP transport and processing; this ultimately results in the production of Aβ oligomers, which are believed to be the major toxic species in Alzheimer disease that leads to synaptic failure and hence neuronal loss. However, there is currently no clear evidence supporting this hypothesis. Therefore, whether CRMP2 or its hyperphosphorylation contributes to the neuronal loss in Alzheimer disease requires further investigation.

CRMP2 has also been implicated in psychiatric diseases such as schizophrenia, which is characterized by impairments in perception, affect, and occupational functioning. A magnetic resonance imaging study suggests that individuals with a high risk of schizophrenia have a smaller prefrontal cortex and temporal lobe than those with a lower genetic risk (Glausier and Lewis 2013). Subtle alterations during development such as dendritic spine defects or transient delayed neuronal migration have been reported in patients with schizophrenia and in experimental animal models (Glausier and Lewis 2013; Niwa and others 2010). Interestingly, the CRMP2 protein level is significantly lower in the frontal cortex of patients with schizophrenia than in normal controls (Johnston-Wilson and others 2000). In addition, a genetic association is reported between the *DPYSL2* gene and schizophrenia. A 2,236T→C single nucleotide polymorphism located in the 3′ untranslated region is genetically associated with schizophrenia including paranoid-type schizophrenia (Nakata and others 2003). However, how reduced CRMP2 levels in the frontal cortex or the genetic

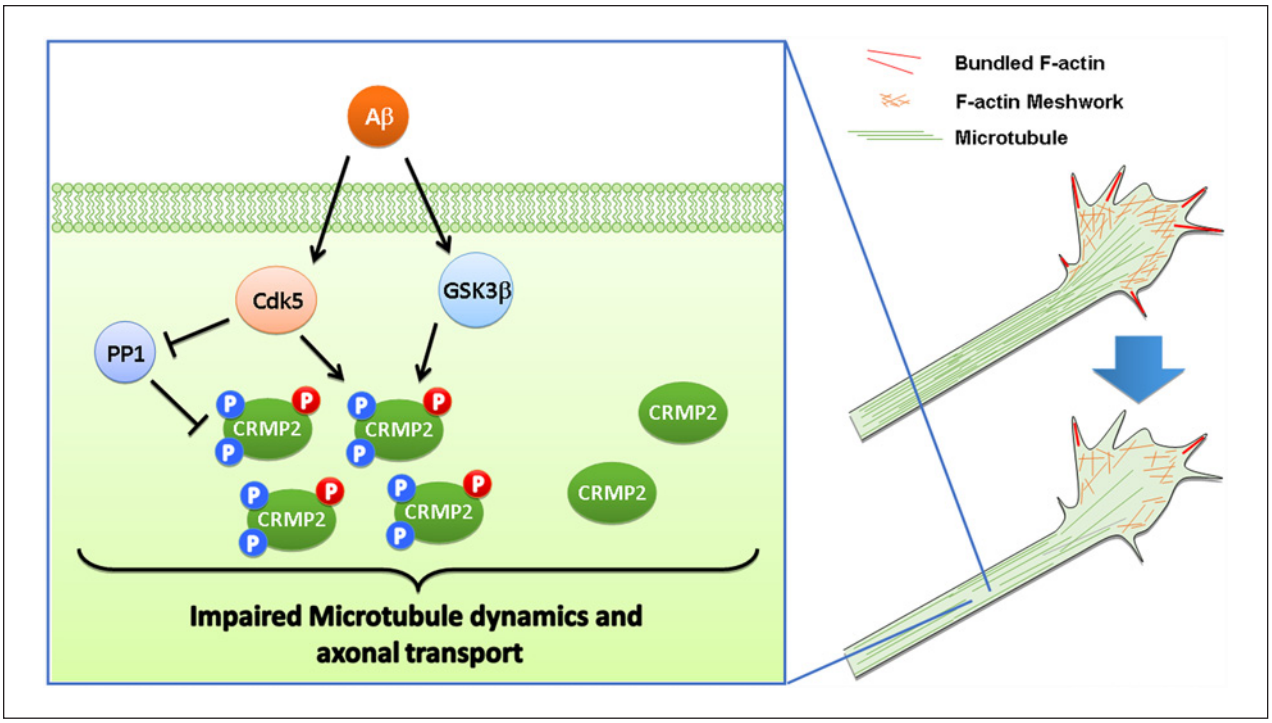

Figure 4. Deregulation of CRMP2 activity in Alzheimer disease models. Aβ induces excessive Cdk5 and GSK3β activation, resulting in CRMP2 hyperphosphorylation. Elevated Cdk5 activity inhibits phosphatase PP1, thereby enhancing CRMP2 phosphorylation. CRMP2 hyperphosphorylation reduces the quantity of functional CRMP2, leading to impaired microtubule dynamics and axonal transport defects. Additional studies are required to clarify the functional consequences of CRMP2 hyperphosphorylation.

association contribute to the pathophysiology of schizophrenia remains unknown. Increasing evidence suggests that the balance of specific circuits is affected in schizophrenia. One possibility is that neuronal migration defects lead to alterations in excitatory/inhibitory balance, contributing to behavioral deficits. An animal model of schizophrenia was recently generated through the in utero knockdown of disrupted in schizophrenia 1 (DISC1) to study how transient migration defects during early development may affect complex brain functions in adulthood (Niwa and others 2010). Future studies using in utero gene transfer systems to manipulate the expressions of CRMP2 or the single-nucleotide polymorphism (SNP) variant in different brain regions may provide functional insight into the disease mechanism of schizophrenia.

Recent studies suggest potential therapeutic approaches targeting CRMP2. For example, multiple sclerosis is a devastating disease that involves demyelination and axon degeneration. CRMP2 phosphorylation at Thr555 has been found to be elevated in degenerating spinal cord neurons in an experimental model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE)–induced mice, and active multiple sclerosis lesions of patients (Petratos and others 2012). One study suggests that axonopathy in EAE can be blocked by reducing CRMP2 phosphorylation; this study further shows that CRMP2 phosphorylation at Thr555 appears to be downstream of the Nogo-A/Nogo-66 receptor 1 pathway (Petratos and others 2012). While Nogo-A is known to potently inhibit axonal regeneration, the deletion of Nogo improves the outcome of the EAE model (Karnezis and others 2004; Petratos and others 2012). Importantly, administration of the Nogo antibody abolishes the up-regulation of CRMP2 phosphorylation at Thr555 in the spinal cord and improves the clinical outcome of EAE. This suggests that the blockade of CRMP2 phosphorylation is a viable therapeutic target for improving the axonopathy in multiple sclerosis (Petratos and others 2012). Another study suggests that CRMP2 can be used to relieve chronic neuropathic pain. CRMP2 was recently shown to interact with CaV2.2 and modulate calcium influx into the presynaptic terminal (Brittain and others 2009). A series of behavioral tests revealed that a short peptide that blocks the CRMP2-CaV2.2 interaction is effective as a mild anxiolytic agent without affecting memory or sensorimotor function (Brittain and others 2011). These exciting findings collectively suggest that the

manipulation of CRMP2 phosphorylation and interaction with its partners are viable therapeutic strategies for developing future treatments.

Concluding Remarks

Since its discovery, the microtubule-binding protein CRMP2 has been implicated in neuronal polarity and growth cone guidance in dissociated neurons. Significant progress has been made towards understanding the cellular and molecular mechanisms underlying neuronal polarization in vivo. This review examined the emerging role of CRMP2 in cortex development. First, CRMP2 activity is required for migrating neurons to undergo multipolar-bipolar transition before glial-guided radial migration towards the pial surface. Further elucidation of the regulation of actin and microtubule dynamics as well as leading process specification by CRMP2 in the context of the multipolar-bipolar transition will enhance our understanding of in vivo neuronal polarization and neurodevelopmental disorders. The growing list of CRMP2 interaction partners corroborates the notion that CRMP2 acts as more than a cytoskeletal regulator, serving diverse neurophysiological roles. CRMP2 activity is altered in several neurological diseases including Alzheimer disease and schizophrenia. However, the precise roles of CRMP2 in these diseases remain to be elucidated. Recent studies suggest that CRMP2 is a viable therapeutic target that may limit multiple sclerosis–related axonopathy and ameliorate chronic neuropathic pain. Therefore, future studies aiming to translate these basic research findings into clinical treatments at each end are warranted.

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