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Opening Pandora's jar: a primer on the putative roles of CRMP2 in a panoply of neurodegenerative, sensory and motor neuron, and central disorders

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Abstract

CRMP2, also known as DPYSL2/DRP2, Unc-33, Ulip or TUC2, is a cytosolic phosphoprotein that mediates axon/dendrite specification and axonal growth. Mapping the CRMP2 interactome has revealed previously unappreciated functions subserved by this protein. Together with its canonical roles in neurite growth and retraction and kinesin-dependent axonal transport, it is now

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known that CRMP2 interacts with numerous binding partners to affect microtubule dynamics; protein endocytosis and vesicular cycling, synaptic assembly, calcium channel regulation and neurotransmitter release. CRMP2 signaling is regulated by post-translational modifications, including glycosylation, oxidation, proteolysis and phosphorylation; the latter being a fulcrum of CRMP2 functions. Here, the putative roles of CRMP2 in a panoply of neurodegenerative, sensory and motor neuron, and central disorders are discussed and evidence is presented for therapeutic strategies targeting CRMP2 functions.

Keywords

Alzheimer's disease; amyotrophic lateral sclerosis; axon elongation; CRMP2; CRMP2/CLN6/KLC4 signaling complex; CRMP2 hyperphosphorylation; excitotoxicity; multiple sclerosis; neuropathic pain; oxidative damage

First identified in chick dorsal root ganglia as a protein responsible for growth cone retraction evoked by negative guidance signals in the Sema3A pathway of the developing CNS [1], CRMP2 analogs were subsequently identified in *Caenorhabditis elegans* (*Unc-33*) [2], in rodents (named *TOAD-64*) in rats and Unc-33 like phosphoprotein [Ulip] in mice) [3–5], in humans (HUlip) [6,7] and in *Drosophila melanogaster* [8]. A nomenclature of TUC (TOAD-64/Ulip/CRMP) was proposed to define this family of proteins but has not been universally adopted [9]. Probing of the genome led to identification of a total of five mammalian CRMP proteins (CRMPs 1–5) of which CRMP2 is the most well studied [10,11]. Pronounced increases in CRMP2 following neurogenesis [4] led to its identification and likely correlate with its canonical roles in axonogenesis, axon outgrowth and neuronal polarity as well as migration [12–15]. These roles are further supported by increased CRMP2 expression in neuronal cell lines and primary cells following differentiation [16–18]. CRMP2 is expressed widely in neuronal tissues, such as the brain, retinal ganglia, spinal cord and dorsal root ganglion [1], with some developmental plasticity that peaks during the middle-embryonic to early-postnatal period followed by reduced but constitutive expression throughout adulthood [1,4,5,10,19]. CRMP2 is also expressed in oligodendrocytes [20] as well as in non-neuronal cells, including fibroblasts [21], which may suggest roles for the protein in proliferation and possibly tumorigenesis; and in T cells [22,23], suggesting roles in immune function (see 'Future perspective' section).

Even prior to crystallization, it was known that CRMP2 forms both hetero- and homo-tetramers with other members of the CRMP family [24] as well as with a variant long form of CRMP2 [25]. Two variants of CRMP2 have been reported: a long (~68–75 kDa) and short (~62 kDa) form, with a common core polypeptide but different N-terminal domains that are products of alternative mRNA splicing [25]. The CRMP-long or 2A form is specifically localized in neuronal soma and/or axons but is absent from dendrites, whereas the CRMP2-short or 2B form is localized in both axons and dendrites [26]. The balance between the expression of the two subtypes is involved in the control of axonal branching and elongation. The crystallization of CRMP2 revealed a 'bilobed lung-shaped' configuration with the majority of CRMP2 packed within this structure [27–29]. Notably, the structure is missing the carboxyl-terminus (amino acids 479–572) residues likely due to their inherent instability. The presumed native confirmation of CRMP2 is tetrameric but how this stoichiometry defines the function of CRMP2 is unknown.

Delineating CRMP2 as an obligatory signaling molecule in Sema3A-induced growth cone collapse resulting from engagement of the neuropilin-1/plexin-A receptor complex [30] has emphasized the importance of phosphorylation as a fulcrum in CRMP2 signaling (Table 1). CRMP2 is a multiphosphorylated protein in neurons [15,31,32]. Phosphorylation, by

GSK-3β, Cdk5 and ROCK [10], as well as CaMKII [33] and the Src family kinase Fyn [34], induces neurite retraction and collapse of growth cones. Phosphorylation by GSK-3β and/or ROCK lowers the ability of CRMP2 to interact with tubulin, leading to axonal growth arrest and growth cone collapse [15,31,32]. CRMP2 phosphorylation is essential for Sema3A-induced growth cone collapse as CRMP2 mutants lacking the Cdk5, GSK-3β or Fyn phosphorylation sites exhibit reduced functionality. In contrast to the Sema3A signaling, lysophosphatidic acid-induced growth cone collapse requires CRMP2 phosphorylation by ROCK only [31]. In an elegant study, Yamashita and colleagues demonstrated that the phosphorylation of CRMP2 at Ser522 by Cdk5 is essential for proper dendritic field organization *in vivo* [35]. As phosphorylation of CRMP2 leads to axon retraction it is not surprising that dephosphorylation of CRMP2 by phosphatases leads to enhanced neuritic growth [36,37]. Two phosphatases, PP1 [36] and PP2A [37], are able to dephosphorylate CRMP2 at GSK-3β sites (i.e., Thr509/514) [38]. The fact that CRMP2 is modified by cytosolic *O*-glycosylation suggests another mechanism for regulating growth cone guidance [39]; however, there is no evidence for how *O*-glycosylation regulates CRMP2 function. Oxidation of CRMP2 has also been demonstrated and is believed to link the redox protein thioredoxin to regulation of CRMP2 phosphorylation and subsequent Sema3A-induced growth cone collapse [40]. Proteolysis of CRMP2 by calpains has been demonstrated [41,42] and, in this truncated state, CRMPs have been linked with neurodegeneration and cell death as reviewed recently by Taghian and colleagues (see also ‘Traumatic injury, excitotoxicity & ischemic stroke’ section) [43]. Nuclear translocation of a cleaved form of CRMP2 has been reported [44]. It is hypothesized that a balance between cytoplasmic full-length CRMP2 and post-transcriptionally processed forms of CRMP2 translocated to the nuclear c ompartment may govern neurite outgrowth [44].

In addition to post-translational regulation, transcriptional control for CRMP2 has been described. Transcriptional suppression of CRMP2 by BMP signaling downstream of the transcription factor SMAD1 has been demonstrated and implicates CRMP2 as playing an essential role at multiple stages of neuronal development [45]. GDNF enhances CRMP2 expression through the GDNF/RET tyrosine kinase signaling pathway, and this CRMP2 induction depends on the ERK pathway [46]. CRMP2 promoter analysis showed that transcription factors, such as Sp1, E2F and GATA-1/2, may play a role in the regulation of CRMP2 transcription [46].

Together with its canonical roles in neurite growth and retraction and kinesin-dependent axonal transport, it is now known that CRMP2 interacts with numerous binding partners to affect microtubule dynamics, protein endocytosis and vesicle recycling, synaptic assembly, calcium channel regulation and neurotransmitter release Table 2 (see also review by Hensley *et al.* [47]). Perhaps unsurprisingly, the first CRMP2 partner identified was the cytoskeletal protein tubulin [48]. In addition to tubulin, CRMP2 binds to the cytoskeletal proteins actin and vimentin [22,32]. CRMP2 acts largely by stabilizing polymerized tubulin at the plus end of microtubules, thus promoting axon extension [1,12,49,50]. Several distinct signaling pathways regulate CRMP2 phosphorylation to change CRMP2–protein binding interactions in a way that either collapses growth cones or promotes axon extension [47]. In the first discovered pathway, Sema3A signaling through its receptors NRP1 and plexin A triggers Rac1 activation, affecting downstream kinases and ultimately activating cyclin-dependent Cdk5 and GSK-3β, which then phosphorylate CRMP2 on Ser 522 and Thr 509/514, respectively [15,27,51]. Phosphorylated CRMP2 loses affinity for tubulin heterodimers, thus reducing microtubule stability, and encouraging axon retraction [1,12,49,50].

A second pathway through which CRMP2 affects neurites is an anterograde axonal transport mechanism by which CRMP2 adapts the microtubule motor kinesin-1 to transport packets. These packets contain various critical synapse-regulating proteins such as TrkB [52] or the

Sra1/WAVE1 complex [53]. At the distal axon and in synapses, WAVE1 activates the Arp2/3 complex, which in turn nucleates actin monomers that would otherwise be kinetically impeded from polymerizing into microfilaments [54]. RNA interference of CRMP2 delocalizes WAVE1 from growth cones, triggering cone collapse [53]. Similarly, knockdown of Sra1 and WAVE1 cancels CRMP2-induced axon outgrowth [53], indicating that proper connection of CRMP2 to Sra1/WAVE1 is essential to preserve the integrity of distal actin networks. In normal neuronal cultures, CRMP2 seems to be a limiting factor in neurite outgrowth because increasing CRMP2 expression is entirely sufficient to stimulate neurite extension [53,55].

Analogous to its role in axonal transport, CRMP2 adapts kinesin motors to endocytic vesicle proteins, such as Numb, involved with cell-surface protein internalization and sorting [13]. Less is known about CRMP2's role in trafficking than about its function in axonal transport, although the two processes are largely analogous in terms of the roles of CRMP2 with regards to kinesin and microtubules. In a recent study, Kaibuchi's group showed that depletion of CRMP2 reduces the amount of TrkB in the axonal membrane and decreases the input signals (ERK1/2 phosphorylation) from BDNF stimulation, suggesting that CRMP2 mediates BDNF signals via two distinct pathways: recruitment of TrkB into the distal part of an axon and enhancement of axon elongation through microtubule formation induced by dephosphorylation of CRMP2 [56]. Thus, CRMP2 is a critical mediator for several essential types of protein trafficking in neurons.

While the majority of CRMP2's characterized interactions make a clear link to its role in axon outgrowth, several recently discovered interactions hint at more diverse functions. For example, CRMP2 interacts with the Ca^{2+}-binding protein CaM [57]. Pharmacological CaM antagonism inhibits CRMP2-mediated outgrowth of neurite-like processes, tetrameric assembly of CRMP2 and reduces calpain-mediated CRMP2 proteolysis [57]. Another example is the protein neurofibromin, a RasGAP protein whose loss results in the disease neurofibromatosis type 1 [58]. CRMP2 was demonstrated to interact with a 558 amino acid domain in the carboxyl terminus of neurofibromin [59] and has been shown to directly regulate CRMP2 phosphorylation or do so indirectly by increasing activity of the kinases that phosphorylate CRMP2 [59]. The functional consequence of this interaction is currently unknown; the authors' laboratory is investigating the molecular mechanisms underlying the signaling of the tripartite complex between Ca^{2+} channel: CRMP2–neurofibromin in *Nf1* haplo-insufficient mice [60].

The authors' laboratory has added the N-type voltage-gated calcium channel (CaV2.2) to the growing list of CRMP2-binding partners [61,62]. While it is well established that presynaptic voltage-gated Ca^{2+} channels trigger release of neurotransmitters at synapses, little is known about the role of CRMPs in presynaptic biology [63]. The authors' results demonstrated that a direct interaction between CRMP2 and CaV2.2 increases cell surface trafficking of CaV2.2 as well as calcium current density, which leads not only to an increased release of the neurotransmitter glutamate and synaptic vesicle recycling but also impacts axonal growth of hippocampal neurons. The CRMP2–Ca^{2+} channel association may serve multiple purposes in neurotransmitter release: to sustain Ca^{2+} influx through functional regulation of Ca^{2+} channels, to target the N-type Ca^{2+} channel to immature synapses during synaptogenesis, to provide a scaffold for the Ca^{2+} channel macromolecular complex and to recruit synaptic vesicles to Ca^{2+} channels [61]. These findings were recapitulated in sensory neurons where CRMP2 also coupled to CaV2.2 to enhance the release of the peptide neurotransmitter CGRP [62]. Thus, these results identify CRMP2 as a novel 'neuromodulator' of Ca^{2+} channels and of synaptic connectivity and strength.

The Wenthold group reported binding of CRMP2 to the NMDA receptor (NMDAR) subunits NR2A/2B [64], which was further mapped by the authors' laboratory to two short domains within the carboxyl terminus of the NR2B subunit [65]. The relevance of this interaction in the context of neuroprotection will be presented in a later section in this review.

Given the breadth of interactions of CRMP2 with motor proteins, kinases, channels, receptors, enzymes and endocytosis/exocytosis-related proteins, it is becoming increasingly clear that CRMP2 may serve as adaptors/scaffold molecules and as traffic 'cops' (see review by Schmidt and Strittmatter [10]). In the next sections of this article, we highlight accumulating evidence for the involvement of CRMP2 in a panoply of disorders will be highlighted: neurodegenerative (neuronal ceroid lipofuscinosis [NCL], also known as Batten disease, Alzheimer's disease [AD] and prion disease), sensory and motor neuron (amyotrophic lateral sclerosis [ALS], multiple sclerosis [MS] and chronic neuropathic pain) and central (excitotoxicity and ischemic stroke, epilepsy and bipolar/schizophrenia/schizoaffective disorders). Understanding the molecular etiology underlying CRMP2's involvement in these disorders has resulted in tremendous progress towards targeting, directly or indirectly, CRMP2 for therapies for AD [66], ALS [67], MS [68], inflammatory and neuropathic pain [69–71], ischemic stroke [42,71], as well as post-traumatic epilepsy (Table 3) [72].

Targeting CRMP2 in neurodegenerative disorders

Could CRMP2-associated signaling complexes serve as potential therapeutic targets for Batten disease?

NCLs are a heterogeneous group of autosomal-recessive disorders that are collectively the most prevalent neurodegenerative disease of childhood. In the vast majority of cases, disease onset occurs in early childhood with visual deterioration, cerebellar ataxia, seizures and mental deterioration. Common disease symptomology and pathology has long suggested a common molecular mechanism underlying NCLs. Although NCL-associated proteins, many of whose function remains elusive, show fairly ubiquitous expression throughout the body, selective neuronal vulnerability suggests that a common link may lie in cellular functions unique to neurons. Recently, studies have suggested that a common NCL pathway, particularly for NCLs associated with membrane-localized proteins (such as CLN3, CLN6, CLN5 and CLN8) may be intracellular transport via disrupted interaction with molecular motors and the cytoskeletal network. Through yeast two-hybrid and proteomic studies, CLN3, CLN6 and CLN8 have all been shown to associate in varying aspects to the microtubule network and neuronal cytoskeleton [73,74]. One conundrum in this theory is that these membrane-associated NCL proteins have been localized to cellular organelles often viewed as nonreliant on the microtubule network for cellular transport such as the Golgi, endoplasmic reticulum, lysosome and mitochondria [75–82]. Unique to neurons, transport of essential building blocks to distal sites within axons and dendrites, often up to 1000–10,000-times the length of the cell body, relies on coupling of cargo to the microtubule network and this cargo often includes mitochondria, endoplasmic reticulum-associated/synaptic vesicles and lysosomes. Both the initial specification and outgrowth as well as the long-term maintenance of neuronal processes is reliant on sustained, efficient transport of cargo and defects in axonal outgrowth and transport are a common theme of neurodegeneration [83].

Mutations in the *CLN6* gene result in the variant late infantile NCL form (vLINCL) with age of onset between 2 and 6 years with death occurring by the third decade of life [84–87] as well as the type A adult-onset form of NCL, or Kuf 's disease [88]. This dual role in infantile and adult forms of NCL underscores the crucial function of *CLN6* both in neuronal

development, but also in the maintenance of the mature nervous system. Naturally occurring animal models with mutations in CLN6 include the Cln6^{nclf} mouse and the New Zealand South Hampshire sheep (OCL6), both of which faithfully recapitulate pathological phenotypes seen in the human form of the disease, including accumulation of storage material, eventual limb paralysis, selective neuronal loss, cortical thinning and glial activation [89–91]. The Cln6^{nclf} mouse harbors an insertion mutation of one additional cytosine (c.307insC, frameshift after P102) resulting in 25 novel amino acids followed by a premature stop codon, which is identical to a common mutation found in vLINCL human patients [79], and thus provides a unique *in vivo* model for exploring the cellular function of CLN6. CLN6 is an endoplasmic reticulum-associated protein that has been shown to complex with CRMP2 [73]. CRMP2's ability to specify axon/dendrite fate and regulate cargo transport during axonal growth/regeneration has been shown to be facilitated and/or antagonized through a complex network of alternative protein–protein interactions, including KLC1, dynein, chimaerin, phospholipase D, calmodulin, neurofibromin and CaV2.2 (see Table 3 and review by Yoshimura and colleagues [92]). Disruption in CRMP2's ability to partner with these proteins has detrimental effects, including disruption in modulation of cytoskeletal dynamics, and has been associated with a whole host of neuropsychiatric disorders [23,33,47,51,93–96]. The common truncation mutation found in Cln6^{nclf} mice and humans, which results in a rapidly degraded truncated protein product, would preclude interaction with CRMP2 [86]. Moreover, recent studies have demonstrated that expression of the Cln6^{nclf} mutation disrupts CRMP's ability to interact correctly with its other partners, such as microtubules, kinesins and NF1 [Weimer JM, Unpublished Data], thus could therapies aimed at stabilizing CRMP's interaction with these partners prove beneficial in the treatment of NCLs? Additionally, the authors' recent studies have shown that CLN6 and CRMP2 can complex with the kinesin light chain protein, KLC4 or KNSL8 [Weimer JM, Unpublished Data], imparting specificity for unique kinesin motors and suggesting a role for this complex in providing a unique mechanism for localized CRMP2/CLN6-dependent signaling and segregation of cellular cargo or packets.

CRMP2 in AD progression & pathogenesis

AD is an age-related neurodegenerative disease and the sixth leading cause of death in the USA [97]. AD is diagnosed using cognitive examinations as well as the exclusion of other possible disease states, while definite diagnosis is still obtained post-mortem by the histopathological hallmarks of AD. Histopathologically, AD is characterized by synapse loss, the presence of abnormal protein deposits such as senile plaques (rich in β-amyloid peptide [Aβ]) and neurofibrillary tangles (NFTs; rich in hyperphosphorylated tau) [98].

The exact mechanism(s) for the development and progression of AD are still unclear. Genetic analysis has demonstrated a link between mutations in *PS1*, *PS2* and *APP* genes, and increased AD risk. In addition, other genes, such as *APOE4*, *CLU* (also known as *APOJ*), *PICALM*, endothelial nitric oxide synthase 3 and α2-macroglobulin, have been suggested as risk factors for AD [99–102].

A number of hypotheses, including the amyloid cascade, excitotoxicity, oxidative stress and inflammation hypotheses, have been proposed to explain the histopathology, biochemistry and clinical symptoms of AD. All these hypotheses implicate, to some extent, Aβ as a common thread. The oxidative stress hypothesis is based on the fact that AD brains have increased markers of oxidation of proteins, lipids, carbohydrates and nucleic acid compared with aged-matched controls [103–106]. The increase in the markers of oxidative stress has been shown to be associated with Aβ, a 40–42 amino acid peptide [107]. $A\beta_{1-42}$ has been shown to aggregate quickly and is proposed to play a central role in AD pathogenesis. Aβ has been shown to exist in at least four aggregated states: monomers, oligomers, protofibrils and fibrils. Furthermore, studies suggest that oligomeric Aβ is the toxic species of this

peptide rather than Aβ fibrils themselves [108–111]. Studies from the Sultana and Butterfield group and others have shown that Aβ-induced oxidative stress markers can be alleviated by free radical scavengers such as vitamin E, estradiol and melatonin, among others [112–115].

The Sultana and Butterfield group proposed the Aβ-induced oxidative stress model for AD pathogenesis. In this model, $A\beta_{1–42}$ can insert itself into the lipid bilayer as toxic oligomers and can therein undergo a one-electron oxidation of methionine, which leads to the formation of sulfuranyl or hydroxysulfuranyl radical cations that remove allylic hydrogen atoms from phospholipid acyl chains, leading to the formation of lipid peroxidation products via catalytic chain reactions [116,117]. Other proposed mechanisms of Met-35 neurotoxicity involve oxidative transfer from methionine to redox metal ions, such as copper or zinc (i.e., Fenton chemistry), which would generate superoxide radical anions and highly reactive hydroxyl radicals and increase Aβ aggregation [107,118–120]. Met-35 is also important in AD pathology because its redox state directly correlates with amyloid fibril formation. When Met-35 is oxidized to methionine sulfoxide, the rate of fibril formation with $A\beta_{1–42}$ is lowered and protofibrils are not formed [121–123]. Oxidized Met-35 exists in high abundance in senile plaques from post-mortem AD patients [124,125]. A recent *in vivo* study showed that Met-35 is critical to Aβ-induced oxidative stress and AD pathogenesis [126].

CRMP2/DRP-2 has been shown to be carbonylated in the inferior parietal lobules and hippocampus of AD patients [127–130]. CRMP2 was also reported to be hydroxynonenal, and 3-NT-modified in AD brains [131,132]. A study by Reiderer and coworkers showed that CRMP2 also undergo *S*-nitrosocysteine modification [133]. This oxidative modification of CRMP2 is expected to affect the cytoskeletal organization and membrane trafficking, consequently leading to the shortening of axons; in addition, it might also affect the functions of other proteins that are downstream to it in its functional proteome set. The oxidation of CRMP2 may provide an explanation for the reported impaired neuronal communication, neuropathological hallmarks: such as NFTs, loss of synapses, and the consequent learning and memory impairment as reported in AD.

In addition to oxidative modif ication, CRMP2 has been reported to be hyperphosphorylated in the AD brain [134]. The hyperphosphorylation of CRMP2 is mediated via the priming kinase Cdk5 and subsequently by GSK-3β (Figure 1). The phosphorylation of CRMP2 is first initiated by Cdk5 followed by GSK-3β, suggesting that Cdk5 activity is important in regulating the GSK-3 activity. Cdk5 has been shown to regulate axonal transport via GSK-3β [135], and it also regulates phosphorylation of APP on the cytoplasmic domain, which may play a role in the increased APP processing to produce Aβ [136,137]. Interestingly, the Ihara group showed that phospho-CRMP2 colocalizes with NFTs, and WAVE protein, a key molecule for actin assembly [138]. Furthermore, they also showed that the brains of 3xTg-AD mice exhibit accumulation of both phospho-CRMP2 and WAVE, suggesting that WAVE accumulation may require both Aβ/APP and tau pathologies, and might contribute to the synaptic alterations induced by disturbances in actin assembly in AD brains [139]. In both AD and mild cognitive impairment brains, the levels of Cdk5 are altered [140]. Therefore, it is possible that Cdk5 dysregulation may play an important role in the Aβ production, synapse loss and NFTs [141].

The existence of CRMP2 along with NFTs points to a likely relationship between these proteins. To date, however, there is no clear evidence to explain whether NFT oxidation affects the CRMP2 function or *vice versa*. Based on the available literature, it is tempting to speculate that through unknown mechanism(s) tau protein is hyperphosphorylated inhibiting

tau function and leading to impaired axonal transportation, which might recruit CRMP2 to axonal sites to promote neuronal sprouting and synapse formation.

The acetylated tripeptide rER (NH2-D-arg-L-glu-D-arg-COOH), derived from the external domain of APP, protects against Aβ-induced memory loss for a passive avoidance task in young chicks and enhances retention for a weak version of the task when injected peripherally up to 12 h prior to training [66]. Anti-CRMP2 antibodies injected intracranially 30 min prior to training induced amnesia for the passive avoidance task, which was rescued by acetylated rER, if injected prior to the anti-CRMP2. The fact that acetylated rER exerts its effects via CRMP2, a protein known to be abnormally phosphorylated in AD, bolsters its potential usefulness as a possible therapy for AD and places CRMP2 as a determinant of memory consolidation [94].

The present evidence demonstrates that Aβ-induced oxidative modification of CRMP2 might lead to impaired axonal transportation and synapse loss (an early feature observed in AD). Moreover, increased phosphorylation suggests that post-translational modifications (i.e., oxidation and phosphorylation) might play an important role in the progression and pathogenesis of AD. Hence, targeting CRMP2 could prevent or delay the progression of this devastating disorder [47,72].

CRMP2 in prion disease

Prion diseases, including Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker syndrome and familial or sporadic fatal insomnia, are rare and fatal neurodegenerative disorders characterized pathologically by neuronal loss, astrocytosis and deposition of PrP aggregates throughout the brain [142]. Approximately one in six human prion diseases are autosomal-dominant and are linked to point mutations or insertions in the gene encoding PrP [143]. Misfolding and oligomerization of mutant PrP are thought to initiate the pathogenic process [144]. Although the molecular neuropathology of prion disease is not fully understood, the axon-related attrition observed following prion infection is likely to involve proteins involved in axonal growth and guidance, such as CRMP2. A recent proteomic study on brains from mice infected with a scrapie prion strain identified an increase in a truncated version of CRMP2 (lacking carboxyl terminal amino acids 518–572, a region harboring several important phosphorylation sites) and a concomitant decrease in full-length CRMP2 [145]. Overexpression of this truncated form of CRMP2 *in vitro* in neurons phenocopied the increased development of neurite tips observed with overexpression of the nonphosphorylated version of CRMP2, suggesting that a lack of regulatory phosphorylation may underlie degenerating neurons in prion diseases. These findings are consistent with the demonstration that phosphorylation of CRMP2 at Ser522 by Cdk5 is critical for maintaining proper dendritic arborization *in vivo* [35]. Hagiwara and colleagues also proposed the idea that the truncated CRMP2 may not oligomerize properly, thus impairing the normal physiological function of CRMP2 [145]. Interestingly, a study with fly *CRMP* showed that circadian activity rhythmicity is impaired in *Drosophila CRMP* mutants [8]; but it is too early to speculate whether this may be linked to diseases such as fatal insomnia.

Targeting CRMP2 in sensory & motor neuron disorders

Evidence for CRMP2 dysfunction as a pathogenic factor & target of therapy for ALS

ALS is a rapidly progressive, invariably fatal, degenerative motor neuron disease with no effective treatments [146,147]. Epidemiologically, approximately 20% of ALS cases are caused by various, mostly dominant genetic mutations (familial ALS [FALS]) whereas the other 80% of sporadic ALS cases have unknown etiology [146]. The most common familial forms of FALS map to mutations in cytosolic Cu, SOD1 and transgenic mice overexpressing these mutations demonstrate a disease very similar to human FALS [147]. ALS appears to

be non-cell autonomous in mice because disease appearance requires the expression of mutant SOD1 in both motor neurons and surrounding non-neuronal cells [148]. In mice and humans, ALS can be described as a progressive distal axonopathy as the lower motor neuron axons first denervate from neuromuscular junctions, then progressively retract toward the neural somas in the spinal cord ventral horns [149]. It is unclear how SOD1 mutations give rise to ALS; however, cellular inclusions are found in both neurons and glia in mutant SOD1-bearing tissue, whereas a variety of other proteinacious inclusions are observed in human sporadic ALS [146,147]. Frequently, these inclusions contain cytoskeletal components, especially neurofilament aggregates [150]. Expression of mutant SOD1 renders glia more prone to neuroinflammatory activation or exacerbates production of glial cytokines and reactive oxygen species [147], and interferes with axonal transport [151,152].

The involvement of axonal protein aggregates coupled with the distal axonopathic nature of ALS motor neuron degeneration and the presence of axonal transport deficits in ALS mice begin to suggest that motor axonal cytoskeletons are a central component to the cellular deterioration that produces ALS clinical presentation. Accordingly, some researchers have begun to test microtubule stabilizing drugs in ALS mouse models. For instance, a 2007 study by Fanara and colleagues reported that the microtubule-stabilizing agent noscapine extended the lifespan of $SOD1^{G93A}$ mice by >10%, restored axonal transport deficits and reduced motor neuron death [152]. This is encouraging when one considers that many attempts to prevent somatic death of motor neurons through antagonism of death pathways and apoptosis has generally been met with poor results [147].

Recent findings suggest that aberrations in CRMP function may contribute to ALS axonopathy in a fashion that is amenable to therapeutic manipulation. Notable among these studies is a 2006 report by Verhaagen's group, which documented expression of the chemorepellant protein Sema3A in specific populations of terminal Schwann cells near fast-fatiguable type-II muscle fibers after nerve injury and in murine ALS [153]. Sema3A is normally expressed during embryonic development where it acts to repel neuritic growth cones through a CRMP2-dependent action (Figure 2). In this mechanism, Sema3A binds NRP1, which dimerizes to its plexin-A coreceptors. This complex recruits intracellular effector kinase cascades. Ultimately, Cdk5 and GSK-3β phosphorylate CRMP2 on Ser 522 and Thr 509/Thr 514, respectively [15,27,51]. Phosphorylated CRMP2 releases tubulin heterodimers, thereby reducing microtubule growth at the distal end of axons, encouraging axon retraction. Conversely, NT-3 and BDNF inhibit GSK-3β via the PI3K/AKT pathway, reducing CRMP2 phosphorylation and promoting axon growth (Figure 1) [154]. Thus, Sema3A expression near the ALS neuromuscular junction could explain one of the initial triggers that begin the process of axon pull-back in ALS motor neurons.

A separate, independent study by Pettman and colleagues has uncovered another mechanism by which CRMP2 dysfunction may contribute to motor neuron disease [155]. These researchers found that a variant of CRMP4 is induced in cultured motor neurons by exposure to nitric oxide. Forced adeno-associated virus-mediated expression of CRMP4 in wild-type motor neurons triggered axon degeneration and cell death, whereas silencing of CRMP4 in mSOD1 motor neurons protected them from nitric oxide-induced death [155]. Thus, ectopic CRMP4 seems to oppose CRMP2 and promote neurodegeneration. If this is the case, then boosting CRMP2 function would be expected to compensate for CRMP4 in order to promote healthy neuritic structure and function.

A serendipitous discovery of endogenous CRMP2-binding sulfur amino acid metabolites may have opened a pathway forward to develop small-molecule, CRMP2-acting therapeutic molecules [67,156]. Lanthionine ketimine (LK) is derived by alternative reactions of the transsulfuration enzyme CβS upon serine and cysteine, followed by metabolism through the

kynurenine pathway enzyme GTK, also known as KATI [67]. The Hensley group found that LK and especially its synthetic cell-penetrating ester derivative, LK ester (LKE), were potent stimuli for growth factor-dependent neurite elongation [156]. Affinity proteomics assays for LK binding partners identified CRMP2 as a plausible candidate for mediation of the neurotrophic activities inherent to LK and LKE [156]. Subsequently, the Hensley group administered LKE to $SOD1^{G93A}$ mice, a transgenic model of FALS [67]. Dosage with 100 mg/kg/day LKE in saline (intraperitoneal) beginning at 90 days of age (clinical stage disease) slowed subsequent functional motor loss and modestly extended animal lifespan [67]. Although other mechanisms of action for LKE cannot be ruled out, this initial data should encourage further investigation for molecules that affect CRMP2 pathways either directly or indirectly, in order to bolster axonal microtubule structure and function, so as to slow the progression of ALS.

Limiting MS-related axonopathy by blocking Nogo receptor & CRMP2 phosphorylation

MS is a severe neurological disorder that involves inflammation in the brain and spinal cord, axonal damage and demyelination. Increased CRMP2 levels were detected in cerebrospinal fluids from patients with MS. Phosphorylated CRMP2 levels were reported to be upregulated in human brain sections containing chronic, active MS lesions and plaques compared with the absence of such reactivity in normal brains post-mortem [157]. Using a well-established animal model of MS, experimental autoimmune encephalomyelitis, which mimics many of the pathophysiological hallmarks of MS, Petratos *et al.* showed that CRMP2 is phosphorylated and hence inhibited during the progression of experimental autoimmune encephalomyelitis in degenerating axons [68]. This seminal work also showed that *in vivo* administration of an antibody against the axonal growth inhibitor, Nogo-A, reduced the levels of phosphorylated CRMP2 (at Thr 555) in the spinal cord and improved pathological outcome. This exciting discovery hints at the potential therapeutic utility of targeting CRMP2 by demonstrating that phosphorylation of CRMP2 results in the activation of Nogo-66 receptor 1 (NgR1) and plays a pivotal role in axonal degeneration in experimental autoimmune encephalomyelitis and MS.

Targeting CRMP2 to curb chronic neuropathic pain

Despite the availability of a variety of analgesics, treatment of chronic pain remains a large unmet medical need. Although some chronic pain conditions may be treated adequately by existing drugs, many patients fail to achieve adequate pain relief. This is especially the case for patients suffering from neuropathic pain due to trauma, disease and neurotoxic antiretroviral treatment, which are often unresponsive to conventional analgesics. Furthermore, the chronic use of many analgesics is limited by side effects or by the development of tolerance. In 2010, analgesics accounted for sales of $22 billion globally and $13 billion in the USA [158,159]. The highest selling were opiates, followed by nonsteroidal anti-inflammatory drugs, antiepileptics, antidepressants and local anesthetics.

Recently, ziconotide (Prialt®, Jazz Pharmaceuticals, CA, USA) was approved by the US FDA for the treatment of severe pain that was refractory to other therapies [160]. This drug is a synthetic version of the naturally occurring cone snail toxin ω-conotoxin MVIIA and is a highly potent and selective peptide blocker of CaV2.2, validating N-type calcium channels as a promising target of novel analgesics. The use of Prialt is limited by the need for it to be administered by the intrathecal route and major side effects, including confusion, somnolence, orthostatic hypotension and nausea [161–163]. Presumably, the side effects occur through a nonselective blockade of N-type channels in all tissues [164,165] and thus, a strategy of modulating N-type channels in injured tissues through a state-dependent mechanism could prove advantageous.

Our laboratory has advanced a strategy for targeting modulators of channel trafficking based on our discovery of the interaction between CaV2.2 and CRMP2, which enhanced channel function by increasing cell surface trafficking of calcium channels [61,62,166]. In a subsequent study, it was found that the interaction between CaV2.2 and CRMP2 can be disrupted by a short peptide (calcium-binding domain 3; CBD3; Figure 3) corresponding to a 15-amino acid region of CRMP2, which results in a reduction in functional channels in the plasma membrane. Fusing CBD3 to the transduction domain of the HIV-1 TAT resulted in a cell permeable peptide, TAT-CBD3, which abrogated the CRMP2–CaV2.2 interaction, reduced CaV2.2-mediated currents *in vitro*, decreased neuropeptide release from sensory neurons and inhibited excitatory synaptic transmission in dorsal horn neurons of the spinal cord [70]. TAT-CBD3 administration *in vivo* reduced pain behavior in a number of pain models, including two animal models of neuropathic pain; antiretroviral drug treatment [70] and focal nerve demyelination [71]. In a battery of rodent behavioral tests to examine off-target effects, TAT-CBD3 was mildly anxiolytic without affecting memory retrieval, sensorimotor function or depression. Importantly, sympathetic activity was not affected by TAT-CBD3 [71]. This is in contrast to Prialt, which is poorly tolerated mainly because of effects on the autonomic nervous system [161,167].

Targeting CRMP2 in central disorders

Traumatic injury, excitotoxicity & ischemic stroke

The expression of modified forms of CRMP2 changes following various kinds of injury. For example, a sciatic nerve crush [4] as well as a hypoglossal nerve injury [168] in rodents result in upregulation of CRMP2, whereas nerves regenerating from a spinal cord injury in chicks exhibit increased levels of phosphorylated CRMP2 [169]. These studies highlight that dynamic regulation of post-translationally modified CRMP2 forms are putative contributors to the maintenance of spinal cord regenerative ability, likely via a transient stabilization of the neuronal cytoskeleton. In the middle carotid artery occlusion model of focal ischemia, expression of a calpain-cleaved and phosphorylated version of CRMP2 are elevated [170–172]; similar cleavage was reported following controlled cortical impact, an *in vivo* model of traumatic brain injury (TBI) [173]; the cytolytic agent maitotoxin or NMDA-mediated neurotoxic injury [173], glutamate-induced excitotoxicity [174] or NGF-induced neurite degeneration [175]. CRMP2 cleavage appears to be neuroprotective [174] as expression of a cleaved CRMP2 construct (mimicking calpain cleavage) increases neuronal survival following excitotoxic challenge and decreases the amount of neurons responding to NMDA challenge [174]. The authors' laboratory recently investigated the mechanism of this neuroprotection and tested the hypothesis that as CRMP2 has been linked to NMDAR trafficking, it may be involved in neuronal survival following excitotoxicity [42]. It was found that lenti-viral-mediated CRMP2 knockdown or treatment with the CRMP2 peptide TAT-CBD3 blocked neuronal death following glutamate exposure likely via blunting toxicity from delayed calcium deregulation. Application of TAT-CBD3 attenuated postsynaptic NMDAR-mediated currents in cortical slices. While exploring the modulation of NMDARs by TAT-CBD3 further, it was also found that TAT-CBD3 induced NR2B internalization in dendritic spines without altering somal NR2B surface expression, probably via disruption of the interaction between NR2B and CRMP2 [42]. Furthermore, TAT-CBD3 reduced NMDA-mediated Ca^{2+} influx and currents in cultured neurons. Importantly, systemic administration of TAT-CBD3 following a controlled cortical impact model of traumatic brain injury decreased hippocampal neuronal death [42]. The authors also showed that an intraperitoneal injection of TAT-CBD3 peptide significantly reduced infarct volume in an animal model of focal cerebral ischemia; neuroprotection was observed when TAT-CBD3 peptide was given either prior to or after occlusion but just prior to reperfusion [65]. Collectively, these data support the utility of TAT-CBD3 as a novel neuroprotective agent that may increase neuronal survival following injury by reducing surface expression of

dendritic NR2B receptors. Another study demonstrated a reduction in brain infarct volume following intracerebroventricular injection of a CRMP2 peptide, partially overlapping (i.e., six of 15 amino acids) with the authors' TAT-CBD3 peptide, in mice just before middle cerebral artery occlusion [176]. These findings validate CRMP2 as a therapeutic target for glutamate-mediated neurotoxicity as well as excitotoxicity-mediated neuronal death.

Targeting CRMP2 for acquired & post-traumatic epilepsy

Acquired epilepsy is the development of a chronic epileptic phenotype following an identifiable insult, such as TBI or stroke [177,178]. As at-risk populations are more readily identifiable, this class of disorders presents a unique opportunity for prevention. Understanding the molecular determinants of epileptogenesis will aid in the identification of novel therapeutic targets. While the major contributing factors in epileptogenesis remain elusive, current literature suggests a combination of increased excitability, loss of inhibition and reorganization of excitatory circuitry, among others (for review see Pitkänen and Lukasiuk [179]). As targeting excitability alone does not prevent the development of chronic epilepsy [180], epileptogenesis is most likely a multifactorial phenomenon. However, the exact causal relationship between circuit plasticity and epileptogenesis has yet to be determined. The most prominent example of such reorganization is the sprouting of mossy fibers within the hippocampus. Mossy fiber sprouting (MFS) is observed in the human epileptic hippocampus as well as in rodent models of acquired limbic epilepsy, including, but not limited to, electrically and chemically induced status epilepticus, kindling and post-traumatic epilepsy [181]. Sprouting of these fibers leads to the formation of recurrent excitatory circuits via aberrant innervation of the inner molecular layer [182]. Interestingly, MFS following induction of status epilepticus is associated with reduced expression of the chemorepellant Sema3A within the entorhinal cortex. In a working model, the Verhaagen group suggests that secretion of Sema3A into the molecular layer by entorhinal stellate cells prevents innervation of the inner molecular layer by granule cell axons [183], which express the receptor neuropilin 1 [184]. Accordingly, loss of this repulsive signal would allow for mossy fiber innervation of the inner molecular layer. As the chemorepulsive properties of Sema3A signaling are mediated by changes in CRMP2 phosphorylation [51], loss of Sema3A signaling could potentially lead to an accumulation of unphosphorylated CRMP2, which can actively promote neurite sprouting and outgrowth. With the intention of targeting CRMP2-mediated sprouting, the authors' laboratory has recently characterized a functional interaction between CRMP2 and the novel antiepileptic, lacosamide (Vimpat®, UCB Inc., NY, USA).

Lacosamide, recently approved for the treatment of partial-onset seizures, impairs voltage-gated sodium channel function through the selective enhancement of slow inactivation (Figure 4) [185]. However, the authors have demonstrated that lacosamide also inhibits the ability of CRMP2 to enhance tubulin polymerization, thereby impairing CRMP2-mediated neurite outgrowth [72]. Lacosamide treatment prevented enhanced excitatory connectivity associated with sprouting of injured laver V cortical pyramidal neurons in an animal model of post-traumatic epileptogenesis. The ability to target this process may prove instrumental in dissecting not only the role of CRMP2, but of aberrant sprouting and outgrowth in the development of acquired epilepsy.

Much attention has recently been bestowed on the involvement of the growth factor receptor TrkB in epilepsy. Increased expression of the ligand BDNF has been observed in the hippocampi of kindled animals and is accompanied by increased levels of TrkB activation [186]. Moreover, expression of a constitutively active receptor facilitated epileptogenesis following status epilepticus [187]. In a landmark study, the McNamara group observed that conditional deletion of the TrkB receptor is one of the only genetic modifications that completely prevents kindling in rodents [188]. TrkB signaling also appears to be of

particular importance in mossy fiber reorganization. Increased levels of activated TrkB are observed within this pathway in kindled animals [189,190]. In experiments with organotypic slice cultures, lesion-induced sprouting is observed in multiple regions within the hippocampus [191], which is prevented by TrkB receptor knockdown [192]. Most interestingly, infusion of BDNF into the hilus of the hippocampus was sufficient to induce MFS, as well as the formation of recurrent excitatory circuits [193]. Aside from its innate ability to promote outgrowth, CRMP2 may also play an important role in MFS as it is involved in forward trafficking of the TrkB receptor. CRMP2 associates with Slp1, forming a complex along with Rab27B, which links TrkB to the motor protein kinesin [56]. Taken together, the ability to regulate axon dynamics (sprouting, guidance and outgrowth) as well as TrkB signaling, position CRMP2 as a potential target in determining the underlying mechanisms associated with circuit reorganization during epileptogenesis.

Evidence for CRMP2 in bipolar/schizophrenia/schizoaffective disorders

Bipolar, schizophrenia and schizoaffective disorders are common, highly heritable psychiatric disorders for which familial co-aggregation, epidemiological and genetic evidence suggests overlapping etiologies. To date, however, no definitive susceptibility genes have been identified for any of these disorders. The expression of CRMP2 in humans was reported to be decreased in the frontal cortex regions of the brains of individuals with schizophrenia and affective disorder [194]. Notably, CRMP2 is located on chromosome 8p21, a region that has been implicated in schizophrenia in genetic linkage studies. A genetic association between five polymorphisms of the *CRMP2* gene and schizophrenia in a Japanese population reported that the frequency of a 2236T>C polymorphism in the 3′ untranslated region was higher in control subjects than in patients with schizophrenia, suggesting that the presence of this allele may reduce the susceptibility to schizophrenia [195]. A single-nucleotide polymorphism genotyping screen of 64 genes among Ashkenazi Jewish individuals demonstrated that CRMP2 met the criterion, using single-nucleotide polymorphism and haplotype-based transmission/disequilibrium tests, to strongly associate with genes implicated in schizophrenia or schizoaffective disorder and bipolar disorders [196]. In rats with pharmacologically induced chronic inhibition of NMDARs (a model for schizophrenia), CRMP2 protein levels were increased by approximately twofold compared with rats with normal NMDAR function [197]. As to how CRMP2 changes or allelic variations contribute towards these neuropsychiatric diseases is presently unknown.

Conclusion

As highlighted throughout this article, the landscape of interactions and functions subserved by CRMP2 is far more complex than previously appreciated. The authors' hypothesize that CRMP2 may serve as an adaptor/scaffold molecule and as a traffic 'cop' directing surface trafficking of several ion channels and receptors. While studies on CRMP2's canonical roles in axon specification and growth still abound, novel roles are being ascribed to CRMP2 from both *in vitro* and *in vivo* studies. Studies examining the mechanistic consequences of post-translational modifications of the CRMP2 protein and CRMP2 interactions with its binding partners in normal physiology and pathophysiology will be invaluable in furthering CRMP2's role as an important neuronal protein.

Future perspective

Basic research into CRMP2 is flourishing with reports ranging from splicing and single-nucleotide polymorphisms, to mechanistic details of CRMP2-mediated axon outgrowth and steering, discovery of novel kinases involved in phosphorylation of CRMP2 and identification of novel binding partners, to reports implicating CRMP2 in a cornucopia of diseases. Despite a largely neurocentric focus of most studies to date with CRMP2, it has

been linked to cancer and immune-related disorders. CRMP2 was selectively detected in a colorectal carcinoma cell line and was found to be increased in patients with colorectal carcinoma identifying it as a potential biomarker for colorectal carcinoma [198]. Recent studies have linked CRMP2 to the migration and polarization of T cells; it is thought that CRMP2 is required for rearrangement of the cytoskeleton required for proper T-cell function [23,199]. T-cell polarization and migration following onset of inflammation is an important aspect of the adaptive immune system. However, aberrant activation and migration of T cells in tissues where there is no pathogen present leads to immune disorders such as Crohn's disease, transplant rejection and airway inflammation. Therefore, strategies targeting CRMP2 to prevent T-cell migration and activation could be vital options to treat these disorders.

In summary, future research should focus on a deeper understanding of the post-translational modifications of CRMP2, particularly phosphorylation, and the functional interconnectivity of the CRMP2 protein network to allow researchers to develop specifically tailored therapeutics for 'CRMPopathies', broadly defined as a collection of diseases linked to CRMP2 dysfunction. Examples of this are already in place with the work of the Petratos group on mitigating MS [68] and the authors' work on the identification of small biomolecules to target CRMP2 interactions with CaV2.2 to ameliorate chronic pain [69–71]. We must also look beyond proteomic-based methods for identification of CRMP2 in nervous system disorders to evaluations of the structure–function relationship of CRMP2 that is likely altered in these disease states. For example, although CRMP2 levels have been reported to be decreased in aborted human fetuses with Down's syndrome, it is unknown how this CRMP2 decrease contributes to the impaired axon outgrowth observed in Down's syndrome [200]. Targeting CRMP2 phosphorylation or interfering with CRMP2 protein–protein interactions may offer some clues to mitigating the abnormalities in neural migration in Down's syndrome brains. One hypothesis is that a common thread in the CRMP2-related disorders may be dysregulation of calcium and this should be investigated further. Translating these basic research discoveries into clinically relevant therapies for some of the nervous system disorders in which CRMP2 is altered is imminent.

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- ■ of interest
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Executive summary

CRMP2

- CRMP2 is a cytosolic protein that functions in axon growth/specification, migration, proliferation and as a trafficking 'cop' for channels and receptors.
- CRMP2 is subject to post-translational modifications, including glycosylation, oxidation, proteolysis and phosphorylation.
- CRMP2 phosphorylation by Cdk5, GSK-3β, RhoK, CamKII and Fyn induces neurite retraction and collapse of growth cones.
- CRMP2 has an extensive network of binding partners that affect microtubule dynamics, protein endocytosis and vesicle recycling, synaptic assembly, calcium channel regulation and neurotransmitter release.

Targeting CRMP2 in neurodegenerative disorders

- Mutations in *CLN6* alter CRMP2/CLN6/KLC4 signaling and presumptive CRMP2 interactions contributing to variant late-infantile as well as adult-onset neuronal ceroid lipofuscinosis.
- Hyperphosphorylated CRMP2 is present in the brains of patients with Alzheimer's disease progression and may contribute to its pathogenesis.
- A truncated CRMP2 lacking phosphorylation site is found in prion-infected brains and may underlie degenerating neurons in prion diseases.

Targeting CRMP2 in sensory & motor neuron disorders

- Aberrations in CRMP2 function may contribute to amyotrophic lateral sclerosis axonopathy, which may be rescued by the CRMP2-acting small molecule lanthionine ketamine ester.
- Experimental autoimmune encephalomyelitis can be mitigated by *in vivo* administration of an antibody against the axonal growth inhibitor, Nogo-A, which reduces the levels of phosphorylated CRMP2 and improves pathological outcome.

Targeting CRMP2 in central disorders

- A peptide (TAT-CBD3) uncoupling CRMP2 from the presynaptic N-type calcium channel suppressed inflammatory and neuropathic pain.
- TAT-CBD3 protects from glutamate-induced excitotoxicity and is neuroprotective in focal cerebral ischemia or a closed-head concussive model of traumatic brain injury.
- The clinically used drug lacosamide, a target of CRMP2, inhibits CRMP2-mediated neurite outgrowth and tubulin polymerization and decreases excitatory synaptic connectivity due to post-traumatic axon sprouting.

Conclusion

- Calcium dysregulation may be a common denominator in 'CRMPopathies' and should be investigated in depth.
- It will be important to investigate the non-neuronal roles of CRMP2 (e.g., in cancer and in immunology).

- CRMP2 post-translational modifications are important determinants of CRMP2 function.

Future perspective

- Future research on CRMP2 phosphorylation and the functional CRMP2 interactome is necessary to allow researchers to develop specifically tailored therapeutics for CRMPopathies.
- CRMP2 phosphorylation or cleavage may be developed as a biomarker for various disorders.
- Genome-wide association studies may unravel CRMP2 polymorphisms, which are predictive of disease susceptibility.

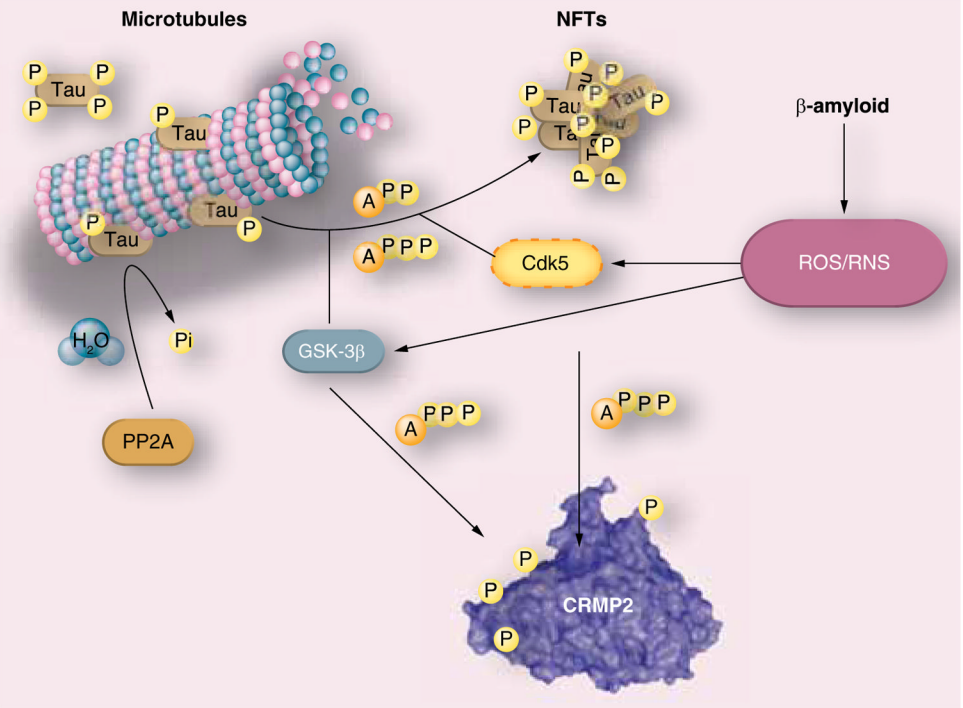

Figure 1. Phosphorylation of CRMP2 and tau proteins is mediated by Cdk5 and GSK-3β, and is key to the Alzheimer's disease pathogenesis

β-amyloid-induced oxidative stress directly/indirectly leads to increased activity of Cdk5 and GSK-3β. Both CRMP2 and tau proteins are phosphorylated by Cdk5 and GSK-3β, consequently leading to impaired neuronal communication, to neuropathological hallmarks, such as neurofibrillary tangles, to loss of synapses and ultimately to the learning and memory impairment observed in Alzheimer's disease.

A: ATP; NFT: Neurofibrillary tangle; P: Phosphate group; Pi: Liberated phosphate group; RNS: Reactive nitrogen species; ROS: Reactive oxygen species.

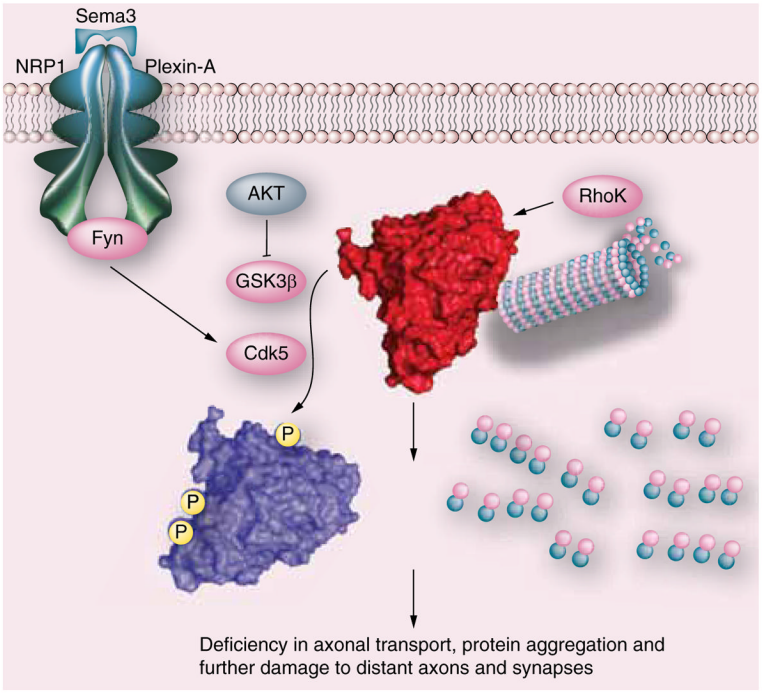

Figure 2. A model of Sema3A signaling to CRMP2 via the NRP1–plexin-A receptor system

This is a regulated pathway for axon microtubule restructuring that appears to be affected in amyotrophic lateral sclerosis, either by virtue of increased Sema3A expression in terminal Schwann cells near amyotrophic lateral sclerosis-sensitive neuromuscular junctions through aberrant CRMP4a expression, or by sequestration of CRMP2 within axonal inclusion bodies.

P: Phosphate group.

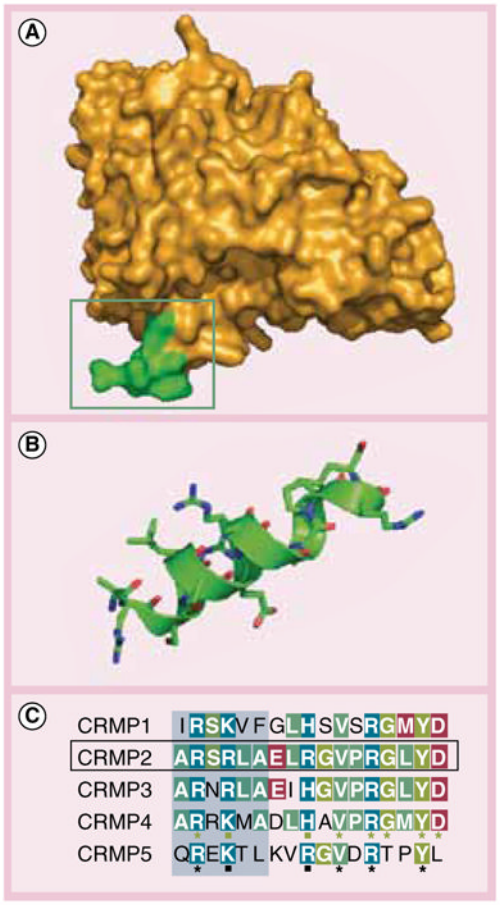

Figure 3. The antinociceptive and neuroprotective CRMP2 peptide CBD3

(A) Surface representations of the 3D structure of the CRMP2 monomer (Research Collaboratory for Structural Bioinformatics databank Protein Data Bank code: 2GSE) [27]. The six amino acids of the CBD3 peptide present in the structure are shown in green. Crystallographic images were rendered using PyMol™ (Schrodinger LLC, MD, USA). **(B)** A helix model of the entire 15-amino acid CBD3 region. **(C)** Amino acid alignment of the region of CBD3 across CRMP1–5. Fully conserved residues are indicated by the asterisk while the period indicates conserved substitutions. The purple dotted line represents the CBD3 peptide present in the crystal structure (boxed region in **[A]**).

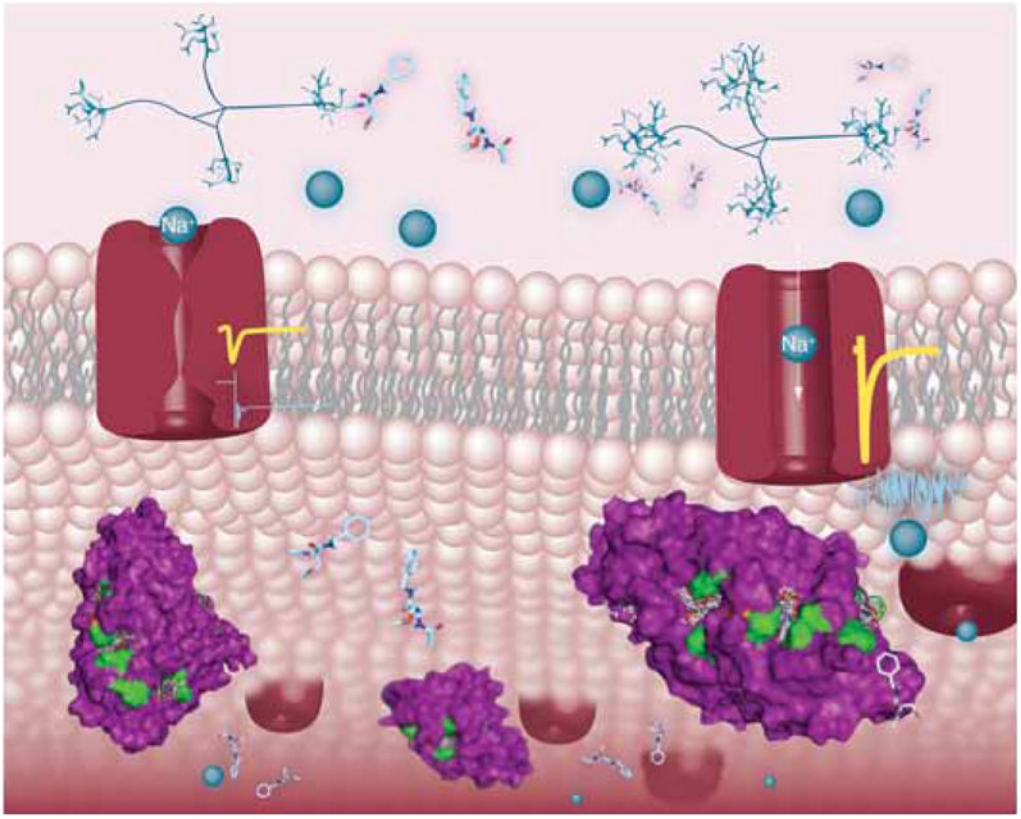

Figure 4. A model of lacosamide targeting of CRMP2 and sodium channels

In silico docking reveals five putative binding pockets for the antiepileptic drug lacosamide on CRMP2 (purple) [212]. Binding of lacosamide to these pockets may alter the efficacy of action of lacosamide on its primary target, the voltage-gated sodium channel (red). Sodium currents (yellow traces) are reduced by lacosamide. Evoked epileptiform events (light blue traces) in the undercut model of post-traumatic epilepsy rats are reduced by lacosamide. Emerging evidence suggests that lacosamide's mode of action involves interactions with CRMP2 to inhibit post-traumatic axon sprouting (dark blue).

Table 1

Summary of CRMP2 phosphorylation.

Phosphorylation site	Kinase	Effect on process growth	Signaling pathway(s)	Ref.
Tyr 32	Fyn, Fes	−	Sema3A	[34]
Tyr 479	Yes	+	CXCL10	[199]
Thr 509	GSK-3β	−	Sema3A	[15,51,93,201]
Thr 514	GSK-3β	−	Sema3A	[15,51,93,201]
Ser 518	GSK-3β	−	Sema3A	[15,51,93,201]
Ser 522	Cdk5	−	Sema3A	[51,201,202]
Thr 555	RhoK	−	LPA	[31,32]
	CaMKII	−	Glutamate	[33]

CRMP2 phosphorylation decreases process growth.

+: Increased cell process growth following CRMP2 phosphorylation (directly or indirectly through cell migration enhancement); −: No data/effect; CaMKII: Ca^{2+}/Calmodulin (CaM) kinase II; Cdk5: Cyclin-dependent kinase 5; CXCL10: C-X-C motif chemokine 10; Fyn: Fes, src-type tyrosine kinases; GSK-3β: Glycogen synthase kinase 3β; LPA: Lysophosphatidic acid; RhoK: Rho-associated protein kinase; Sema3A: Semaphorin 3A; Ser: Serine; Thr: Threonine; Tyr: Tyrosine; Yes: A src-type tyrosine kinase.

Table 2

Currently known interaction partners of CRMP2.

CRMP2 interaction partner	Validation		Effect of interaction	Ref.
	Biochemical	*Functional*		
Abl	Y	N	Weak binding partner; role not determined	[199]
Actin	Y†	Y	CRMP2 regulates actin dynamics	[25,31,32,40,156]
α-actinin	Y†	N	Role not yet determined	[156]
AP-2	Y	Y	Binding reduces endocytosis	[13]
sAPP	Y	Y	Phosphorylated CRMP2 reported in neurofibrillary tangles may deplete neurons of necessary CRMP, leading to abnormal neuritic and/or axonal outgrowth, thereby accelerating neurodegeneration	[203]
Blk	Y	N	Weak binding partner; role not determined	[199]
CaM	Y	Y	Binding prevents CRMP2 proteolysis and enhances process growth	[57]
CaV2.2	Y	Y	Binding regulates trafficking of CaV2.2 and downstream neurotransmitter release	[61,62]
α2-chimaerin	Y	Y	Binding decreases axon growth and neuronal migration; regulates bipolar transition and neuronal migration by modulating CRMP2 activity	[201,204]
CLN6	Y	Y	Disruption of binding decreases CRMP2 expression and likely contributes to neuronal dysfunction and pathology in vLINCL	[73]
CRMP1	Y	Y	Overexpression of CRMP1 and CRMP2 opposes the effects of RhoA on neurite retraction	[205]
CRMP5	Y	Y	CRMP5 acts a dominant-negative in preventing neurite outgrowth promotion induced by CRMP2	[206,207]
Dynein	Y	Y	Binding regulates dynein by linking it to cargo	[139,208]
αIN	Y†	N	Role not yet determined	[156]
Kinesin-1	Y	Y	Binding regulates kinesin by linking it to cargo	[53,209]
MICAL-L1	Y	Y	Links to intracellular dynein motors	[139]
MBP	Y†	N	Role not yet determined	[156]
NFL/NFM	Y†	N	Role not yet determined	[156]
Neurofibromin	Y	Y	Changes in neurofibromin expression alters CRMP2 phosphorylation	[59]
Neuropilin 1	Y	Y	Couples Sema3A to CRMP2 signaling	[27,201]
Numb	Y	Y	Binding regulates endocytosis	[13,32]
NMDARs	Y	Y	CRMP2 expression regulates surface expression of NR2B subunit	[64,65]
PIPP	Y	Y	Binding reduces neurite growth	[36]
PI3Kβ	Y	N	Moderate strength binding partner; role not determined	[199]
PLCγ	Y	N	Strong binding partner; role not determined	[199]
PLD_2	Y	Y	Binding inhibits PLD_2 activity	[199]
Plexin-1	Y	Y	Couples Sema3A to CRMP2 signaling	[27,201]
PP2A	Y	Y	Dephosphorylation of CRMP2 enhances axon growth	[37]

CRMP2 interaction partner	Validation		Effect of interaction	Ref.
	Biochemical	*Functional*		
ROCK-II	Y	Y	CRMP2 prevents ROCK-II from aiding in cell migration	[25]
Slp1	Y	Y	Links TrkB to CRMP2 for TrkB trafficking	[56]
α- and/or β-spectrin	Y†	N	Role not yet determined	[156]
Sra1	Y	Y	CRMP2 links Sra1/WAVE complex to kinesin for trafficking	[53]
Tau	Y	N	CRMP2 binds to both phosphorylated and nonphosphorylated forms; phosphorylated CRMP2 accumulation is observed in brains of Alzheimer's disease transgenic mice models	[96]
Thioredoxin	Y	Y	Binding induces CRMP2 phosphorylation causing growth cone collapse	[40]
TrkB	Y	Y	Links TrkB to kinesin for anterograde trafficking	[56]
α-/β-tubulin	Y‡	Y	Binding enhances microtubule formation	[48]
VAV1	Y	N	Moderate strength binding partner; role not determined	[199]
Vimentin	Y	Y	CRMP2 phosphorylation at Tyr 479 alters vimentin mobilization	[22,199]
WAVE1	Y	Y	CRMP2 links Sra1/WAVE complex to kinesin for trafficking	[53]
Yes1	Y	Y	Moderate strength binding partner; CRMP2 phosphorylation at Tyr 479 regulates CXCL12-induced T lymphocyte migration	[199]

† Identified by mass spectrometry to co-immunoprecipitate with CRMP2 [156].

‡ Cdk5/RhoK/CamKII phosphorylation decreases interaction [32].

CaM: Calmodulin; CaV2.2: N-type voltage-gated calcium channel; CLN6: Ceroidlipofuscinosis neuronal protein 6; MICAL1: Molecule interacting with CasL; NMDARs: NMDA receptors; N: No; PI3Kγ: Phosphoinositide-3-kinase p85 regulatory β-subunit; PIPP: Proline-rich inositol polyphosphate 5-phosphatase; sAPP: Amyloid precursor protein secreted from (sAPP) RERMS (328–332) neurotrophic domain; VAV1: Vav proto-oncogen SH2 domain 1; vLINCL: variant late-onset neuronal ceroidlipofuscinosis; Y: Yes; Yes 1: Yamaguchi sarcoma virus oncogene homolog 1.

Table 3

Current experimental and clinical drugs targeting CRMP2.

Compound	Disorder targeted	Rationale or mechanism of action
Experimental		
Ac-rER [66] (D-arg-L-glu-D-arg)	Alzheimer's disease	Mimic of soluble APPα, binds to CRMP2
Lanthionine ketamine ester [67]	Amyotrophic lateral sclerosis	Binds to CRMP2, likely confers increased axonal microtubule structure and function to CRMP2
Tianeptine [55]	Depression	Increases CRMP2 protein levels
TAT-CBD3 [70,71] (YGRKKRRQRRRARSRLAELRGVPRGL)	Inflammatory and AIDS therapy-induced neuropathic pain Migraine Glutamate-induced excitotoxicity Anxiety TBI MCAO	Inhibits functional interaction between CaV2.2 and CRMP2 and reduces transmitter release; blocks neuronal death following glutamate exposure likely via blunting toxicity from delayed Ca^{2+} deregulation; attenuates postsynaptic NMDAR-mediated currents in cortical slices
TAT-CBD3A6K [71] (YGRKKRRQRRRARSRLKELRGVPRGL)	Migraine	Inhibits functional interaction between CaV2.2 and CRMP2 and reduces transmitter release
TAT-CBD3G14F [69] (YGRKKRRQRRRARSRLKELRGVPRFL)	Migraine AIDS therapy-induced neuropathy	Inhibits functional interaction between CaV2.2 and CRMP2 and reduces transmitter release
TAT-CRMP2 peptide [176] (YGRKKRRQRRRGVPRGLYDGPVCEV)	MCAO Autohypoxia-induced hypoxic preconditioning	Prevents the cleavage of endogenous CRMP2 and may inhibit CRMP2 dephosphorylation
(R)-lacosamide ((2R)-2-(acetylamino)-*N*-benzyl-3-methoxypropanamide (lacosamide, LCM)	Post-traumatic epilepsy	Inhibits CRMP2-mediated neurite outgrowth and tubulin polymerization; decreases excitatory synaptic connectivity due to post-traumatic axon sprouting [72]
anti-Nogo(623–640) antibody [68]	Experimental autoimmune encephalomyelitis, a model of MS	Blocks CRMP2 T555 site phosphorylation
Clinical		

Compound	Disorder targeted	Rationale or mechanism of action
Tianeptine (Stablon®)	Depression Anxiety	Increases CRMP2 protein levels
Lacosamide (Vimpat®) [210,211]	Epilepsy	Enhances slow inactivation of sodium channels; CRMP2 expression likely modifies this effect

The TAT sequence (YGRKKRRQRRR) is appended *N*-terminal to the wild-type and point mutant CBD3 peptides.

MCAO: Middle cerebral artery occlusion; MS: Multiple sclerosis; NMDAR: NMDA receptor; TAT: Transduction domain of the HIV-1 trans-activator of transcription; TBI: Traumatic brain injury.