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Collapsin Response Mediator Protein-2: An Emerging Pathologic Feature and Therapeutic Target for Neurodisease Indications

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Abstract Collapsin response mediator protein-2 (DPYSL2 or CRMP2) is a multifunctional adaptor protein within the central nervous system. In the developing brain or cell cultures, CRMP2 performs structural and regulatory functions related to cytoskeletal dynamics, vesicle trafficking and synaptic physiology whereas CRMP2 functions in adult brain are still being elucidated. CRMP2 has been associated with several neuropathologic or psychiatric conditions including Alzheimer's disease (AD) and schizophrenia, either at the level of genetic polymorphisms; protein expression; post-translational modifications; or protein/protein interactions. In AD, CRMP2 is phosphorylated by glycogen synthase kinase-3β (GSK3β) and cyclin dependent protein kinase-5 (CDK5), the same kinases that act on tau protein in generating neurofibrillary tangles (NFTs). Phosphorylated CRMP2 collects in NFTs in association with the synaptic structure-regulating SRA1/WAVE1 (specifically Rac1-associated protein-1/WASP family verprolin-homologous protein-1) complex. This phenomenon could plausibly contribute to deficits in neural and synaptic structure that have been well documented in AD. This review discusses the essential biology of CRMP2 in the context of nascent data implicating CRMP2 perturbations as either a correlate of, or plausible contributor to, diverse neuropathologies. A discussion is made of recent findings that the atypical antidepressant tianeptine increases CRMP2 expression, whereas other, neuroactive small molecules including the epilepsy drug lacosamide and the natural brain metabolite lanthionine ketimine appear to bind CRMP2 directly with concomitant affects on neural structure. These findings constitute proofs-of-concept that pharmacological manipulation of CRMP2 is possible and hence, may offer new opportunities for therapy development against certain neurological diseases.

Keywords CRMP2 · Alzheimer · Schizophrenia · Epilepsy · Lanthionine · Tianeptine · Lacosamide

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Structure, Expression Pattern, and Protein Interactome of DPYSL2/CRMP2

Collapsin response mediator protein-2 (formally, dihydropyrimidinase-like protein-2 (DPYSL2) but also referred to by the abbreviations DRP-2, UNC-33, TOAD-64 or ULIP-2) is a cytosolic protein with primary sequence homology to the dihydropyrimidinase enzyme (DPYS) responsible for uracil and thymine catabolism. CRMP2 itself has no known enzymatic activity but interacts with its binding partners to affect microtubule dynamics, neurite outgrowth and retraction, neural differentiation, dendrite/axon fate specification, kinesin-dependent axonal transport, Ca^{2+} homeostasis, neurotransmitter release, and other essential neurophysiology still being elucidated [1–13]. Because the acronym CRMP2 best describes the predominant phenotypic behavior of the protein with which this review concerns itself, i.e., neurite length modulation, and the formal DPYSL2 nomenclature specifies no enzymological relationship of CRMP2 to DPYS, this review will refer to the protein as CRMP2 with the exception of

instances where the CRMP2 gene itself is referred to by the formal abbreviation *DPYSL2*.

CRMP2 is the most abundant and best-studied of five mammalian CRMP homologs [6, 7]. As might be expected from their role in central nervous system (CNS) patterning, CRMPs are developmentally regulated but only CRMP2 expression is retained at high levels in adult neurons and oligodendrocytes [6]. CRMP1 and CRMP3–5 expression in the adult CNS is maintained at low levels in restricted neuronal populations of the cerebellum, olfactory lobe, or hippocampus and in subsets of oligodendrocytes distributed along a distinct rostral-caudal gradient [6]. CRMP2 is expressed most heavily in highly plastic areas of the adult brain such as the hippocampus, olfactory bulb and cerebellum [6]. In neurons, CRMP2 normally is concentrated within the distal portions of neurites, in synapses and in growth cones [3, 4, 14]. Multiple CRMP2 isoforms result from alternative splicing of the N terminus, with predominant forms being a 75 kDa CRMP2A variant that is important during embryonic development and a 62–66 kDa CRMP2B variant (multiple sub-isoforms) that appears most commonly expressed in adult CNS [15]. CRMP2A appears localized to neural soma and axons whereas CRMP2B is localized to both axons and dendrites [15].

Structurally, CRMP2 is a homotetramer whose stability is enhanced by Ca^{2+} or Mg^{2+} [16]. At least 26 distinct CRMP2 post-translational modifications have been identified in normal rat hippocampus, including phosphorylations on Ser-Pro or Thr-Pro sites [16], Asn deamidation [16], Met oxidation [16], isoaspartyl conversion [17], and *O*-glycosylation (in synaptosomes) [18]. Interestingly, and perhaps especially relevant to the pathogenesis of Alzheimer's disease, recent proteomics analyses find CRMP2 to be one of the predominant targets for adduction by the lipid oxidation product 4-hydroxynonenal (HNE) in brains of patients with incipient AD [19, 20] (discussed further below).

Pull-down studies have created a diverse list of proteins that bind CRMP2 directly or coalesce with CRMP2 in multimeric complexes [6, 11–13]. Table 1 provides a summary of the growing list of CRMP2-interacting proteins. Although CRMP2 is best known for its role in organizing neuritic structure, the diverse array of interaction partners suggests the likelihood of other neurophysiologic functions for this protein.

Table 1 CRMP2-interacting proteins

Interaction partner	Reference
Actin	[13]
α-Actinin	[13]
Adaptor-related protein-2 (AP-2)	[28]
α-Internexin intermediate filament protein	[13]
Amyloid precursor protein secreted form (sAPP) RERMS (328–332) neurotrophic domain	[44]
Calmodulin (CaM)	[71]
Dynein	[29]
Kinesin	[28]
Molecule interacting with CasL (MICAL-L1)	[29]
Myelin basic protein	[13]
Neurofilament L and M	[13]
Nuerofibromin-1 (NF-1)	[11, 13]
Numb	[28]
N-type voltage-sensitive Ca^{2+} channel (CaV2.2)	[12]
α- and/or β-Spectrin	[13]
Tau (total and hyperphosphorylated)	[10]
TrkB neurotrophin receptor tyrosine kinase	[7, 25, 26]
α- and/or β-Tubulin	[13, 21, 23]
Wiskott–Aldrich syndrome protein family verprolin-homologous protein-1 (WAVE1)	[10, 41]

The CRMP2 primary interactome includes a number of proteins involved with cytoskeletal structure, dynamic cytoskeletal reorganization, axonal transport, or vesicle trafficking. Additional proteins with functional significance to ion channel function (CaV2.2), GTPase regulation or synaptic vesicle targeting (NF-1 and tubulin), or endocytosis (MICAL-L1 and AP-2) recently have been evidenced as CRMP2 binding partners. This list is not intended to be comprehensive and includes only proteins evidenced to bind CRMP2 directly, as opposed to secondary interactions within an extended protein complex, such as a neurofibrillary tangle

CRMP2 Dynamically Regulates Neurite Structure with Additional Roles in Vesicle Trafficking and Ion Channel Function

CRMP2 was first identified by Goshima and colleagues in chick dorsal root ganglia (DRG) cultures as a signal transducer responsible for axon growth cone retraction evoked by negative guidance signals in the semaphorin 3A (Sema3A) pathway of the developing nervous system [1]. Subsequent work largely performed by the Strittmatter, Kaibuchi, Goshima, and Charrier groups demonstrated that CRMP2 serves functions as a dynamic structure regulator of post-mitotic mammalian neurons as well, and clarified several mechanisms of this function [2–6]. CRMP2 acts largely, but not solely, by binding and stabilizing tubulin at the plus end of microtubules (Fig. 1, upper inset) thus promoting axon extension [1–7]. Thus, over-expressing CRMP2 in neuroculture causes general increase of neurite length, and can result in supernumerary axons [2, 8, 9]. Although CRMP2 serves some microtubule stabilizing functions superficially similar to microtubule-associated proteins (MAPs), CRMP2 is phylogenetically distinct from MAPs and has more diverse functions mediated through proteins besides tubulin [10–13].

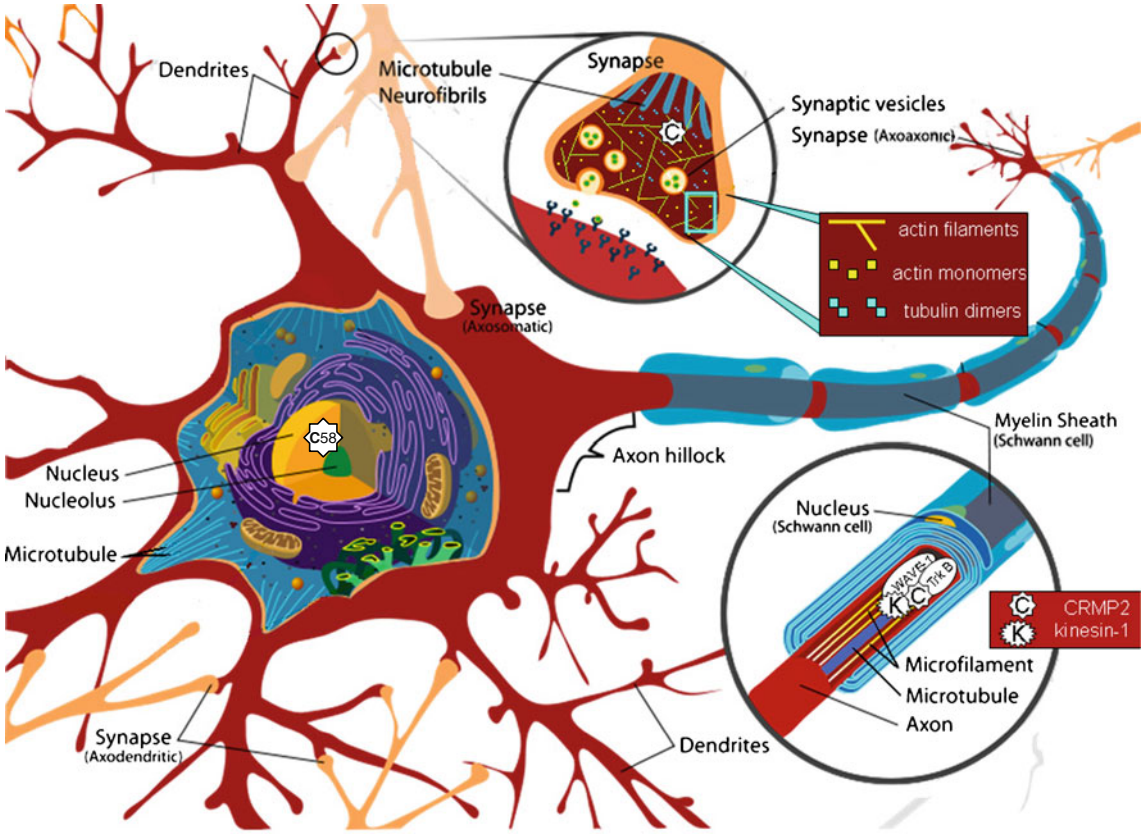

Fig. 1 Mechanisms by which CRMP2 affects neural structure. The cytoskeletal structure of a neuron is determined by tubulin-based microtubule networks that provide rigidity inside axons, by actin-based microfilament networks that provide flexibility near curvilinear branch points and synapses, and by intermediate (neuro)filaments that set axon diameter. Full-length CRMP2 promotes neurite growth through mechanisms involving all three types of cytoskeletal networks, as discussed in the text. Additionally, a 58 kDa CRMP2 proteolytic fragment (C58 in this figure) translocates to the nucleus under certain stress conditions and functions to inhibit neurite outgrowth. Neuron schematic modified with permission from [72]

The exact physical-biochemical mechanism by which CRMP2 stabilizes microtubules remains uncertain, but recent findings by Ryu and colleagues suggest that CRMP2 acts as a GAP (GTPase activating protein) to stimulate tubulin GTPase activity in vitro, in a manner that is subject to conformational properties of CRMP2 [21]. Though GTPase activation might be expected, based on first principles, to destabilize microtubules, this finding by Ryu's group may begin to shed light on the essential molecular nature of CRMP2: tubulin interaction. Alternatively, CRMP2 may largely affect microtubule stability in the growth cone by a mass-action affect mediated through CRMP2-dependent transport of tubulin dimers into, or out of, the distal axon [10, 15, 21].

CRMP2 interaction with tubulin is dynamically regulated during CNS development. In the now classic pathway for CRMP2-mediated growth cone collapse, semaphorin-3A (Sema3A) signaling through its receptors neuropilin-1 (NP-1) and plexin A (PlexA1–3) triggers Rac1 activation, affecting downstream kinases and ultimately activating glycogen synthase kinase 3β (GSK3β) which phosphorylates CRMP2 on Thr-509 and Thr-514 [3–8, 22]. The GSK3β-mediated CRMP2 phosphorylation is prerequisite upon prior phosphorylation of CRMP2 Ser-522 by cyclin dependent kinase-5 (CDK-5) [3, 4, 22, 23]. In a situation analogous to that of tau protein phosphorylation [24], this CDK-5 "priming" is necessary to allow subsequent GSK3β-mediated phosphorylation events. Phosphorylated CRMP2 loses affinity for tubulin heterodimers thus reducing microtubule growth at the distal end of axons, encouraging axon retraction [3–5]. Conversely, neurotrophin-3 and brain-derived neurotrophic factor inhibit GSK-3β via the phosphatidylinositol-3-kinase (PI3K)/Akt pathway, reducing CRMP2-(pThr-509/pThr-514) and promoting axon growth [4, 25].

Other extracellular-regulated signaling pathways have since been discovered that affect CRMP2-dependent neuritic structural dynamics. For instance, a second and sempahorin-independent pathway through which CRMP2 affects neurites is an indirect anterograde transport mechanism (Fig. 1. lower insert, axon schematic). During this process, CRMP2 adapts the microtubule motor kinesin-1 to ante-

rograde transport vesicles carrying the neurotrophin receptor tyrosine kinase TrkB [7, 25, 26]. After insertion into the cell membrane and activation by its cognate ligand, TrkB promotes axon growth by signaling for accumulation and polymerization of F-actin in distal axon shafts and growth cones [7, 25]. GSK3β-phosphorylated CRMP2 releases kinesin-1, impeding TrkB function and reducing structural integrity of the actin-based cytoskeleton in distal axons, growth cones, and synapses [26].

A third pathway of CRMP2-dependent neuritic remodeling and axonogenesis operates through kinesin-1-dependent transport of the Sra1/WAVE1 (specifically Rac1-associated protein-1/WASP family verprolin-homologous protein-1) complex [10]. Analogous to the case with TrkB, CRMP2 links kinesin-1 to Sra1/WAVE1 for transport to distal axons and synapses (Fig. 1). There, WAVE1 activates the Arp2/3 complex which in turn nucleates actin monomers which otherwise would be kinetically impeded from polymerization into microfilaments [27]. RNA interference of CRMP2 delocalizes WAVE1 from growth cones, triggering cone collapse [10]. Similarly, knockdown of Sra1 and WAVE1 cancels CRMP2-induced axon outgrowth [10] indicating that proper connection of CRMP2 to Sra1/WAVE1 is essential to preserve integrity of distal actin networks. In normal rodent neuron culture CRMP2 concentration seems to be limiting in neurite outgrowth because increasing CRMP2 by transfection or treatment with the CRMP2 expression-inducing compound tianeptine (discussed below) is sufficient to stimulate neurite extension [1–3, 14].

In yet a fourth known pathway of CRMP2-regulated growth cone retraction, lysophosphatidic acid activates RhoA-GTPase affecting downstream Rho kinase which phosphorylates CRMP2 on Thr-555 [6, 8]. This event triggers growth cone collapse separately from the Rac1/GSK3β-regulated pathway [6, 8].

The pathways described above operate in a normal, uninjured CNS. Recently, it has emerged that limited C-terminal proteolysis after neurotrauma, ischemic injury or excitotoxicity can produce a 58 kDa truncated CRMP2 with an unmasked nuclear localization signal that acts in a dominant fashion to inhibit neurite outgrowth [15] (Fig. 1). The degree to which this short CRMP2 functions in regulated, normal developmental neurobiology or in spontaneous neurodisease versus instances of brain trauma remains to be determined.

The growing list of CRMP2-binding partners (Table 1) suggests other functions of CRMP2 outside the scope of neuritogenesis, synaptogenesis, and growth cone retraction. The known CRMP2 interactome includes such diverse species as the neurofibromatosis-associated Ras-GAP protein neurofibromin-1 [11, 13] and the N-type voltage-sensitive Ca^{2+} channel CaV2.2 [12] (Table 1). In some cases novel CRMP2 binding has been shown to have functional significance. For instance, CRMP2 overexpression increases Ca^{2+} current density via CaV2.2 whereas lentiviral knockdown of CRMP2 reduces these densities with concomitant increase or decrease in vesicular neurotransmitter release under a depolarizing stimulus [12]. In another example, Kaibuchi and colleagues report that CRMP2 binds the protein Numb thus regulating Numb interaction with the clathrin-coat protein α-adaptin, such that siRNA knockdown of CRMP2 or expression of truncated, dominant-negative CRMP2 inhibits endocytosis at axon growth cones [28]. In non-neuronal cells, CRMP2 has been further implicated as a key regulator of endocytosis and vesicle recycling through its action as a linker between dynein and MICAL-L1 [29]. Thus, CRMP2 is a versatile adaptor protein that serves diverse purposes in cell physiology.

CRMP2 is Intimately Associated with Cardinal Neuropathologic Features of AD

AD is defined neuropathologically by the presence of both β-amyloid (Aβ) peptide-rich plaques and neurofibrillary tangles (NFTs, composed of abnormally phosphorylated microtubule-associated tau protein) in specific brain regions including the entorhinal cortex, hippocampus and isocortex; combined with loss of neural arborization and synaptic connections (i.e., frank neural atrophy) [30–32]. NFTs are located throughout the grey matter, but dystrophic neurites (axons) form in a distinctive corona about the plaques [30–33]. Although plaques and tangles are histologically dramatic, synapse loss is most consistently and significantly correlated to clinical dementia [30, 34]. Synapse loss is evident even in mild cognitive impairment (MCI), a prodromal state of AD [35]. Ultrastructural stereology indicates an approximate 20% loss of synapses in hippocampal CA1 of MCI patients progressing to 55% loss in mild–moderate AD, with a generally consistent correlation to local NFT density [34, 35]. The most striking synaptic changes occur in close apposition with Aβ plaques where loss of dendritic spines (orthogonal presynaptic termini) is remarkable [35]. Loss of dendritic arborization in human AD also is well documented, for instance, manifesting as decreased average length per cell of Golgi-stainable branches and numbers of branches in particular amygdaloid and hippocampal neurons [31, 32, 34]. Although neuritic atrophy and synaptic structural pathology is subtler in AD mouse models than in human AD, synaptic deficits are inherent to the "triple transgenic mouse" [presenilin-1 (PS1)(M146V)KI; Thy1.2-amyloid precursor protein (APP)(swe); Thy1.2tau(P301L); "3xTg-AD"] that develops AD-like amyloid plaques and NFTs [36].

CRMP2 is well documented to be hyperphosphorylated in the AD brain in close association with NFTs, though total, *extractable* CRMP2 is not affected (the amount of

functionally available CRMP2 in AD vs. normal brain is not known) [3, 23, 37–41] (Fig. 2). CRMP2 is phosphorylated by the same CDK5 and GSK3β kinases responsible for pathological tau phosphorylation [24]. Thus, drugs that decrease CRMP2 phosphorylation might also beneficially affect tau aggregation. CRMP2 localization to NFTs has been confirmed by immunohistology, co-immunoprecipitation experiments and by laser micro-dissection-assisted proteomics analyses [37–41]. In fact, CRMP2 phosphorylation in AD is so pronounced that it has been used as a marker of NFTs: In the 1990s, an antibody called 3F4 (not related to the 3F4 antibody commonly used to visualize prion proteins) raised against partially purified tau filaments was characterized by Ihara et al. who identified its cognate antigen as dual-phopshorylated CRMP2 (pT509 and pS522) [37, 39]. Ihara suggested that CRMP2 collection in NFTs might deplete neurons of functional CRMP2, thus predisposing their neurites to degeneration [37], an idea that has neither been directly tested nor used as a basis for pharmacotherapy because, until very recently, there has been no pharmacological means of manipulating CRMP2.

A similar CRMP2 hyperphosphorylation is observed in cortex and hippocampus of the 3xTg-AD mouse. In 2009, Kitamura and colleagues reported that CRMP2 co-aggregates with both tau and WAVE1 in NFTs of the 3xTg-AD mouse but not in Tg2576 or JNPL3 mouse strains that have only plaques or tangles, respectively [41]. The 3xTg-AD mouse is unique amongst these mouse models in that it demonstrates synapse loss and electrophysiological deficits [36, 41]. In other words, amongst these three mouse strains, the protein component unique to the triple-transgenic (synapse-sensitive) animal is WAVE1 trapped in the Tau^{+}, $CRMP2^{+}$ deposits. CRMP2 therefore offers evidence in favor of its mechanistic linkage between two very distinct aspects of AD, namely plaques and tangles: some interaction between the plaques and tangles appears necessary to trap CRMP2 and its cargo proteins in the NFTs, at least in murine models. WAVE1 and 3F4 co-localization was observed in human AD brain as well, and furthermore direct binding of WAVE1 to CRMP2 was evidenced through co-immunoprecipitation experiments performed on human AD brain lysates [41], thereby confirming the relevance of the murine observations to the human pathology [41].

Thus, considering the role of CRMP2 in maintaining neurite cytoskeletal integrity particularly through anterograde transport of actin regulators toward the synapse (discussed above) these discoveries begin to suggest that Aβ and tau pathways might converge to bring about AD synapse loss through a CRMP2-dependent mechanism. Such a hypothesis functionally relates the three cardinal neuropathologic features of AD: Aβ, NFTs, and synapse loss. Although plausible this hypothesis requires much further investigation of human AD specimens, and further studies of appropriate animal models.

The processes that encourage co-aggregation of CRMP2 and its binding partners within NFTs may be oxidative in nature because redox stress is a major recognized pathocomponent of AD which is commonly thought to arise, in part, from various amyloid-dependent processes [42]; and because oxidative stress is a well-known factor that can initiate or propagate protein aggregation phenomena through both covalent cross-linkage and increased hydrophobic protein: protein interac-

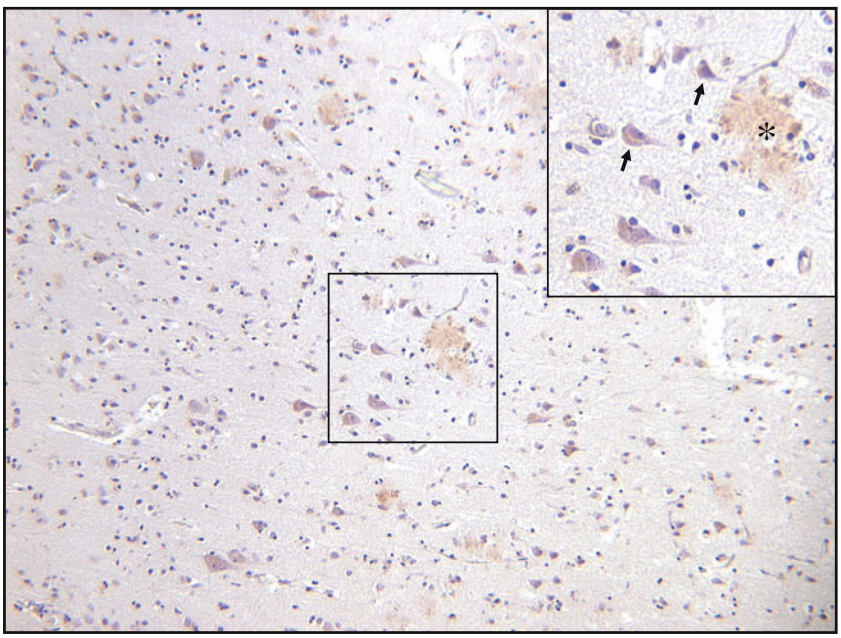

Fig. 2 CRMP2 is hyperphosphorylated in human AD brain. A histological section from the frontal pole of the brain of an elderly female with post-mortem neuropathologically confirmed Alzheimer's disease was immuno-reacted with anti-CRMP2-pThr-514 which recognizes a GSK3β-targeted phosphorylation site. The figure represents a 20x image with the *boxed* region expanded to highlight salient histopathologic features. Note reactivity in tangles (*arrows*) and in the neuritic corona of amyloid plaques (*asterisk*)

tions [43]. Consistent with such a possibility, CRMP2 reportedly is heavily adducted by the lipid oxidation product HNE in the AD brain ([19, 20] and personal observations). This oxidative modification happens by the clinically definable early stage of AD (early AD (EAD)). EAD is the intermediate stage between mild cognitive impairment and frank AD [20] which is becoming appreciated as an appropriate clinical stage for targeted therapy development.

In addition to the logic described above for considering CRMP2 as a pathologically-involved protein and potential therapeutic target for AD, independent lines of evidence suggest that CRMP2 binds a specific amino acid sequence within secreted forms of amyloid precursor protein (APP) that mediate neurotrophic actions of sAPP [44]. This interaction necessarily would require the sAPP or a fragment thereof to be internalized by the target cell before presentation to CRMP2. Agents that mimic the sAPP: CRMP2 interaction might mimic some of the beneficial natural actions of APP, for net benefit in AD.

CRMP2 Associations with Neuropsychiatric Conditions, Particularly Schizophrenia

Emerging data suggest that CRMP2 may be pathologically associated with a variety of neurodegenerative or psychiatric disorders, not restricted to AD. Currently, the neuropsychiatric case is strongest for CRMP2 association with schizophrenia. CRMP2 has been linked to schizophrenia both at the genetic level and at the level of protein expression. Nakata et al. first reported [45] that a *2,236 T>C polymorphism in the 3′ untranslated region of *DPYSL2* occurred with significantly lower frequency in Japanese patients with schizophrenia (p= 0.0097) and paranoid schizophrenia (p=0.0083) than in psychiatrically normal subjects [45]. Subsequent studies failed to confirm this association in a large Japanese population but found suggestive evidence in a subgroup [46]. In separate work, Pulver and colleagues reported results of a single nucleotide polymorphism screening study of Ashkenazi Jewish persons that indicated a CRMP2 association with either schizophrenia, bipolar disorder, or both [47]. At the protein level, seven of 13 relevant proteomics studies have identified significant changes in CRMP2 protein level in schizophrenic brain (reviewed in [48]). The protein is variously described to be increased or decreased depending on the study and the brain region investigated [48]. In another unbiased brain proteome-wide search for differentially expressed proteins, CRMP2 was reportedly one of only six proteins whose expression was changed (for CRMP2, decreased) in frontal cortex of patients with bipolar disorder or depression without schizophrenia [49]. The relevance of *DPYSL2* polymorphisms to schizophrenia is currently being debated in the literature, with most emphasis on identifying and understanding possible gene×environment or gene×gene interactions [50].

If CRMP2 does prove contributory to subsets of schizophrenia, the mechanism is likely to be either neurodevelopmental, or else allosteric via CRMP2-dependent ion channel modulation. Neurodevelopmental patterning of cognitive circuits certainly could create an organic basis for later expression of behavioral phenotypes, perhaps in combination with independent genetic factors or particular environmental exposures. In this case, pharmacological manipulation of CRMP2 expression or function may not prove beneficial for adults suffering schizophrenia. Alternatively, emerging ionotropic functions for CRMP2 suggest a reason that CRMP2-targeting therapies might alter the clinical presentation of the disorder. Primary disorders of schizophrenia, such as altered dopaminergic and glutamatergic neurotransmission, are being increasingly linked to dysregulated neuronal Ca^{2+} homeostasis [51]. Simultaneously, dopamine receptor interacting proteins, including neuronal Ca^{2+} sensors, are upregulated in schizophrenia [52]. Amongst other functions, dopamine regulates neuron excitability through both ligand- and voltage-gated ion channels [51, 52]. As discussed above, CRMP2 overexpression increases Ca^{2+} current density via CaV2.2 whereas lentiviral knockdown of CRMP2 reduces these densities with concomitant increase or decrease in vesicular neurotransmitter release [12]. If CRMP2 proves to dynamically regulate either schizophrenia-associated Ca^{2+} transients or downstream neurotransmitter vesicle trafficking, then drugs that either support or antagonize these aspects of CRMP2 function could prove useful either in treating schizophrenia, or in the preclinical investigation of schizophrenia model systems.

CRMP2 is A Credible Target for Experimental Therapeutics

In recent years there have been at least one neurotrophic protein and three small molecules reported to either bind CRMP2 or induce CRMP2 expression, with potential therapeutic implications. Takahashi first reported (2004) that glial-derived neurotrophic factor (GDNF) could increase CRMP2 expression in human neuroblastoma cell culture through a pathway involving extracellular regulated kinase but independent of PI3K and Src family kinases [53]. This GDNF effect appeared dependent upon a 214-48 bp region of the CRMP2 promoter upstream from the transcription start site, containing SP1-, E2F-, and GATA1/2-binding sequences [53]. Unfortunately from the standpoint of experimental therapeutics, proteins such as GDNF are generally poor candidates for treating neurodisease because of their size and limited ability to cross the blood–brain barrier.

Very recent serendipitous discoveries have revealed that small molecule neuroactive substances can also induce CRMP2 protein expression, at least in cell culture. In a proteomics study intended to uncover protein changes in primary mammalian neurons exposed to the atypical antidepressant and anxiolytic drug tianeptine [(*RS*)-7-(3-chloro-6-methyl-6,11-dihydrodibenzo[*c, f*][1, 2]thiazepin-11-ylamino) heptanoic acid *S*,*S*-dioxide; Stablon or Coaxil], Chu et al. found a highly selective drug-induced increase in CRMP2. Tianeptine increased CRMP2 expression up to 4-fold, with a concomitant promotion of neurite elongation and especially, axon extension [14]. Our group has independently observed and confirmed this phenomenon (Fig. 3). Additionally, we find that tianeptine at low micromolar concentrations is able to potently promote the outgrowth of neurites in chick dorsal root ganglia cultures, a classic model system for the study of CRMP2-dependent neural remodeling (Fig. 4).

The discovery by Chu et al. suggests that it might be possible to pharmacologically increase CRMP2 with brain-accessible small molecules in order to preserve neural function in the face of pathophysiological stress. Tianeptine is particularly compelling to consider for AD because of its benign safety profile and well-studied pharmacodynamics including studies in emotionally depressed elderly [54]. For this reason, a slightly more detailed discussion of tianeptine is justified.

Tianeptine is an atypical antidepressant produced and marketed by Laboratoires Servier SA. Tianeptine does not share the pharmacological properties of tri-cyclic antidepressants, monoamine oxidase inhibitors, or selective serotonin reuptake inhibitors (SSRIs) leading to major unresolved questions about tianeptine's essential mechanism-of-action (MOA) [55]. Tianeptine acts as an acute selective serotoin reuptake enhancer rather than an SSRI, though chronic treatment may down-regulate serotonin transporters [55]. Although tianeptine has been used for two decades in Europe for treating human depression, and numerous clinical studies document that tianeptine efficacy is at least comparable to SSRIs while generally possessing better tolerance and lesser drug interactions [55–57], no studies have yet been undertaken to specifically assess effects on cognition in patients with AD or its prodromal state, MCI. A detailed literature search revealed only one report of tianeptine use in AD. A small Polish study of tianeptine vs. fluoxetin in treating *depression complications* of AD observed $N=16$ patients on tianeptine vs. 19 patients on fluoxetin [57]. Tianeptine appeared better-tolerated and produced more

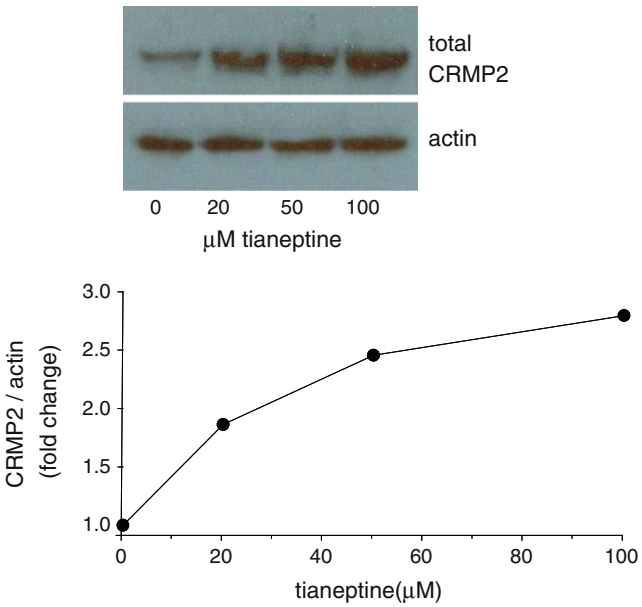

Fig. 3 Tianeptine upregulates CRMP2 in mammalian neurons. Rat embryonic cortical neurons were treated 24 h with tianeptine, lysed, and western blotted for CRMP2 or actin. CRMP2 expression level was normalized to actin for graphical purposes

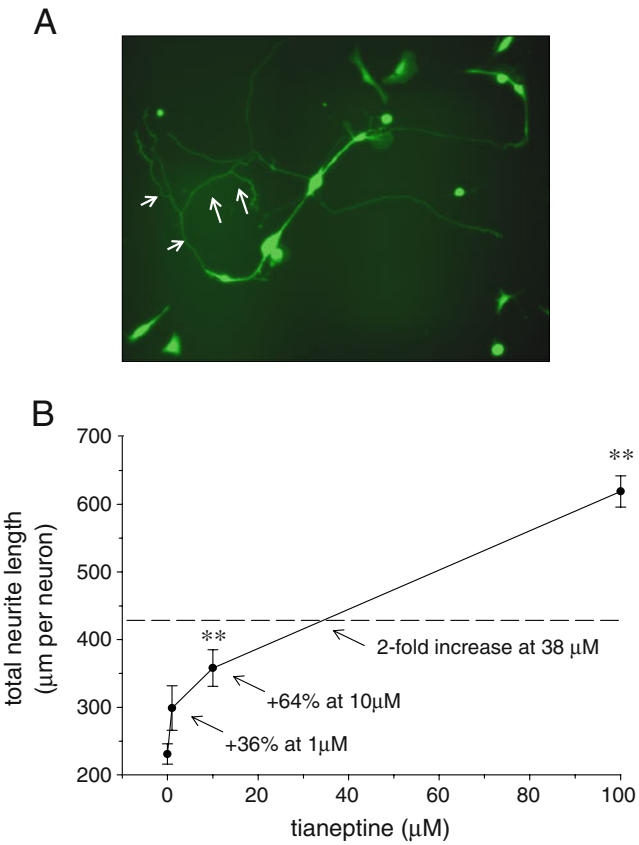

Fig. 4 The CRMP2 expression-inducing small molecule tianeptine promotes neurite outgrowth. **a** A typical chick DRG neuron (DIV4), showing ample neuritic arborization (arrows). Cells were cultured as described previously [73], labeled with Promega Live/Dead® reagent and imaged as described previously [13, 67]. Neurites (*arrows*) were measured by a blinded analyst using MetaMorph® software (molecular devices). **b** Neurite length as a function of tianeptine. Each point is mean ± SEM of 45–70 neurons from three experiments; $^{**}p<0.01$ by Student's *t* test. The *dashed horizontal line* represents the point of 2-fold increase of mean neurite length

rapid alleviation of depression on a clinical assessment tool, though the report did not mention any outcomes on cognitive exams [57]. This study was neither designed nor powered to assess effects on dementia [57]. Taken together these findings raise the question: Why has tianeptine not been developed for dementia or approval sought in the USA? In a thorough review of tianeptine pharmacology, Preskorn opines "…its [tianeptine's] patent life may have run our before the company [Servier] could develop resources to more actively pursue development of the drug in the United States…" [55]. Preskorn notes that tianeptine's MOA has never been proven but that "If confidence in tianeptine's MOA were sufficiently high, its structure could provide a platform for the development of analogs with sufficient patent life to warrant more extensive development efforts" [55].

Despite the dearth of human clinical studies of tianeptine in the context of neurodegenerative disease or cognitive neuropathology (as opposed to studies focusing purely upon depressive indications), there is considerable preclinical literature about the neurotrophic, neuroprotective, or neuroregenerative potential for tianeptine against stress-induced anxiety and stress or trauma-induced brain injury. Indeed, several French studies report that tianeptine treatment of rodents can reduce, or even reverse atrophy of hippocampal neurons caused by restraint stress or corticosterone administration *in vivo* [58, 59]. Thus, the behavioral action of tianeptine to reduce anxiety correlates with a physical protection of neural structure, at least in rodents. Tianeptine improved rodent memory performance of non-diseased, non-stressed animals on discrimination tasks such as T-maze, and radial arm water maze, whereas other antidepressants tend to suppress performance [55, 58]. These tianeptine effects have lately been ascribed to drug-induced increases in synaptic plasticity (long-term potentiation and primed burst potentiation) [55] though the molecular and cellular targets have yet to be identified that would explain the memory-enhancing (noötropic) action of tianeptine. Given the role of CRMP2 in modulating neural structure, synaptic architecture and possible interactions with ion channels, it is obligatory to consider CRMP2 as a plausible molecular explanation for the neurotrophic and/or noötropic features of tianeptine.

The two small molecules that so far have been evidenced to bind CRMP2, thus possibly explaining their mechanisms-of-action in humans or animal models, are lacosamide ((*R*)-2-acetamido-*N*-benzyl-3-ethoxypropionamide; Vimpat) [60–63] and lanthionine ketimine [63–65]. Lacosamide is a recently registered, FDA approved compound marketed by UCB Pharma as an adjunctive agent for treatment of refractory partial-onset epileptic seizures [60]. Lacosamide is most frequently discussed with reference to its ability to selectively enhance slow inactivation of voltage-gated sodium channels without affecting fast inactivation, thereby stabilizing neuronal firing patterns [60, 61]. Lacasomide was identified as a CRMP2-binding ligand through proteomics experiments, and subsequent radioligand-binding studies indicated a binding affinity of approximately 5 μM [61]. These findings led to experiments in which lacosamide was found to inhibit neurotrophin-mediated axonal outgrowth of cultured hippocampal neurons at low micromolar concentrations of the drug [61]. Since epilepsy is associated with abnormal neurite sprouting in the hippocampus, this apparent CRMP2 functional antagonism could explain some of lacosamide's utility against epileptic seizures. Lacosamide also has been evidenced to provide anti-nociceptive action [61] or anxiety relief [63] in animal models. Despite the apparent antagonism of CRMP2 by lacosamide, lacosamide's anxiolytic effects are reminiscent of anxiolytic activity inherent to the aforementioned CRMP2 expression inducer, tianeptine. It is currently unclear to what extent the neurophysiological affects and clinical benefits of lacosamide map to its CRMP2-binding properties vs. more direct action on sodium channels, however considering the recent finding that CRMP2 can bind voltage-sensitive Ca^{2+} channels [12] and otherwise indirectly influence organization of synaptic peri-membranous cytoskeletal structures (discussed above), further research in this direction is justified.

Lanthionine ketimine (LK; 2*H*-1,4-thiazine-5,6-dihydro-3,5-dicarboxylic acid) is a natural brain sulfur amino acid metabolite probably derived from non-canonical reactions catalyzed by the transsulfuration enzyme cystathionine-β-synthase [14, 64–66]. Our group recently discovered that LK possesses various biological activities including the ability to potentiate growth factor-mediated neurite outgrowth, and another ability to antagonize the activation of microglia and macrophages by pro-inflammatory cytokines [13, 66–68]. In an effort to identify binding partner candidates responsible for these activities, LK was covalently attached to a solid-phase matrix which was then used to capture LK-binding proteins. One of the principal identified LK-binding candidates was CRMP2 [13]. The LK interaction with CRMP2 was corroborated by treatment of mouse brain lysate with LK, followed by immunoprecipitation and mass spectrometric identification of CRMP2-binding partners [13]. LK altered the spectrum of CRMP2/protein interactions, generally decreasing the number of proteins that co-immunoprecipitated with CRMP2 but notably increasing the degree of interaction between CRMP2, and specific proteins including neurofibromin-1 and neurofilament-M [13]. These findings demonstrate that at least one small molecule experimental therapeutic can interact with CRMP2 in a manner that has functional consequences to the CRMP2 interactome.

The neuritogenic (neurite growth-promoting) effects of LK have been observed when either NSC-34 mouse motor neuron-like cells [13] or primary chick DRG cultures ([13]

and this work) were treated with a cell-penetrating ethyl ester derivative (LKE) ([67] and this work). Our initial experiments using 100 μM of LKE showed approximately 2-fold increase in mean neurite length of DRG neurons following 24-h treatment [67]. However, more thorough experiments exploring a wider concentration range and a 48-h treatment period demonstrated that the effect of LKE is actually much more potent (Fig. 5). Compared with tianeptine, which is effective in the micromolar range, only nanomolar concentrations of LKE are required to induce a significant increase in mean neurite length in DRG cultures. These concentrations approximate the physiological concentrations of bovine brain LK (0.5–1 nmol/g) as measured by Ricci, Cavallini, and colleagues [69] suggesting that the neuritogenic properties of LK may serve a natural physiological function in the mammalian CNS. Despite the potency of LKE in promoting neurite extension, the compound displays no toxicity to at least 100 μM in DRG cultures (data not shown) or 500 μM in NSC-34 cells and, in fact, higher concentration of LKE protect NSC-34 cells from oxidative stress insults [13, 67, 68]. Thus, in terms of cell culture effects vs. tolerability, the therapeutic window for LKE spans at least three orders of magnitude, a highly desirable feature of any experimental therapeutic. Because of the CRMP2 function in growth factor-mediated neurite extension (described above), coupled with the direct observation that LK binds CRMP2 in brain lysates [13], the hypothesis is credible that the neuritogenic action of LK is mediated through one or more CRMP2-dependent pathways.

LKE is attractive as an experimental therapeutic because of its apparent lack of toxicity in cell culture. Moreover, our group has demonstrated that administration of LK-ethyl ester slows disease progression in the $SOD1^{G93A}$ mouse model of the neurodegenerative disease amyotrophic lateral sclerosis (ALS) [67, 68] in which, incidentally, prognosis is also beneficially affected by tianeptine [70]. These in vivo findings begin to validate the potential of LK derivatives as novel pharmacophores for therapeutic intervention against certain neurodegenerative disorders. Further research is needed to better define the spectrum of neurodisease indications that might be amenable to treatment with LK derivatives.

Conclusions

CRMP2 is a functionally important protein in neurophysiology whose expression and/or post-translational modifications are demonstrably altered in Alzheimer's disease, schizophrenia, and possibly other neurological conditions. Both the expression of CRMP2 and its interaction with other proteins can be pharmacologically manipulated by small molecule drug candidates such as tianeptine, lacosamide and lanthionine

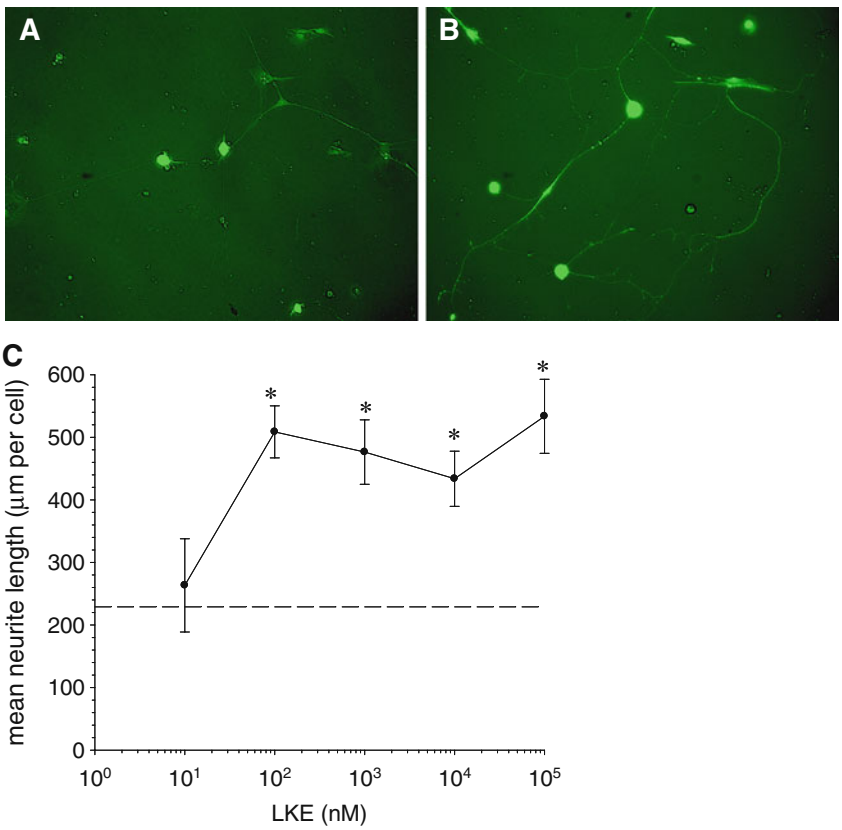

Fig. 5 The CRMP2-interacting central nervous system metabolite lanthionine ketimine promotes neurite outgrowth in chick dorsal root ganglia cultures. DIV3 neurons were treated 48 h with vehicle (**a**) or with LKE (**b**, **c**) then treated with Promega Live/Dead® fluorescent reagent [13, 67], photomicrographed, and quantitatively assessed for morphometry by an analyst blinded to treatment group. Data indicate mean ± SEM for ten to 30 microscopic fields at each LKE concentration. Control neurite length (no drug) was 239±23 μm (*dashed line*). *$p<0.05$ by *t* test

ketimine. These molecules offer proof of the concept that CRMP2-targeting drugs can offer therapeutic benefit at least in specific preclinical models including the $SOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis. Further research is needed to understand the full spectrum of CRMP2 functions both in healthy and diseased adult brain; to better elucidate the precise molecular mechanisms by which tianeptine, lacosamide and LK affect CRMP2-dependent processes of neuron growth and neurophysiology; to test CRMP2-acting drugs in appropriate preclinical models of neurodisease; and to identify other small molecules that can selectively alter CRMP2 expression or affect CRMP2-binding interactions. It may prove to be the case that certain neurodegenerative pathologies (e.g., Alzheimer's disease, ALS, spinal cord injury, etc.) or neoplasms (e.g., neurofibromatosis) could benefit from functional support of CRMP2 whereas other pathologies of abnormal neuritic sprouting or synaptogenesis (e.g., epilepsy and neuropathic pain) could benefit from functional CRMP2 antagonism. Thus it will be important that future research into CRMP2-directed pharmacology ascertain when CRMP2 support may be indicated vs. contraindicated.

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