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Collapsin Response Mediator Proteins (CRMPs)

Involvement in Nervous System Development and Adult Neurodegenerative Disorders

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Abstract

The members of the collapsin response mediator protein (CRMP) family—five cytosolic phosphoproteins—are highly expressed throughout brain development. The first member to be cloned, CRMP2, was identified as an intracellular messenger required for the growth cone-collapse induced by semaphorin 3A (Sema3A). A rapidly expanding body of study indicates that the functions of CRMPs are not solely limited to the signaling transduction of the Sema3A guidance cue. They are probably involved in multiple cellular and molecular events involved in apoptosis/proliferation, cell migration, and differentiation. In the adult brain, the expression of CRMPs is dramatically downregulated. However, they remain expressed in structures that retain their capacity for differentiation and plasticity and also in a subpopulation of oligodendrocytes (CRMP2 and CRMP5). Moreover, the expression of CRMPs is altered in neurodegenerative diseases, and these proteins may be of key importance in the physiopathology of the adult nervous system.

Index Entries: CRMP; nervous system development; growth-cone collapse; brain plasticity; oligodendrocytes; neurodegenerative disorders.

Introduction

The collapsin response mediator protein (CRMP) family is composed of five cytosolic phosphoproteins that are strongly expressed in the developing nervous system. The first member of this family was identified in 1995 as an intracellular component of the transduction pathway of the extracellular semaphorin 3A signal. Four new members of the family have since been discovered, and numerous studies indicate that their functions are not restricted to the transduction of a single extracellular signal

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in neurons during nervous system development. Moreover, it appears that CRMPs may be involved in adult neurodegenerative disorders. A better understanding of their roles and mechanisms of action may be crucial both in normal and pathological brains.

Discovery of the CRMP Family

CRMPs (CRMP1 to CRMP5) were discovered by several groups that work in parallel in different fields of research. First, in 1995, Goshima and collaborators identified a new protein required for the transduction of the extracellular signal semaphorin 3A (Sema3A) in chick dorsal root ganglia (DRG) *(1),* known as an inhibitory protein for axonal guidance *(2).* This protein was cloned and named CRMP-62 for Collapsin Response Mediator Protein of a relative molecular mass of 62 kDa (corresponding to CRMP2). At the same time, a 64-kDa protein referred to as TOAD-64 for Turned On After Division and showing a marked increase in abundance over the period of corticogenesis was identified *(3,4).* The cDNA sequence of TOAD-64 corresponds to rat CRMP2 *(4),* but the protein recognized by the antibody directed against TOAD-64 is CRMP4 *(5–7),* antibody known now as anti-TUC-4 *(8).* In 1996, Byk and colleagues used a rabbit polyclonal antiserum directed against stathmin, which recognized a 64-kDa mouse-brain specific phosphoprotein, and identified a protein with similarities with the product of the *unc-33* gene from the nematode *C. elegans (9).* This protein, designated Ulip for *Unc-33* LIke Phosphoprotein, corresponds to mouse CRMP4. During the same year, Wang and Strittmatter isolated four rat sequences that were strongly related to CRMP-62, and identified the corresponding rat CRMPs: CRMP1, 2, 3, and 4 *(10).* In parallel to the cloning of human liver dihydropyrimidinase (DHPase, an enzyme involved in the uracil and thymine catabolism), three additional sequences homologous to DHPase were isolated from the human fetal brain and named DRP 1, 2, and 3 Dihydropyrimidinase Related Protein *(11).* These proteins correspond to human CRMP1, 2, and 4. In 1998, after the identification of mouse CRMP4, three additional complete coding sequences of the murine CRMP family were isolated *(12).* Finally, CRMP5 was cloned in 2000 after two-hybrid screenings of brain libraries *(13,14)* or purification from a proteic complex *(15).* Our group identified the CRMPs, particularly CRMP5 *(16),* as target antigens for autoantibodies that were present in the serum of patients who suffered from paraneoplastic neurological syndrome, an autoimmune neurodegenerative disorder associated with cancer *(17–21).*

Table 1
Reciprocal Homologies Between Mice CRMPs (according to refs. *12,14*).

	CRMP1	CRMP2	CRMP3	CRMP4	CRMP5
CRMP1		76%	69%	74%	49%
CRMP2	76%		75%	76%	50%
CRMP3	69%	75%		69%	50%
CRMP4	74%	76%	69%		49%
CRMP5	49%	50%	50%	49%	

All CRMP genes are highly homologous (50–75%) *(10–16)* (Table 1), and this family of proteins is conserved throughout evolution (about 95% between mouse and human) *(12,16).* The cDNAs display consensus phosphorylation sites for several protein kinases *(4,5,9,12,14–17),* a high similarity (30%) with the *unc-33* gene from the nematode *C. elegans (1,4,9,11,13,15–17)* and with the human dihydropyrimidinase (50–60%) *(11,14,15)* or the bacterial enzyme D-hydantoinase (40%) *(1,5,9,11).* However, no enzymatic activity (hydantoinase or dihydropyrimidinase) has been detected for CRMPs until now *(1,9,11,15,22,23).* In *C. elegans,* two additional *unc-33* related proteins, displaying 46–49% identity with vertebrate CRMPs, have been isolated and named CeCRMP/DHP-1 and 2 *(24).* The authors suggest that they could represent a common ancestral state before gene duplication between CRMPs and DHPase.

Table 2
Regulation of CRMPs mRNA Expression in the Developing Nervous System

	E 12–E 16	E 18	P 0	P 5–P 7	P 14	P 21	Ad
CRMP1	+	+++	+++	+++	++	+	+/−
CRMP2	++	+++	+++	+++	++	++	++
CRMP3	+	++	++	++	+	+	+
CRMP4	+	+++	+++	+++	+	+/−	+/−
CRMP5	+	+++	+++	+++	++	+	+

Ei = embryonic day i, Pi = postnatal day i, Ad = adult, +/− = very weak expression, + = weak, ++ = average, +++ = strong (according to refs. *3,4,9,10,12,14,15,17,23).*

Regulation of CRMP Expression

Although some biochemical experiments have demonstrated an association with the particulate fraction of cells *(1,4),* in vivo CRMP immunoreactivity is localized mainly in the cytoplasm *(1,4,9,17,23).*

In the Developing Nervous System

All CRMPs are developmentally regulated proteins. They are strongly expressed in postmitotic neural cells from early embryonic life, reach a peak around the first postnatal week, and are dramatically downregulated in adults *(1,3,4,9,10,12–19,21,23,25–27).* However, each CRMP displays spatiotemporal expression patterns, which are precisely and differently regulated during brain development *(10,12)* (Table 2). For example, we have clearly shown that in the developing cerebellum, CRMP2 is highly expressed in the external granular layer (EGL), in which mitosis of future cerebellar granular neurons occurs, whereas CRMP5 is never detected in the EGL *(16).* However, CRMP2 and CRMP5 are co-expressed in postmitotic granular neurons that migrate toward the internal granular layer (IGL), and are present in the fasciculi of neuronal fibers *(16)* (Fig. 1). Because CRMP expression peaks during the first postnatal week, when maturation of neurons and synaptic connections is highly active, many authors have suggested their involvement in neuronal migration and/or differentiation and axonal growth *(3,4,9,16,25).* Moreover, in *Xenopus,* neuronal inducers trigger early CRMP2 expression, suggesting its involvement in the commitment to a neuronal phenotype *(28).* As their expression is differentially regulated during ontogenesis, CRMPs may play distinct but complementary biological roles during nervous system development *(12).* For example, it has been proposed that different CRMPs could be associated with similar or distinct intracellular events stimulated by separate semaphorins *(11).* Their diversity of actions may be a result of the interaction of CRMPs with each other to form multimers, and particularly heterotetramers *(14,15,22).*

In the Adult Nervous System

In the adult brain, the CRMPs are dramatically downregulated and are mainly expressed in areas that retain plasticity and/or neurogenesis such as the olfactory bulb, hippocampus, and cerebellum. Indeed, CRMP1 mRNA is mainly detected in Purkinje cells of the cerebellum *(10,29).* CRMP2 is the most highly expressed CRMP in the adult brain *(10,29),* in postmitotic neurons *(23)* and is most often detected in the olfactory system *(4,30,31),* cerebellum *(10,29),* and hippocampus *(4,10,29).* CRMP3 mRNA is restricted to the cells of the granular layer of the cerebellum, inferior olive, and dentate gyrus of the hippocampus *(10,21,29).* CRMP4 appears to be the least expressed CRMP in the adult brain. It is con-

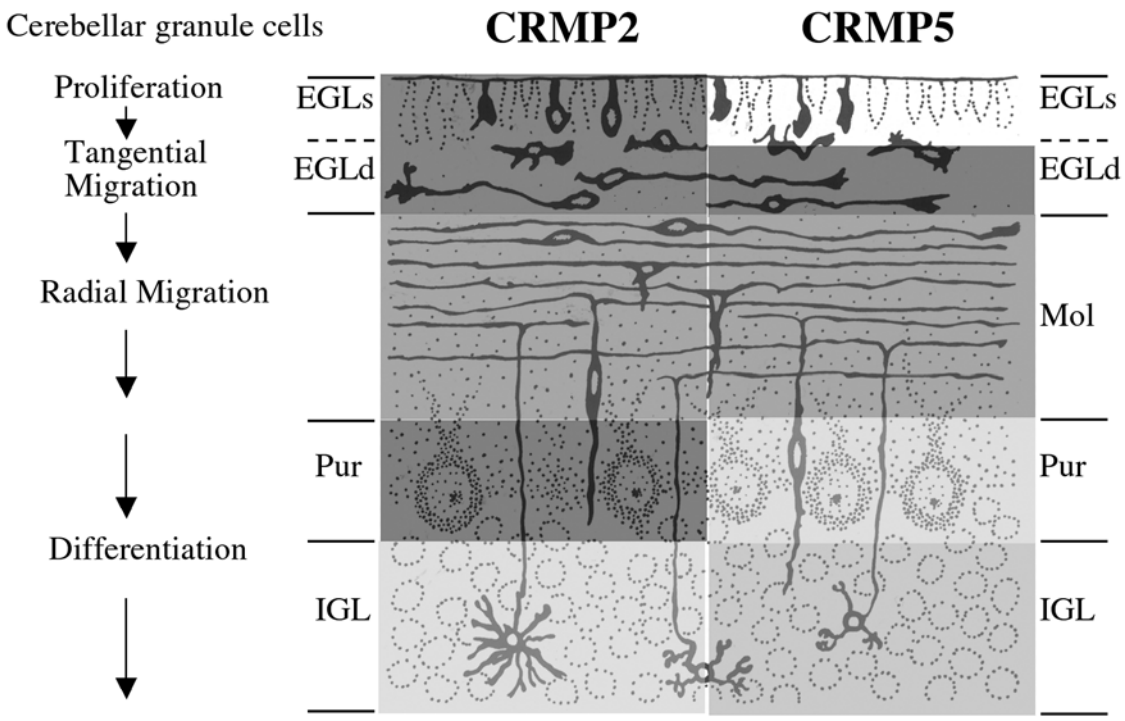

Fig. 1. Expression of CRMP2 and CRMP5 in cerebellar granule cells during cerebellum development. EGLs, superficial zone of external granule layer; EGLd, deep zone of external granule layer; Mol, molecular layer; Pur, Purkinje cell layer; IGL, internal granule layer. Intensity of the immunostaining is represented by different intensities of gray tones: white = no expression, darkest grey = strongest expression.

fined to the olfactory bulb *(7)* and hippocampal formation *(7,8,29)* and to sparse cells in the IGL of the cerebellum *(7,29)*. CRMP5 is detected in a few postmitotic neurons in the olfactory bulb, olfactory epithelium *(31)*, and dentate gyrus of the hippocampus *(16)*. Our group demonstrated CRMP5 expression in peripheral nerve axons and sensory neurons *(32)*. Intriguingly, the strongest expression of CRMP2 and CRMP5 proteins is detected in subsets of oligodendrocytes distributed according to an increasing rostro-caudal gradient *(16,19,23,26,29)*. CRMP2 and CRMP5 are co-expressed in some oligodendrocytes, but some CRMP2-expressing oligodendrocytes do not express CRMP5 *(16)*, which has enabled us to identify different subtypes of these glial cells that may display specific properties.

In Peripheral Tissues

Although CRMPs are mainly expressed within the nervous system, they can be detected in peripheral tissues. CRMP1 *(33)*, 4 *(9,34)*, and 5 *(16)* have been described in the adult testis, restricted in the cell spermatid stage *(33,34)*. Interestingly, *unc-33*, which has a sequence that displays high homologies with CRMPs and other genes that are known to affect multiple aspects of axonogenesis, are essential for sex myoblast migration in *C. elegans* hermaphrodites *(35)*. This could be related to the testicular expression of the three CRMPs. The presence of CRMP2 mRNA has been reported in lung tissue of the fetal mouse *(10,36)* and adult human *(11)*. CRMP2 has also been identified as a gene, which is induced in maturing

monocytes and downregulated to pre-activated levels during further maturation *(37).* However, the functional significance of CRMP2 in monocytes is not understood.

Functions and Mechanisms of Action in the Nervous System

In the Developing Nervous System

Neuronal localization of CRMPs in neurites and axonal growth cones *(1,4,9,23,38,39)* and regulation of CRMP expression in vivo and/or in vitro *(4,5,12,15,23)* has led several authors to suggest that they may be involved in neuronal differentiation and axonal outgrowth *(9,23),* as *unc-33* gene in *C. elegans (40).* Recent reports have begun to reveal CRMP's mechanisms of action. CRMP2 could be an intracellular component of the transduction cascade initiated by Sema3A *(1),* which is known as a repulsive signal in axonal guidance and to induce neuronal growth-cone collapse *(41–43)* through its receptor complex formed by neuropilin-1 and plexinA1 *(44–49).* Fes, a cytoplasmic protein tyrosine kinase expressed in developing neurons, interacts with and phosphorylates some CRMPs and plexin-A1, and could therefore link Sema3A/plexinA1 signal to CRMPs *(50).* Another enzyme, phospholipase D2 (PLD-2, which catalyzes the hydrolysis of phosphatidylcholine to phosphatidic acid and choline) may also be involved in this transduction pathway *(39).* PLD-2 is located in the growth cone, where its activity may be important for actin cytoskeleton rearrangement. Interestingly, CRMP2 can specifically inhibit PLD-2 activity by direct interaction in a Sema3A-dependent manner. Inhibition of PLD-2 may play a role in axonal growth-cone collapse resulting from actin depolymerization *(39).* CRMPs may also be part of transduction pathways of other molecules that are also known to cause neuronal growth-cone collapse. Among these, extracellular lysophosphatidic acid (LPA), a bioactive phospholipid, is capable of inducing axonal growth-cone collapse *(51,52)* via CRMP2 in chick DRG neurons *(53).* Interestingly, the transduction of Sema3A and LPA signals during axonal elongation involves two different intracellular pathways, Rac1 *(54,55)* and RhoA *(56–60)* respectively (Fig. 2). During LPA-induced growth-cone collapse, CRMP2 is phosphorylated on Thr-555 by Rho-kinase, downstream of RhoA *(53).* In contrast, CRMP2 mediates Sema3A signaling independently of phosphorylation by Rho-kinase *(53).* Moreover, CRMP2 may reverse the morphological effects of RhoA and Rac1 signaling, thus permitting a dynamic modulation of axonal growth-cone guidance *(61).* CRMP1 is also involved in the RhoA pathway through interaction with Rho-kinase, leading to regulation of RhoA signaling *(62).* Post-translational modifications of CRMPs other than phosphorylations may also mediate their effects during axonal growth. Thus, CRMP2 has been shown to be particularly modified by O-GlcNAc (β-*N*-acetylglucosamine linked to hydroxyls of serine or threonine) in nerve-terminal synaptosomes *(63).* This post-translational modification may block CRMP2 phosphorylation, thereby regulating CRMP2 action *(63).* Downstream of these signaling pathways, the effects of CRMPs on axonal growth may be related to modulatory actions on cytoskeletal—namely microtubules—organization. The CRMPs share 30% homology with *unc-33,* which was proposed to be a component of the axonal cytoskeleton that directs axonal outgrowth *(40,64).* CRMP2 has been shown to bind to tubulin heterodimers and modulate microtubule assembly *(65,66),* which could be the basis of its regulatory effect on axonal branching and axonal/dendritic fate in hippocampal cultures *(38,67).* Moreover, Sema3A can modulate axonal transport *(68),* a mechanism in which microtubules are involved. In this context, CRMPs could regulate axonal flow by transducing the Sema3A signal. CRMPs may also play a role in cellular events other than neurite dynamics. CRMP2 may participate in transduction pathways leading to apoptosis,

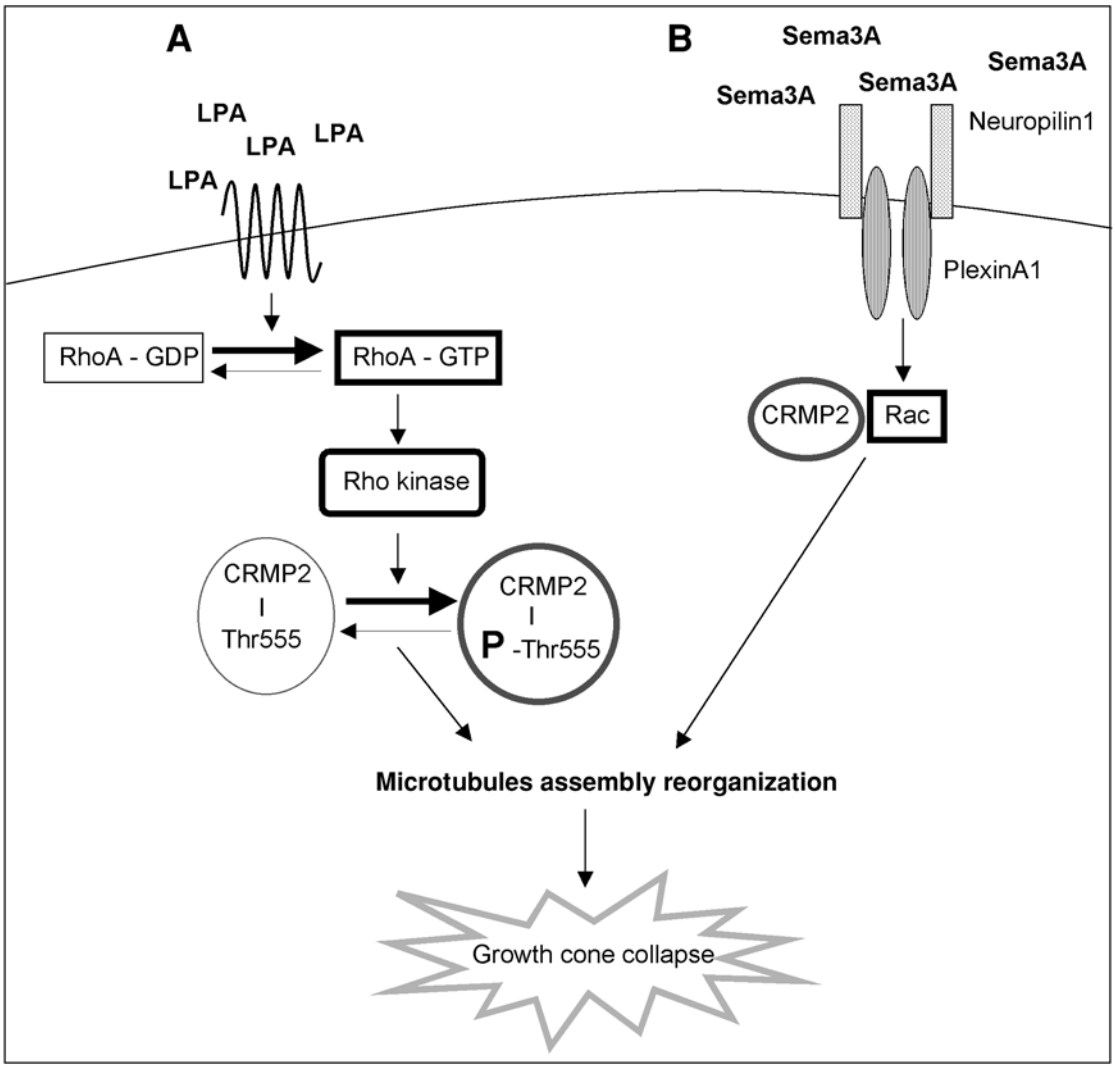

Fig. 2. Schematic representation of CRMP2 involvement in signal transduction pathways triggering axonal growth-cone collapse. CRMP2 participate in different signaling cascades induced by extracellular molecular cues via their respective transmembrane receptors. **(A)** LPA-induced growth-cone collapse signal through a seven-transmembrane receptor that triggers activation of RhoA, which leads to phosphorylation of CRMP2 on Threonine-555 by activated Rho-kinase. **(B)** Sema3A signaling initiates clustering of the receptor plexin and neuropilin. In a CRMP2-dependent process, this clustering leads to alterations of a Rac1-dependent pathway that modulates the actin filament assembly in the growth cone (according to refs. *1,53–55,65–67*).

induced or not by Sema3A *(65,69–72)*. In addition to their roles as cytosolic signal transduction molecules, CRMPs could also act as ligands of the extracellular matrix (ECM) in the neonatal brain *(73)*.

In the Adult Nervous System

CRMPs in Oligodendrocytes

CRMPs expression is dramatically downregulated in the adult brain. Interestingly, some

CRMPs (CRMP2 and CRMP5) are expressed in a subpopulation of oligodendrocytes *(16,29).* We have shown that Sema3A, through its binding to neuropilin1, induces a dramatic reduction of oligodendrocytes' processes extension in vitro *(16).* This effect is mediated by CRMP2 and CRMP5 *(16).* These data show that, in the adult brain, Sema3A could modulate the extension of oligodendrocytes' processes via CRMP2 and CRMP5, as in neurons of the developing nervous system. Interestingly, CRMP4 could also be involved in process formation of Schwann cells *(74).* Thus, CRMPs might play a role in myelination/demyelination mechanisms in the central and peripheral adult nervous system. Interestingly, mature oligodendrocytes and Schwann cells express one LPA receptor, edg-2 (endothelial differentiation genes-2) *(75–78)* and display responsiveness to LPA application *(77–80).* We can speculate that CRMPs mediate LPA signaling (as CRMP2 does in neurons) in oligodendrocytes and Schwann cells.

CRMPs and Brain Plasticity

In the adult, CRMPs are expressed especially in areas that undergo neurogenesis and/or plasticity, such as the hippocampus, olfactory system, and cerebellum *(4,9,10,29–31).* CRMP4 is expressed in postmitotic neurons of the dentate gyrus *(7,8),* and the number of CRMP4-expressing cells is increased following epileptic seizures *(6).* CRMP4 has also been reported to be expressed in synaptic sites at the adult neuromuscular junction, where it could maintain neuronal stability at the junctional level *(9).* In the adult olfactory system, CRMP2, CRMP5, and neuropilin1 are expressed in olfactory neurons of the epithelium, whereas Sema3A is detected in the olfactory bulb *(30,31).* The cellular distribution of these molecules suggests that in the intact olfactory bulb, Sema3A could create a molecular barrier, which helps to restrict ingrowing olfactory axons to the nerve and glomerular layer *(30).* The expression pattern of CRMP2, but also Sema3A and neuropilin1, is spatially and temporally modulated during regeneration of the olfactory nerve after axotomy or bulbectomy *(30,31).* CRMP4 expression is also regulated in motoneurons after the appearance of sciatic nerve lesions *(4).* Thus, CRMPs may participate in the regulation of the neuron regeneration process. Finally, CRMPs may also be involved in synaptic plasticity, since CRMP2 can be phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) in postsynaptic densities (PSD) *(81).* Indeed, both PSD—which is located beneath the postsynaptic membrane—and CaM kinase II, are known to participate in synaptic plasticity. In particular, CaM kinase II is implicated in structural modification of the cytoskeleton and long-term potentiation (LTP). Therefore, CRMP2 as a substrate of CaM kinase II, could be involved in synaptic-plasticity mechanisms. In the adult nervous system, CRMP expression in migrating and/or differentiating cells and regulation following injury or neuronal activity strongly suggest their involvement in neuronal plasticity and regeneration.

Possible Roles in Nervous System Diseases

Until now, CRMPs have been mainly described as critical for nervous system development, and a growing body of evidence indicates that they may also participate in the pathogenesis of neurological disorders. CRMP expression is dysregulated in hypothyroidism and Down syndrome, diseases characterized by abnormalities of central nervous system (CNS) development, such as incomplete maturation of neuronal and glial cells or deteriorated wiring of the developing brain *(82,83).* Neurodegenerative disorders are also associated with CRMP induction. In Alzheimer's disease (AD), CRMP2 expression in the brain is higher than in controls, and is associated with neurofibrillary tangles (NFT) *(27).* It has been suggested that the incorporation of CRMP2 into NFT may deplete soluble, cytosolic CRMP2, and may lead to abnormal neuron process outgrowth, thus accelerating neuronal degeneration. Increased expression of CRMP2 could also explain extensive abnormalities of

neurites in the AD cortex *(27,84).* Interestingly, NFT-associated CRMP2 is highly phosphorylated on Thr509, Ser518, and Ser522 *(84).* These phosphorylations may play an important role in regulating CRMP2 activity, and may thus be involved in the formation of degenerating neurites in the AD brain. However, high levels of phosphorylated CRMP2 in the AD brain could also reflect the attempt of the remaining neurons to compensate for neuronal loss *(84),* as phosphorylated CRMP2 is more abundant in the fetal than the adult brain *(84).* Moreover, CRMP2 is significantly more oxidized in the AD brain *(85)* and oxidative stress appears to be involved in the progression of the disease. The oxidation-related decrease in CRMP2 function may increase the potential for neuritic degeneration because of a deficiency of the repair system *(85).* It is very interesting to link this study with the fact that CRMP2 belongs to a complex of proteins with redox activity, named *trans*-plasma-membrane oxidoreductase (PMO) *(86).* This PMO activity is known to be related to the redox control of receptor function and the receptor-mediated signal-transduction pathway. PMOs also act as a redox sensors to modulate cell proliferation in response to external pro- or antioxidants *(86).* In another neurodegenerative disease, Parkinson's disease, CRMP2 could be involved in dopamine-induced neuronal apoptosis, possibly via Sema3A *(71).* Moreover, CRMPs and especially CRMP5 are the target of autoantibodies produced during paraneoplastic neurological syndrome *(16,20).* Paraneoplastic neurological syndromes are autoimmune neurodegenerative disorders associated with cancer. The most frequently associated tumor is small-cell lung carcinoma *(19,87,88).* Cells of this type of tumor express CRMPs *(88).* However, the functional significance of CRMP expression by lung-cancer cells and the implication of anti-CRMPs autoantibodies in the neurological disorder are not understood. Interestingly, the expression of CRMP1 in human lung adenocarcinoma is negatively associated with cell invasiveness in vitro, and expression of CRMP1 mRNA in lung cancer specimens is inversely associated with disease progression, lymph node metastasis, and early postoperative recurrence and survival *(89).* In addition, the expression of neuropilin1 and 2 is positively associated and expression of CRMP1, as Sema3A, is negatively associated with the invasiveness of tumorous cells *(89).* Therefore, in lung cancer, CRMP1 could act as an inhibitor of cell migration, perhaps in relation to Sema3A, which has already been shown to play a role in neuronal *(72,90)* and non-neuronal *(91)* cell migration. Finally, decreased expression of CRMP2 levels has been reported in the frontal cortex of patients who suffer from psychiatric disorders (schizophrenia, bipolar, or major depressive disorders) *(92).*

Conclusion

In conclusion, although much is still unknown about the functions of these proteins, CRMPs probably play a central role in normal and pathological events in the nervous system. CRMPs have been found to be regulated in many diversified contexts—whether normal or pathological—both in the developing and the mature brain. This suggests that they may stand at the junction of many different cellular and molecular events of brain development, myelination, and adult neuronal plasticity. Despite their high homology, CRMPs are probably differentially regulated at the post-translational level, which could increase the specificity of action of each one. Finally, as the knowledge of CRMP functions progresses, the adequacy of CRMP terminology may be questioned. Collapsin is the former and now obsolete name of Sema3A, and CRMPs do not solely transduce the semaphorin signal. Among the names previously given to CRMPs (e.g., TOAD-64, Ulip, DRP, TUC), an international consensus to select a name for these proteins would be desirable.

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