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The CRMP Family of Proteins and Their Role in Sema3A Signaling

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Abstract

The CRMP proteins were originally identified as mediators of Sema3A signaling and neuronal differentiation. Much has been learned about the mechanism by which CRMPs regulate cellular responses to Sema3A. In this review, the evidence for CRMP as a component of the Sema3A signaling cascade and the modulation of CRMP by plexin and phosphorylation are considered. In addition, current knowledge of the function of CRMP in a variety of cellular processes, including regulation of the cytoskeleton and endocytosis, is discussed in relationship to the mechanisms of axonal growth cone Sema3A response.

The secreted protein Sema3A (collapsin-1) was the first identified vertebrate semaphorin. Sema3A acts primarily as a repulsive axon guidance cue, and can cause a dramatic collapse of the growth cone lamellipodium. This process results from the redistribution of the F-actin cytoskeleton^{1,2} and endocytosis of the growth cone cell membrane.^{2–4} Neuropilin-1 (NP1) and members of the class A plexins (PlexA) form a Sema3A receptor complex, with NP1 serving as a high-affinity ligand binding partner, and PlexA transducing the signal into the cell via its large intracellular domain. Although the effect of Sema3A on growth cones was first described nearly 15 years ago, the intracellular signaling pathways that lead to the cellular effects have only recently begun to be understood. Monomeric G-proteins, various kinases, the redox protein, MICAL, and protein turnover have all been implicated in PlexA transduction. In addition, the collapsin-response-mediator protein (CRMP) family of cytosolic phosphoproteins plays a crucial role in Sema3A/NP1/PlexA signal transduction. Current knowledge regarding CRMP functions are reviewed here.

Properties and Expression of CRMPs

A number of CRMP genes were identified independently in different species around the same time, and were named according to their method of discovery. CRMPs are also known as turned on after division (TOAD-64),⁵ dihydropyrimidinase related protein (DRP),⁶ unc33 like protein (Ulip),⁷ and TUC (TOAD64/Ulip/CRMP).⁸ Five vertebrate CRMP genes (CRMP1–5) have been identified, while the *Drosophila* genome appears to encode for only a single CRMP. CRMP1–4 share ~75% protein sequence identity with each other, however CRMP5 (also referred to as CRAM) is only 50–51% homologous. CRMPs share a high sequence homology with the *C. elegans unc-33* gene,^{5,7,9,10} although two other nematode genes, CeCRMP1 and 2, have been classified in the CRMP family.¹¹ In addition, mammalian CRMP1, 2, and 4 appear to undergo alternative splicing.^{12,13} CRMP isoforms strongly interact with each other and exist as heterotetramers when purified from brain.¹⁴ Specificity exists for the hetero-oligomerization in that different isoforms have varying affinities for each other.¹⁴ Information obtained from the examination of the crystal structure of CRMP1 homotetramers reveals that this specificity is likely due to differential polar and hydrophobic residues between isoforms at the two oligomerization interfaces.¹⁵ CRMP1–4 genes share a high sequence homology (60%) with the liver dihydropyrimidinase (DHPase) and structural similarity with members of the metal-dependent amidohydrolases, both of which form stable tetramers. However, none of the CRMP

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isoforms demonstrate any enzymatic activity, likely due to the fact that they lack crucial His residues which coordinate binding of a metal atom at the active site of amidohydrolase enzymes.^{6,14,15}

CRMPs were discovered to be one of the first proteins expressed in newly born neurons in the developing brain,⁵ and CRMP2 expression has been shown to be induced by factors that promote neuronal differentiation such as noggin, chordin, GDNF, and FGF.^{16–18} Not surprisingly, CRMPs are most highly expressed during the neurogenic period of brain development and expression peaks during the period of axon growth.¹⁹ In addition, CRMP1, 2 and 5 are expressed in immature interneurons in the adult olfactory bulb,²⁰ a site of ongoing neurogenesis in adulthood.²¹ The expression of CRMPs is restricted primarily to the nervous system, however some isoforms show a differential pattern of expression in various nervous system structures.^{19,22} CRMP2, and to some extent CRMP3, are expressed in mature neurons at low levels. These expression patterns, when taken together with the fact that CRMPs form heterotetramers, imply that oligomers consisting of different combinations of monomeric isoforms may have different functional effects in various cell types.

The significant sequence similarity of CRMPs with the worm *unc-33* gene implies a role for CRMP in axon growth and morphology, since *unc-33* mutants display severe axonal abnormalities.^{23,24} Also, overexpression of CRMP2 induces ectopic axon formation in cultured hippocampal cells.²⁵ Although CRMP is a cytosolic protein, a significant fraction has been shown to be tightly associated with the cell membrane.^{5,26} This membrane-associated pool of CRMP is enriched at the leading edge of the growth cone lamellipodium and filopodia, further supporting a role in axon outgrowth and guidance.⁵ All CRMP isoforms continue to be expressed through the period of axon pathfinding and synaptogenesis,¹⁹ and CRMP expression is induced in sprouting fibers after injury in both the central and peripheral nervous system.^{5,27,28}

Evidence For CRMPs in Sema3A Signaling

The first evidence for the involvement of CRMPs in Sema3A signaling came from the identification of chick CRMP-62 (CRMP2) in an expression cloning screen looking for Sema3A signaling components.¹⁰ Injection of CRMP-62 (CRMP2) RNA rendered *Xenopus* oocytes electrophysiologically responsive to Sema3A (collapsin-1). Further, treatment of DRG neurons with antibodies developed against an N-terminal region (a.a. 30–48) of CRMP-62 blocked the ability of Sema3A to collapse their growth cones.

Sema3A signaling can be reconstituted in a nonneuronal heterologous system.^{29,30} COS7 cells overexpressing PlexA1 and NP1 contract when Sema3A is applied to the media. This effect is easily quantified using alkaline phosphatase (AP)-tagged ligand and measuring the surface area of the cells with bound AP. AP-Sema3F treatment fails to cause contraction of PlexA1/NP1-expressing cells, demonstrating the specificity of the assay. COS7 cells overexpressing CRMPs in addition to the PlexA1/NP1 receptor components undergo contraction at a much more rapid rate than cells in which the receptors are expressed alone.¹⁵ Therefore, CRMP proteins are able to facilitate Sema3A-mediated morphological changes in nonneuronal cells, further suggesting that they play a role in the signaling pathway (Fig. 1).

In addition, CRMP1–4 are all able to form a complex with the Sema3A receptor PlexA1 in transfected nonneuronal cells.¹⁵ Although the presence of NP1 attenuates this interaction, stimulation with Sema3A reestablishes the complex. This is a specific effect since treatment with Sema3F is unable to promote PlexA1-CRMP interactions in the presence of NP1. Together with the fact that CRMPs facilitate COS7 contraction, these data demonstrate that PlexA1 and CRMP are able to initiate a Sema3A signaling cascade in nonneuronal cells.

What happens to CRMP upon Plexin binding? CRMPs may undergo a conformational change that leads to CRMP activation. Evidence for this is found in the observation that mutation of residues at the N-terminal region of the murine CRMP1 (a.a. 49-56) causes the protein to become constitutively active.¹⁵ Overexpression of this mutant CRMP1 leads to the contraction of COS7 cells in the absence of PlexA1 and/or Sema3A stimulation, and the reduction of neurite outgrowth and Sema3A responsiveness in DRG neurons and explants. The expression of this mutant protein therefore mimics the cellular responses to Sema3A. Mutation of nearby residues (a.a. 38, 39, 41, 43) causes partial activation such that Plexin, but not Sema3A, is required for cell contraction (Fig. 2). Consistent with the mutation studies, antibody binding to residues 30-48 blocks Sema3A growth cone collapse,¹⁰ suggesting that the N-terminal region between residues 30-56 plays an important role in mediating the activity of CRMPs in Sema3A signaling.

Phosphorylation of CRMP proteins at the C-terminal region may also be important in Sema3A signaling. A number of studies have shown the activity of a variety of kinases, including fyn, cdk5, GSK3 β , LIMK, and Fes are important mediators of cellular responses to Sema3A³¹⁻³⁴ (Fig. 1). PlexA1 and A2 are constitutively bound to the src family tyrosine kinase, fyn, in a kinase-independent manner. Stimulation with Sema3A causes fyn activation and leads to the recruitment of the serine/threonine kinase, Cdk5, into the complex and activation of its kinase activity.³⁴ The blockade of Cdk5 kinase activity attenuates DRG growth cone collapse in response to Sema3A.³⁴⁻³⁶ CRMP1, 2, 4, and 5 are all phosphorylated by Cdk5 at Ser522,³⁶ and this can occur in response to Sema3A in vitro and in vivo.^{35,36} The growth cones of neurons overexpressing a mutant CRMP2, in which Ser522 is replaced by alanine (S522A), fail to collapse when Sema3A is applied.^{35,36}

The phosphorylation of CRMPs at Ser522 allows for the subsequent phosphorylation of CRMP2 and CRMP4 at Ser518, Thr509, and Thr514 by the serine/threonine kinase GSK3 β .³⁵⁻³⁸ It was previously shown that Sema3A activates GSK3 β at the leading edge of growth cones, and this activation is required for proper responses to SemaA.³² Overexpression of CRMP2 mutants T509A, T514A, S518A in neurons resulted in moderate decreases in Sema3A-induced growth cone collapse,^{35,36} suggesting that single mutations are not sufficient to completely block Sema3A signaling. This may be due to the fact that the other phosphorylation sites are intact, or that CRMPs are not the primary targets of Sema3A-dependent GSK3 β signaling. However, the regulation of CRMP by GSK3 β phosphorylation may play an important role in Alzheimer's disease (AD), since CRMP2 is hyper-phosphorylated at Thr509, Ser518, and Ser522 in neurofibrillary tangles³⁹ and in amyloid precursor protein intracellular domain (AICD) transgenic mice.⁴⁰

The nonreceptor tyrosine kinase, Fes, was copurified with CRMP5 (CRAM) from mouse brain.³³ Fes was able to phosphorylate all 5 CRMP proteins and PlexA1 in a constitutive manner, moreover, this effect was enhanced by Sema3A. The coexpression of Fes and PlexA1 in COS7 cells led to cell contraction, suggesting that Fes may be able to activate PlexA1. NP1 was able to block Plexin-Fes interactions, similar to the observations with CRMP-Plexin interactions, and this effect was overcome by Sema3A.³³ Further work needs to be done to identify the tyrosine residues on CRMP and Plexin that are putatively targeted by Fes, and to determine the functional consequences of Fes kinase activity.

The function of CRMP in axon guidance may not be restricted to Sema3A signaling. The growth cone collapsing factor LPA as well as the inhibitory guidance cue ephrin-A5 are both able to stimulate phosphorylation of CRMP2 at Thr555 by Rho kinase (ROCK), however Sema3A fails to do so.^{41,42} Rho kinase is known to regulate neurite outgrowth and growth cone motility downstream of the GTPase, RhoA.⁴³ The kinase activity is specific for CRMP2 since Thr555 is not conserved in CRMP1,3,4, or 5.⁴¹ Inhibitors to Rho kinase and expression

of a kinase-dead mutant attenuate LPA mediated growth cone collapse, but have little to no effect on *Sema3A* growth cone collapse.⁴¹ Further, the nonphosphorylated CRMP2 mutant T555A protects growth cones from the collapsing activity of both LPA and ephrinA5.^{41,42} These observations demonstrate that CRMPs may be differentially regulated by multiple axon guidance cues, and thus be an important mediator of cellular responses to a variety of signals.

Examination of the crystal structure of CRMP1 reveals two separate regulatory regions of the protein (Fig. 2). The residues involved in constitutive activity and those targeted by the function blocking antibody all map the N-terminal “upper lobe” of the CRMP1 monomer, termed the “activation loop” (Fig. 2B, top; ref. ¹⁵). CRMP tetramers are assembled such that the activation loops are situated on the outer surface of the complex, allowing for regulation of other factors in the cytosol (Fig. 2B, bottom). The sites of phosphorylation by Cdk5, GSK3 β , and ROCK are all localized to the C-terminus of the protein. Although the crystal structure does not include the C-terminal 80 amino acids, it seems likely that these residues also are located at the surface of the tetramer, supporting the hypothesis that phosphorylation may regulate tetramerization or that phosphorylation may be a reflection of CRMP oligomeric state (Fig. 2B, bottom).

The Regulation of the Cytoskeleton by CRMPs

Although it is clear that CRMP proteins are important for normal *Sema3A* responses in developing neurons, it is still not known what the downstream effectors of CRMP signaling may be. Rearrangement of the cytoskeleton is a well-characterized phenomenon resulting from *Sema3A* treatment.^{1,2} Therefore one possibility is that CRMP2 may link *Sema3A* receptors to the cytoskeleton either directly or via factors known to regulate cytoskeletal dynamics (Fig. 3).

A growing body of literature suggests that CRMP2 plays a role in neuronal polarity by regulating microtubule dynamics.^{38,44–46} CRMP2 is highly enriched in the growing axons of dissociated hippocampal cells, and when overexpressed, induces the formation of multiple axon-like processes.²⁵ Truncation of 24–191 amino acids at the C-terminus of CRMP2 prevents this axogenesis. CRMP2 colocalizes with microtubules in neuronal cells lines and fibroblasts, and CRMP1–4 can all bind to tubulin in vitro and in brain.^{44–46} Furthermore, CRMP2 can induce microtubule assembly by binding to α - and β -tubulin heterodimers, an effect mediated by residues 323–381.⁴⁴ CRMP2's effect on axon formation is dependent upon the ability to promote microtubule assembly, since the overexpression of a mutant CRMP (Δ 323–381) and the disruption of CRMP-microtubule interactions both fail to promote axon specification.⁴⁴

A possible mechanism of *Sema3A* signaling may be an alteration of microtubule dynamics mediated by CRMP. Phosphorylation of Thr514 by GSK3 β abolishes the ability of CRMP2 to bind to tubulin heterodimers.³⁸ Using a pThr514-specific antibody, the authors show that unphosphorylated CRMP2 is enriched at the leading edge of the growth cone. Expression of a nonphosphorylated CRMP2 mutant (T514A) causes an increase in axon length and branching relative to untreated or WT CRMP2 expression. The constitutively phosphorylated mutant T514D behaves similarly to WT CRMP2.³⁸ As discussed earlier, the phosphorylation of CRMPs by GSK3 β is dependent on prior phosphorylation of Ser522 by Cdk5. Not surprisingly, the nonphosphorylated CRMP2 mutant S522A also shows enhanced association with microtubules and an increased ability to induce multiple axons.³⁶ Since the overexpression of S522A and T514A CRMP2 mutants leads to a reduction in *Sema3A*-induced growth cone collapse,^{35,36} a model linking *Sema3A* to microtubule dynamics via CRMPs is supported. Interestingly, the phosphorylation of CRMP2 by Rho kinase also decreases the ability of CRMP2 to bind to tubulin dimers, supporting the idea that other guidance cues may converge on this pathway.⁴²

Although microtubules regulate growth cone steering in response to axon guidance cues,⁴⁷ their polymerization state remains relatively unchanged immediately following Sema3A treatment.¹ It is possible that the CRMP2-dependent regulation of microtubules mediates longer term responses to Sema3A, such as growth cone turning.

During Sema3A-mediated growth cone collapse, a dramatic rearrangement of the actin cytoskeleton occurs.^{1,2} A few studies have shown that a pool of CRMPs colocalize with actin in growth cones and neuronal cell lines.^{13,24,46,48} It is therefore possible that CRMP activation modulates changes in actin dynamics. It is unlikely that such an effect is mediated by a direct binding of CRMP to actin, since there is no evidence that such an interaction occurs. However, it is possible that CRMP may be linked to the actin cytoskeleton by other proteins.

Key regulators of the actin cytoskeleton are Rho family GTPases. G protein signaling has been shown to play a large role in the cellular response to semaphorins.⁴⁹ Rac1, Rnd1, and RhoD have all specifically been shown to mediate class A plexin signaling.^{50–53} CRMP2 is able to modulate the activities of Rac1 and RhoA. Expression of constitutively active RhoA (RhoA V14) in fibroblasts generally leads to the formation of stress fibers and focal adhesions. However, when CRMP2 is coexpressed with RhoA V14, there was a loss of stress fibers and increased formation of ruffles and microspikes, features usually associated with Rac1 activity. Conversely, when CRMP2 was coexpressed with the constitutively active Rac1 mutant (Rac1 V12), the cells lacked ruffles and developed stress fibers and some focal adhesions.⁵⁴ A similar switch in morphology was observed in neuroblastoma cells. Therefore, CRMP2 may act as a toggle between Rho GTPases. However, this activity of CRMP2 was dependent on phosphorylation of Thr555 by Rho kinase, which does not occur during Sema3A signaling.^{41,54} It is not clear whether the phosphorylation of CRMP2 and/or other CRMP family members by Cdk5 and GSK3 β can promote this activity.

The Sra1 protein is a downstream effector of Rac1 signaling, linking activated Rac1 to actin polymerization.⁵⁵ CRMP2 was shown to recruit a complex consisting of Sra1 and WAVE, another mediator of actin dynamics, to growth cones of cultured hippocampal neurons to promote axonal differentiation.⁵⁶ It will be interesting to determine whether this process can be regulated by Sema3A since it provides a direct link between Plexins, CRMP, and Rac1 regulation of the cytoskeleton. Since Sra1/WAVE promotes axon outgrowth, a possibility is that Sema3A attenuates the affinity of CRMP2 to associate with this complex.

The activity of Rho GTPases is dependent upon their nucleotide-binding state such that they are active when bound to GTP and inactive when bound to GDP.⁵⁷ Three classes of proteins exist that regulate this status: guanine nucleotide exchange factors (GEFs), which facilitate the exchange of GDP for GTP, GTPase activating proteins (GAPs), which promote GDP binding, and guanine dissociation inhibitors (GDIs), which also promote the GDP-bound state.⁵⁷ Recently, it was found that Sema3A promotes the dissociation of PlexA1 from the Rac1 GEF FARP2, and thus activation of Rac1 signaling.⁵¹ CRMP2 has been shown to interact with the SH2 domain of the Rac1 GAP α 2-chimaerin.³⁵ The significance of this interaction on the regulation of the GAP activity remains unclear, although active α 2-chimaerin promotes CRMP2 association and the overexpression of GAP-inactive mutants block Sema3A-mediated growth cone collapse. These observations contradict studies showing the requirement for active Rac1 for Sema3A collapse of growth cones.^{50,51} However, an initial, transient inactivation of Rac1 was seen immediately following an ephrinA2 or Sema3A challenge, followed by an increase in activity.⁴ It is possible that the GAP activity of α 2-chimaerin may mediate the initial decrease of Rac function, or that separate pools of Rac are differentially regulated (see below).

CRMP, Endocytosis and Other Signaling Events

In addition to disassembly of the cytoskeleton, *Sema3A* and other growth cone collapsing factors promote endocytosis of the growth cone membrane.^{2–4} Within 10 minutes after treatment with *Sema3A*, *PlexA1* and *NP1* are recruited to vesicles inside the growth cone that also label with *Rac1* and F-actin.² This process is dependent on the cell adhesion molecule *L1* and *Rac1* activation.^{3,4} It is possible that the pool of *Rac1* mediating endocytosis is separate from that regulating actin dynamics, partially explaining the discrepancies of the requirement of both *Rac1* GEF and GAP activities in *Sema3A* signaling.

The long form of CRMP4 (TUC4b) was observed to be enriched in SV2 immunoreactive vesicles in the body of the growth cone.¹³ Further, CRMP proteins were found to bind to the SH3A and SH3E domains of intersectin. Intersectin is a GEF for *cdc42* and has been shown to promote N-WASP dependent actin polymerization.⁵⁸ However, it also associates with the AP2 adaptor complex of clathrin-coated vesicles in neurons⁵⁹ and may mediate vesicle dynamics. Therefore, CRMP appears to link Plexin signaling to the endocytotic pathway. This hypothesis is further supported by the observation that CRMP2 also interacts with Numb and α -adaptin, both of which are a part of the AP2 adaptor complex and involved in clathrin-dependent endocytosis.^{60,61} The binding of CRMP2 to Numb was required for normal endocytosis of *L1* in growing hippocampal neurites. Overexpression of a CRMP2 mutant unable to bind to Numb, or knocking down expression of CRMP2 by RNAi was sufficient to attenuate *L1* endocytosis.⁶¹ The intersectin and Numb studies demonstrate that CRMP proteins provide a direct link between the intracellular domain of *PlexA1* and the endocytotic machinery.

A number of other proteins have been shown to interact with CRMP, although the implication of these interactions in the context of *Sema3A* signaling needs to be elucidated. It was recently shown that CRMP2 negatively regulates phospholipase D2 (PLD2) activity, and *Sema3A* is able to inhibit PLD2 activity in PC12 cells.⁶² Although the functional significance of this in growth cones is elusive, a number of intriguing possibilities exist since PLD2 is localized at the distal tips of neurons and has been implicated in regulating actin dynamics and receptor-mediated endocytosis.^{62–64}

The flavin monooxygenase molecule interacting with *CasL* (MICAL), binds directly to *Drosophila* *PlexA* and is required for *Sema1a* signaling.⁶⁵ The proper axon guidance of fly motor neurons and *Sema3A*-mediated repulsion of mammalian DRG neurons is dependent on enzymatic activity.^{65,66} The C-terminal half of MICAL has a number of conserved protein-protein interaction domains and signaling motifs, suggesting a complex role in cell signaling. Although no direct substrates for MICAL have been identified, it has been speculated that cytoskeletal proteins are potential targets.^{65,67} Our unpublished observations indicate that CRMP1-4 can bind to mammalian MICAL1. Whether CRMP is a substrate for MICAL or regulates MICAL activity is not yet clear. An intriguing possibility is that CRMP binds to and presents substrates to MICAL, since CRMP tetramers structurally resemble dihydropyrimidinase but lack enzyme activity. Regardless, the possibility for CRMP-MICAL interactions suggests a large signaling complex may exist to mediate Plexin signaling.

CRMP and Disease

Understanding CRMP signaling has significant clinical implications. The formation of appropriate axonal pathways and synaptic connections during nervous system development is critical for normal function in adults. Disruption of *Sema3A* signaling or CRMP activity may lead to devastating developmental neurological disorders. In addition, it has been shown that *SemaA* and CRMP are upregulated and play a role in axon growth and regeneration in response to nervous system injury.^{5,27,28}

CRMP proteins have also been implicated in a more common adult neurological disease. A highly phosphorylated form of CRMP2 was identified as a component of the neurofibrillary tangles associated with Alzheimer's disease.^{39,68} Transgenic mice overexpressing amyloid precursor protein intracellular domain (AICD), the protein implicated in the pathogenesis of Alzheimer's disease (AD), demonstrate an elevated level of phosphorylated CRMP2, likely due to increased GSK3 β activity.⁴⁰ It will be important to determine whether normal CRMP activity or disfunction of CRMP proteins play a role in AD pathogenesis. Interestingly, a processed form of *Sema3A* as well as *PlexA1*, *PlexA2*, and CRMP2 were copurified in a complex from the brains of AD patients, leading to the possibility that dysregulation of semaphorin signaling in general may play a role in disease phenotypes.⁶⁹

CRMPs are autoantigens for some paraneoplastic neurological disorders. Auto-antibodies against CRMP3 (anti-CV2) and CRMP5 (anti-CRMP5 IgG) have been found in patients with certain types of cancer.^{70,71} Patients who are producing these antibodies commonly suffer from peripheral neuropathy, cerebellar degeneration, and optic neuritis, among other neurological pathologies.^{72,73} It is unclear whether the neuropathies are a direct result of disruption of CRMP function by the antibodies or a secondary effect. Surprisingly, CRMP1 was shown to be expressed by breast cancer cells, although high levels of expression actually led to decreased motility, and therefore abrogated invasiveness.⁷⁴

Concluding Remarks

The CRMP family of proteins plays a central role in nervous system development and pathology. It is clear that CRMP is regulated by *Sema3A* signaling, and this is likely to occur both by phosphorylation of certain residues and by conformational changes in CRMP structure. Evidence links CRMP to cytoskeletal dynamics, G-protein signaling, and endocytosis, all of which are essential steps leading to growth cone responses to semaphorins (Fig. 3). The relative degree to which CRMP contributes to each of these processes remains to be elucidated, however it is quite evident that CRMP is able to tether these events to semaphorin receptors. Finally, interactions of CRMP family members with a variety of other signaling molecules and the pathogenesis of CRMP regulation in disease imply a role for CRMP in numerous cellular processes in addition to axon outgrowth and guidance. Therefore, further understanding of CRMP function is likely to elucidate numerous aspects of physiology and pathology.

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Figure 1.

Schematic depicting the regulation of CRMP by Sema3A signaling. A) CRMP is a cytosolic phosphoprotein that exists as a heterotetramer and can bind to the kinase Fes. In the absence of Sema3A, NP1 blocks CRMP-PlexA interactions. B) Upon Sema3A stimulation, the CRMP tetramer is recruited to the cytoplasmic domain of PlexA, undergoes a conformational change (oval shape), and is phosphorylated by Cdk5, GSK3β, and Fes. These events lead to a change in the CRMP activation state subsequently mediating cellular responses to Sema3A.

Figure 2.

Mapping regulatory residues onto the crystal structure of CRMP1. A) The crystal structure of a CRMP1 monomer (top) and tetramer (bottom). The surface residues contributing to tetramerization are highlighted in orange (interface 1) and blue (interface 2) on a GRASP diagram of the CRMP1 monomer (top left). A ribbon diagram depicting α -helices and β -strands are shown for the CRMP1 monomer (top right). In the tetramer structure individual monomers are colored in blue, pink, cyan, and yellow with oligomerization interfaces 1 and 2 indicated. B) Residues involved in the regulation of CRMP activity are mapped onto the CRMP1 structure. (B, top left) Ribbon diagram of CRMP1 monomer is reflected 180° from that in A, and the C-terminal 80 amino acids are represented by the oval. The N-terminal “activation loop” is highlighted in pink on the GRASP diagram (upper right). All of the phosphorylation sites of CRMP are located on the C-terminal 80 amino acids that are not included in the crystal structure. A color version of this figure is available online at www.Eurekah.com.

Figure 3.

A model for CRMP-mediated cellular responses to Sema3A. 1) CRMP binds to the AP2 vesicle adaptor complex proteins intersectin (ITSN) and Numb to facilitate endocytosis of the growth cone membrane and PlexA/NP1 Sema3A receptor complex. 2) Phosphorylation of CRMP by GSK3 β blocks the ability of CRMP to bind to tubulin dimers and subsequently prevents microtubule polymerization. 3) The Sra-1/WAVE complex, effectors of Rac1, regulate actin dynamics and are recruited to the growing axon by their interaction with CRMP. Sema3A may prevent the interaction of CRMP with the Sra-1/WAVE complex, thus attenuating recruitment and polymerization of actin in the growth cone. 4) CRMP interacts with the Rac1 GAP α 2-chimaerin. The GAP activity may then lead to the inactivation of a pool of Rac1 mediating actin dynamics and an inhibition of the Sra-1/WAVE complex. Another pool of Rac1 is activated by PlexA and promotes endocytosis (top). 5) CRMP binds to the Plexin-interacting protein, MICAL, and may modulate its oxidoreductase enzymatic activity. A red "X" indicates interactions or processes that may be blocked by Sema3A signaling. Refer to text for details. A color version of this figure is available online at www.Eurekah.com.