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Unconventional Signaling by Extracellular CRMP2: Possible Role as an Atypical Neurotransmitter?

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Collapsin Response Mediator protein 2 (CRMP2) is an abundant protein, comprising ~0.05% of embryonic brain (Goshima et al., 1995). It was first discovered as a protein necessary for mediating Semaphorin 3A (Collapsin)-evoked calcium currents in oocytes and growth cone collapse in dorsal root ganglia (DRG) neurons (Goshima et al., 1995). Since 1995, CRMP2 has been reported to be a nuclear, juxtamembrane and cytosolic protein, wherein it plays a role in physiological processes including, lymphocyte chemotaxis (Vincent et al., 2005), cancer cell proliferation (Moutal et al., 2017), axonal guidance, (Kamata et al., 1998) or intracellular trafficking (Brittain et al., 2011;Dustrude et al., 2016). The report by Castillo and colleagues in this issue of *Neuroscience* (Castillo et al., 2018) challenges the field's dogmatic view of CRMP2 as a solely intracellular protein serving intracellular functions.

In this groundbreaking study (Castillo et al., 2018), Castillo and colleagues identified CRMP2 as a component of conditioned media (CM) from degenerated sciatic nerve, optic nerve, and Schwann cells. However, whether CRMP2 was actively secreted from degenerating nerves or originated from cellular debris was not explored. Notwithstanding the source, CRMP2 elicited a Ca^{2+} influx through presynaptic N-type voltage-gated calcium (CaV2.2) channels and postsynaptic ligand-gated N-methyl-D-aspartate (NMDA) receptors (Fig. 1). Inhibition of CM-evoked Ca^{2+} influx was observed with a cell-impermeant blocking peptide derived from CRMP2 (i.e., CBD3), while extracellular application of purified CRMP2 was sufficient to elicit Ca^{2+} influx, congruent with an extracellular function for CRMP2. A possible extracellular role for CRMP2 had been suggested by prior reports demonstrating that extracellular application of antibodies against CRMP2 were able to affect both neurite outgrowth of DRG neurons (Goshima et al., 1995;Quach et al., 2004) and Semaphorin 3A-induced branching of oligodendrocytes (Ricard et al., 2001). The presence of CRMP2 in the extracellular milieu may explain the perplexing findings of auto-antibodies targeting presumptively *cytosolic* CRMP2 in patients with autism (Piras et al., 2014) or autoimmune retinopathy (Adamus et al., 2013).

The findings by Castillo and colleagues identify potential pathophysiological roles for secreted CRMP2 (Castillo et al., 2018). Notably, the secretion of CRMP2 from a degenerated sciatic nerve or from Schwann cells could be of high relevance in demyelinating diseases like multiple sclerosis or in chronic pain. Excessive CRMP2 secretion by degenerating Schwann cells could alter neuronal signaling by increasing activation of NMDARs thus leading to unabated Ca^{2+} influx that may culminate in cell death. CRMP2 was found to bind to and control the trafficking of both CaV2.2 channels and NMDA receptors (Brittain et al., 2011; Brustovetsky et al., 2014). Since both membrane-delimited proteins are involved in nociceptive signal transmission (Bourinet et al., 2014), the ability of secreted CRMP2 to facilitate CaV2.2- and NMDAR-mediated Ca^{2+} influx suggests a link to nociception. Application of a cell-permeable, CRMP2-derived peptide directly onto the spinal cord resulted in anti-nociception in models of inflammatory and neuropathic pain (Brittain et al., 2011). The novel finding of an extracellular CRMP2 calls into question the provenance of this beneficial effect – i.e., was it intracellular or extracellular? These lines of evidence raise the possibility of an extracellular CRMP2 participating in chronic pain signaling through positive regulation of CaV2.2 and NMDAR.

The idea that CRMP2 can be secreted and act on pre- and/or post-synaptic sites to elicit a Ca^{2+} influx is exciting and raises numerous novel questions: (*i*) does extracellular CRMP2 contribute to neurodevelopmental disorders like bipolar disorders (Tobe et al., 2017)?; (*ii*)

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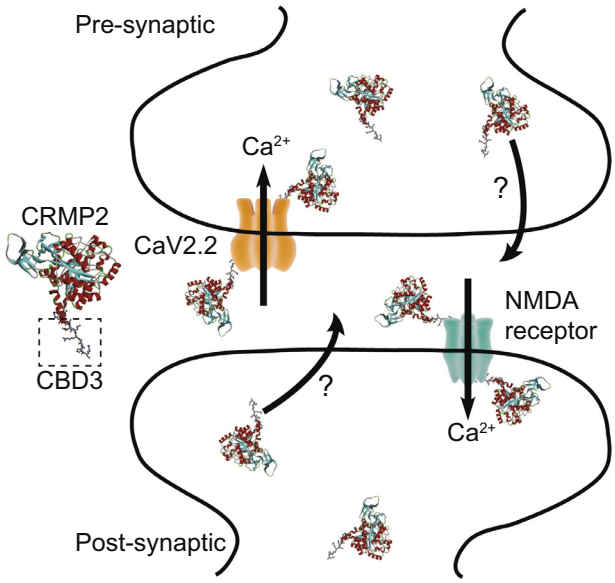

Fig. 1. Synaptic effects of a secreted form of CRMP2. Schematic representation of the salient findings of Castillo and colleagues (Castillo et al., 2018) demonstrating convergent regulation of calcium signaling via actions on intracellular as well as extracellular domains of voltage- (CaV2.2) and ligand- (NMDA) gated calcium channels. An intracellular pool of CRMP2 could positively regulate membrane localization of CaV2.2 channels and NMDA receptors. An extracellular pool of CRMP2 could synchronize presynaptic vesicle release gated by calcium influx via CaV2.2 to postsynaptic facilitation of NMDA receptors; however, not all cells respond to extracellular CRMP2. Together, both intracellular and extracellular CRMP2 pools may coordinate unconventional synaptic transmission, placing CRMP2 as a possible atypical neurotransmitter. At present, exactly how CRMP2 journeys to the outside of the cell is not known.

could extracellular CRMP2 participate in NMDAR loss of function in autism spectrum disorders (Hu et al., 2016) by triggering production of anti-CRMP2 auto-antibodies?; (*iii*) what would be the effect of secreted CRMP2 on the establishment of long-term potentiation?; (*iv*) could secretion of CRMP2 following a nerve injury elicit chronic pain?; (*v*) which post-translationally modified state of CRMP2 (phosphorylation, oxidation, or SUMOylation (Dustrude et al., 2016)) is secreted?; and (*vi*) how does secretion of CRMP2 occur, i.e. does it involve an active process or could it be a leak from the cytoplasm of damaged cells?.

Finally, an exciting and provocative question arises from the demonstration that CM-CRMP2 triggered opening of NMDARs. Other ligands doing so have been classified as agonists and/or neurotransmitters. Thus, future studies aimed at defining the biological relevance of secreted CRMP2 will need to consider whether CRMP2 could be classified as an atypical neurotransmitter.

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CONFLICT OF INTEREST

There is no conflict of interest for any of the authors.

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