Phosphorylation of Collapsin Response Mediator Protein 1 by Semaphorin 3A-Fyn signaling regulates basal dendritic growth and arborization

Aoi Jitsuki-Takahashi[1], Takeshi Kawashima[2], Susumu Jitsuki[3], Yoshio Goshima[2], Fumio Nakamura[1]

[1]Dept. Biochem, Tokyo Women's Medical Univ., [2]Dept. Mol Pharmacol and Neurobiol, Grad. Sch. Med., Yokohama City Univ., [3]Dept. Physiol, Grad. Sch. Med., Yokohama City Univ.

Collapsin Response Mediator Protein 1 (CRMP1) is an intracellular phoshoprotein that mediates Semaphorin3A (Sema3A) intracellular signaling. Upon Sema3A stimulation, Fyn, a Src-type tyrosine kinase, phosphorylates and activates Cycline-dependent kinase 5 (Cdk5), which subsequently phosphorylates serine 522 of CRMP1. In addition, it has been shown that Fyn directly phosphorylates tyrosine 504 (Y504) of CRMP1 (Buel et al., 2010). Then, we investigated the functional role of this phosphorylation in Sema3A signaling. We found that Fyn phosphorylated Y504 but not other tyrosine residues of CRMP1. A dominant negative mutant of CRMP1 Y504F, substitution of tyrosine 504 to phenylalanine (F), suppressed Sema3A-induced growth cone collapse of chick E8 DRG neurons. We next tested the role of Fyn and CRMP1 in Sema3A-mediated dendritic growth *in vivo. CRMP1-/-* or *Fyn -/-* single-homozygous mice as well as $Fyn^{+/-}$; $Crmp1^{+/-}$ double-heterozygous mice exhibited aberrant development of cortical layer V basal dendrites. Finally, we examined the dominant negative effect of CRMP1 Y504F on cortical dendritic morphogenesis. CRMP1 Y504F or CRMP1 WT with tdTomato was transfected in the mice cortical layer V neurons at E15 by *in utero* electroporation. CRMP1 Y504F-expressed layer V neurons showed poor development of basal dendrites compared with CRMP1 WT-expressed neurons at 5-weeks old mice. These results suggest that Fyn-induced phosphorylation of CRMP1 Y504 may participate in Sema3A-regulated axon pathfinding and cortical dendritic development.