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**The interaction of plexin-B family members with Rho Guanine Nucleotide Exchange Factor proteins: mechanisms and functions of Sema4D induced RhoA activation**

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In the present thesis project, I have identified and characterized a new signalling mechanisms employed by plexins of the B-family. I was able to show that B-family plexins interact with the guanine nucleotide exchange factors PDZ-RhoGEF and LARG and that this interaction is crucial for plexin-B signalling. The stimulation of the plexin-B1/RhoGEF complex with the plexin-B1 ligand, Sema4D, leads to RhoA activation. Moreover, PDZ-RhoGEF/LARG were found to be crucial for proper localization of B-plexins at the plasma membrane as well. Upon ligand activation, plexin-B1 but not its downstream mediators, PDZ-RhoGEF/LARG, become tyrosine phosphorylated. A series of experiments using various pharmacological inhibitors of common effectors followed by *in vitro* experiments with dominant negative proteins revealed that a member of the ErbB receptor tyrosine kinase family ErbB-2 is an important part of the plexin-B signalling complex. Further investigation revealed that B-plexins are able to form stable complexes with both PDZ-RhoGEF/LARG and ErbB-2 and that Sema4D activates the tyrosine kinase activity of ErbB-2 which then leads to phosphorylation of both plexin-B1 and ErbB-2 itself, followed by the RhoGEF-dependent RhoA activation. In parallel, phosphorylated ErbB-2 recruits the adaptor proteins Shc and Grb2 what in consequence leads to the activation of the MAP kinase cascade and finally to activation of Erk. Plexin-B1, PDZ-RhoGEF/LARG and ErbB-2 colocalize in various parts of the brain and the

coimmunoprecipitation experiments using brain lysates revealed that plexin-B1 and PDZ-RhoGEF/LARG also form complexes *in vivo*. Finally, provided data demonstrate that the plexin-B1 receptor complex is an important mediator of the Sema4D-induced collapse of growth cones in hippocampal neurons and that this effect involves PDZ-RhoGEF as well as ErbB-2.

Taken together, these data show that PDZ-RhoGEF and ErbB-2 are part of the plexin-B receptor complex. Both proteins are required for Sema4D induced plexin-B1-mediated cellular effects. In addition to the RhoA pathway, signalling processes downstream of ErbB-2, like the MAP kinase pathway, may be involved in effects induced by Sema4D.