SUMMARY

Synaptogenesis is a collaborative effort of activity-independent processes specifying neuronal connections, and activity-dependent processes refining these initial synaptic connections. Several families of molecules are known for establishing initial connections. Studies have hinted that proteins modulated by neuronal activity are promising candidates for refining synapses. We were interested in finding such molecules. A preliminary screen using KCl incubated chick retina cultures, recognized CALEB as an activity-dependent regulated molecule.

The aim of my work was to investigate the mechanism of CALEB down regulation. I showed that cell surface CALEB down regulation was enhanced within five min, in biotinylated chick retina cultures incubated with KCl or agonist of glutamate receptors in comparison to untreated cultures. Modulation of extracellular calcium concentration and experiments using blockers of calmodulin revealed the involvement of calcium in the mechanism of down regulation. Since, CALEB belongs to the EGF family of growth and differentiation factors like HB-EGF and TGF-α which undergo cell surface cleavage, I hypothesized a similar phenomenon responsible for the cell surface down regulation of CALEB. Isolation of a 18 kD soluble component in supernatants of KCl treated cultures and a remaining membrane-tethered part of 38 kD in KCl incubated chick retina cultures confirmed my hypothesis. I showed in membrane fraction incubation experiments, that cleavage of CALEB ectodomain was caused by the catalytic action of a membrane protease and that shedding of CALEB ectodomain was prevented in the presence of hydroxymate broad spectrum metalloprotease inhibitor. For a detailed analyzation of CALEB shedding two ADAM (A disintegrin and metalloprotease) proteases, ADAM 10 and ADAM 17 were selected, based on their ability to be activated by calcium influx and to cleave other EGF-family members (Han and Fischbach, 1999;Ito et al., 1999b;Sahin et al., 2004). Blockade of ADAM 10 and ADAM 17 with pharmacological inhibitors prevented shedding of CALEB. Furthermore, increased expression of ADAM 10 resulted in a decrease of total chick CALEB in comparison to COS-7 cells coexpressing dominant negative ADAM 10 and chick CALEB. These results supported my idea that metalloproteases ADAM 10 and ADAM 17 cleave cell surface CALEB. Furthe experiments showed the involvement of Erk kinase in ectodomain shedding of CALEB.

I assumed that inhibition of ectodomain shedding for longer duration would result in stagnation of CALEB synthesis, arising of a feed back mechanism resulting from the accumulation of uncleaved CALEB. My assumptions were disproved when, the results revealed an upregulation of total CALEB after 4 hr, in cultures incubated with KCl and TAPI (metalloprotease-inhibitor) with respect to untreated cultures or cultures incubated with TAPI alone. This upregulation could be prevented by blocking protein translation.

In another part of my project, in order to predict a plausible role of CALEB during development I investigated the expression and localization of CALEB in the visual system. The appearance of the membrane attached fragment of CALEB in Western blots starting from E13 chick retina might suggest ectodomain shedding having a role in synaptogenesis begining at E12 . In mouse, deglycosylation experiments showed CALEB as a highly glycosylated brain specific protein. Subcellularly, CALEB was enriched in the synaptic junction fraction obtained from post-synaptic density preparation, pointing to a possible role of CALEB in synapses. The developmentally regulated expression of CALEB in mouse retina with a steep decrease at P10-12 and in superior colliculus with a peak expression at the same stage of development (P10-12), suggested involvement of CALEB before eye opening.

My findings suggest that neuronal activity induced by depolarization leads to ectodomain shedding of CALEB by the catalytic action of metalloproteases. The membrane attached EGF-domain of CALEB could act as a receptor for an unknown ligand, thereby activating signalling cascade. The expression profile and localization confirms CALEB to be a brain specific, glycosylated transmembrane protein, which might have a role during the formation or maintainence of synapses.