F. FUTURE PROSPECTS

The most interesting way to go ahead with this project would be to identify the missing molecules in the cascade for ectodomain shedding. In our study we demonstrated ADAM 10 responsible for ectodomain shedding of CALEB. Following experiments in similar lines, role of ADAM 17 in CALEB shedding could be investigated i.e., knock-out cell lines of ADAM 17 could be transfected with CALEB cDNA and analyzed for ectodomain shedding. Silencing the enzymes in primary culture or in-*ovo* and analysis of cell surface CALEB would confirm the role of enzyme (s) in *invivo* situation. The role of Erk kinase, which has been implicated in ectodomain shedding, can be studied in more details by investigating the phosphorylation state or looking for the involvement of other molecules belonging to the MAP kinase pathway.

In scope of this study I did not look at the function of the released fragment. It would be interesting to look at the released fragment and the membrane attached part with respect to either stablization or maturation of synapses. Based on the available electrophysiological data from Dr. René Jüttner, implication of shedding could be investigated towards it role in maintainence of synapses. Electrophysiological stimulus could be used to induce electric activity in a local area of cell culture to induce shedding followed by measurement of physiological properties such as frequency, amplitude etc. This would give a direct outcome of ectodomain shedding on physiological function of the cell as well as it would give information about the effect of shedding on the functionality of synapses, specifically release of neurotransmitters, number of post-synaptic receptors, number of contacts between pre and post-synaptic cell.

The presence of an intact membrane attached remaining fragment of CALEB after shedding would prompt to look in detail for the functionality of the cytoplasmic domain, which can be performed by attaching a tag (GFP), transfecting and fluorescent microscopy. It is known that EGF domain containing molecules are affected in tumorigenic processes. Therefore, it would be of great interest to look if the exposed EGF domain on the membrane after CALEB shedding would have consequences on the occurrence and progression of cancer. Assays on cell migration, adhesion can also be performed to investigate the outcome of ectodomain shedding in cell lines.