B. AIM AND HYPOTHESIS

The initial synaptic connections are dependent on the presence of so-called axonal guidance cues whereas the established connections are most likely refined by an activity-dependent process (Raper and Tessier-Lavigne, 1999). The activity-dependent step leads to the elimination of weak synaptic connections and strengthening of the strong connections, thus stabilizing neuronal connectivity (Katz and Shatz, 1996). While neuronal guidance cues have been characterized, less is known about the proteins which are involved in refinement processes. Our lab has been interested in identifying cell surface proteins which are regulated by neuronal activity. These proteins might be candidates for synapse refinement (Wong et al., 1995).

1. Part-I: Is CALEB an activity-dependent regulated molecule

1.1 Hypothesis:

An initial screen was performed to identify cell surface proteins that were regulated upon long term potassium chloride (KCl) treatment by Prof. Rathjen. CALEB was fished out in this screen and appeared down regulated, whereas the expression of other cell surface proteins such as NgCAM and F11 remained unaffected. Chicken acidic leucine rich EGF-like domain containing brain (CALEB) protein was identified originally by combining ELISA binding assays with immunological screens (Schumacher et al., 1997). I hypothesized that CALEB down regulation should be observed not only by long term KCl treatment, but also by shorter incubation with KCl and by application of agonists of neurotransmitter receptors. I was also interested to study the kinetics of CALEB down regulation.

1.2 Objective:

To analyze this phenomenon I used biotinylated chick retina cell cultures. Effect of depolarization induced by KCl and agonists of excitatory neurotransmitters was studied on cell surface CALEB expression. Western blotting was used as a tool to analyse the expression of CALEB on the cell surface.

2. Part-II: What is the mechanism of the activity-dependent down regulation

2.1 Hypothesis:

Depolarization of cells leads to a shift in the membrane potential inducing a cascade of events (Li et al., 1996). One of the initial effects of depolarization is opening of the calcium

channels followed by an influx of calcium ions which plays crucial role directly or as a second messenger in various signalling cascades (MAP kinase pathway) (Corriveau, 1999). I hypothesized that activity-dependent down regulation of CALEB could be due to endocytosis or proteolytic processing based on the facts that CALEB is a cell surface molecule like the AMPA receptors which undergo endocytosis upon depolarization or CALEB belongs to the EGF family of proteins like TGF-α, HB-EGF, neuregulin that undergo proteolytic processing (Pandiella et al., 1992;Dempsey et al., 1997;Sunnarborg et al., 2002;Sahin et al., 2004)

2.2 Objective:

The aim was to investigate the mechanism of cell surface CALEB down regulation as an outcome of endocytosis or proteolytic processing. To prove proteolytic processing, the presence of a released fragment of CALEB in the cell supernatant, and a possible membrane bound remaining fragment after proteolytic processing was analysed. For detection of a membrane remaining fragment, antibodies against the cytoplasmic domain of CALEB were used.

3. Part-III: Is the Ectodomain shedding of CALEB caused by a protease

3.1 Hypothesis:

EGF-family of proteins like EGF, TGF-α, HB-EGF and neuregulin undergo ectodomain cleavage by the activation of metalloproteases (Fischbach and Rosen, 1997;Fan and Derynck, 1999;Hirata et al., 2001;Ozaki et al., 2004). The most common proteases have been the ADAM group of enzymes (Moss et al., 2001). These are membrane proteases which can be activated by calcium influx and by the Erk kinase (Pandiella and Massague, 1991;Phong et al., 2003).I hypothesized that CALEB undergoes ectodomain shedding most likely due to the action of ADAM metalloproteases.

3.2 Objective:

In order to characterize the protease responsible for shedding and to analyze down stream signalling pathways that result in shedding of CALEB, chick retina cultures were incubated with blockers of membrane metalloproteases and activators/blockers of signalling pathways and. Membrane fractions were isolated to determine whether the nature of the protease responsible for CALEB shedding. To further strengthen the findings, cDNA of CALEB was co-transfected with cDNA of the ADAM protease, and the results were verified by Western blot.