Summary

The CAP family of proteins belongs to the actin binding proteins and is found in all analysed eukaryotic species. The first member of the family was discovered as a component of the adenylate cyclase in *S. cerevisiae*. The later CAP homologues were identified in a wide variety of animals, plants and fungi. Mammals express two homologues, CAP1 and CAP2.

This work included the construction of a knockout vector for mouse CAP1. In addition, the expression pattern of CAP1 in different organs and the subcellular localization of CAP1 in different cell lines was tested.

Genomic ES-cell DNA as a template and the proofreading enzyme *Pfu*Turbo®-DNA-Polymerase were used to amplify the arms of the ko vector by PCR. Together with the neomycin resitance cassette these arms were cloned into a pBS vector. The homologous recombination of the knockout vector with the chromosomal target sequence will result in a frameshift mutation. This will lead to disruption of the function of one CAP1 allele. Putative alternative splice sites could have produced a truncated protein missing the N-terminal domain. After the transfection of the ES-cells with the ko vector, 450 clones were isolated and screened by Southernblot analysis. So far no positive clone was detected.

In a Westernblot analyses of homogenized tissues the biggest amounts of CAP1 were detected in thymus, spleen and lung followed by testis, liver and stomach. The lowest expression levels were revealed in heart, skeletal muscle, brain, skin and kidney. The expression of CAP1 was more precisely tested in the brain. In newborn mice the expression level of CAP1 was higher in the cortex and the olfactory bulb than in the homogenates of thalamus/ striatum, brainstem and *Cerebellum*. In adult mice the thalamus and striatum were tested separately and in addition to the brain areas tested in the newborn mice, *Medulla oblongata* was tested. For most brain areas a similar expression level of CAP1 was detected. Just in the thalamus and the *Medulla oblongata* a slightly elevated CAP1 level were observed. In addition to the westernblot analysis, CAP1 could be detected in the olfactory bulb, cortex, hippocampal formation, striatum, *Cerebellum* and the *Capsula interna* by indirect immunofluorescence.

Northernblot analysis showed an equal expression of CAP1 from embryonic day 7 to day 17. At the same time indirect immunofluorescence studies revealed a restricted expression of CAP1 to several organs.

The subcellular localization of CAP1 was examined by indirect immunofluorescence and by expression of GFP-CAP1 fusion protein in cells. Additionally cell homogenates were checked by subcellular fractionation.

In fibroblasts and keratinocytes the cytosolic protein CAP1 locallized with F-actin at the leading edges of the cells. In differentiated neuroblastoma cells GFP-CAP1 was concentrated in the growth cone. In wound healing experiments CAP1 is found to be enriched at the leading edge of fibroblasts and keratinocytes too. Taken together CAP1 was enriched in all cases at sites with highest actin dynamics.

In the subcellular fractionationing CAP1 couldn´t be shown as associated to the membrane via actin. Instead of this it was shown that CAP1 is forming multimers.