

## Summary

The available work describes studies for the function of the bifunctional enzyme GNE, the key enzyme of the biosynthesis of sialic acids. The GNE and/or sialic acids are essential for the organism, since GNE deficient (KO-) mice are embryonic lethal. This work focuses on the elucidation of the regulation of this enzyme over its different oligomeric states and on the identification of interacting proteins. Additionally murine embryonic stem cells, which are deficient in GNE were isolated and characterized. Using Y2H system the kinase domain of the GNE was identified as important component for oligomerization. In addition by screening a cDNA gene bank from fetal human brain four interacting proteins were isolated: CRMP1, PLZF, RIF1 and KIAA1549. The interaction of CRMP1 with GNE could be confirmed by coimmunoprecipitation. Likewise the interaction of PLZF with GNE could be verified by pull down assays. After the isolation of murine embryonic stem cells these were characterized first genotypically using PCR and biochemically using radioactive enzyme assay. Subsequently, they were examined for their stem cell markers, in order to guarantee the pluripotency of these cells. WT and KO cells were cultivated under FCS containing and serum-free conditions and were compared concerning their sialic acid content (resorcinol assay) and cell surface sialylation (FACS). Subsequently, KO stem cells were incubated with the sialic acid precursor ManNAc, in order to adjust the deficit at sialic acids. It was found out that a concentration of 1-3 mM ManNAc is sufficient, to raise the sialylation level of WT stem cells. The proliferation of stem cells was examined on different culture conditions, whereby no difference between WT and KO stem cells could be determined.