7. Summary

This work describes the investigation of protein S-acylation as a hydrophobic posttranslational modification by different *in vitro* experiments. The main interest was on the mechanism of fatty acid transfer onto the protein with a special emphasis on the enzymology of the modification, which still remains mostly unknown. In order to find evidence for an enzymatic process it will be necessary to isolate and identify an enzyme at the molecular level. In this regard purifying an enzyme with protein fatty acyl transferase (PAT) activity was the aim of this work. Establishing an enzyme assay using peptides and recombinant proteins as substrates for PAT, the central focus of the investigations, served both to develop new tools for purifying PAT and to further characterize S-acylation. Biochemical and cell biological characterizations were also gained from the experiments which aimed for localizing the enzyme within the cell and cell membranes. The results can be summarized as follows:

1. Immobilized peptides which mimick sequences of palmitoylated proteins did not function as substrates (fatty acid acceptors) in the tested *in vitro* PAT-assays. Palmitoyl-CoA was transferred onto these synthetic peptides but the results did not match the results given in experiments with the corresponding protein. The PAT-assay using a peptide of 10 amino acids of the acylation site of E1 (SFV) as substrate showed contrary results to the assay performed with deacylated E1 isolated from SFV envelope membranes. On the other hand the results of the assays using these peptides agree with *in vitro* PAT assays described in literature for the myristoylated peptide representing myristoylated protein tyrosine kinase Yes (Bañó *et al*., 1998). But for this investigation there is no comparison to the original protein which questions their meaningfulness in site of our results.

2. No *in vitro* PAT-assay was established using SNAP-25-His_6 or GAP43-GFP-His_6 recombinant proteins expressed in *E.coli* as substrates. The fusion protein GAP43-GFP-His_6 carries 10 amino acids of the amino terminus of GAP43 or neuromodulin. Interestingly another group (L. Berthiaume and his colleagues) showed in GFP localization experiments that the fusion protein gets palmitoylated with functional relevance *in vivo*. So we hypothesize that the expression of the 10 amino acid fusion protein in *E. coli* is not sufficient to confer palmitoylation.

3. In the purification process of the PAT-enzyme from human placenta microsomes, we were able to advance the purification by adding further chromatographic steps. E1 (SFV) substrate served as indicator for PAT-activity. For the enrichment of the activity in the microsomes compared to the placenta homogenate a factor of 225 could be calculated.

From these microsomes PAT activity was solubilized by the nonionic detergent TX-100, before chromatographic steps with DEAE fast flow and blue-Sepharose could follow. After these steps the activity was very labile which prevented further purification.

4. The investigation of the subcellular distribution of PAT-activity which palmitoylates $p59^{fyn}$ gave interesting results. Plasma membrane and Golgi apparatus and endoplasmatic reticulum fractions from rat liver where tested for PAT-activity. Only Golgi and plasma membrane showed PAT activity towards the Fyn substrate and not the endoplasmic reticulum. Further comparison revealed differences in the solubility of the activity from plasma membrane and Golgi. Golgi PAT activity could only be extracted from membranes by 100 mM Na_2CO_3 but was resistant to extraction by various NaCl concentrations. Plasma membrane PAT activity could be solubilised by a shaking incubation of one hour without increased salt concentration or detergent present. These evidences of different membrane extraction properties suggest the existence of different PAT-enzymes.

Even though the goal of purifying the PAT enzyme to a single band as analyzed by electrophoresis was not reached, the reported results of this research contributes new scientific information on the ongoing investigations of palmitoylation. They show that the choice of enzymatic assays plays a critical role and that the comparison of the synthetic substrate to the original or native protein is an important control to critically assess biochemical palmitoylation data. In the data provided by our results the membrane bound PAT-activity shows characteristics of a protein related, enzymatic activity. Therefore it supports the theory of an enzymatic mechanism of palmitoylation. The various subcellular distributions of PAT activity and the difference in the solubilization properties of these PAT activities suggest the existence of different members of a PAT family.