## Principal Investigator: Dr. Richard Strasser Grant Title: Identification of the Arabidopsis N-acetylglucosaminyltransferase I receptor

N-glycosylation is an essential modification of secretory proteins. The correct localization of N-glycan processing enzymes within the Golgi apparatus dictates N-glycan structures and therefore glycoprotein function. Hence, it is important to unravel the underlying mechanisms that concentrate Golgi resident glycosyltransferases and glycosidases distinct in Golgi compartments. While different models have been suggested to



control the localization of mammalian and yeast glycosylation enzymes, comparable little is known about these mechanisms in plants.

<u>1. Objectives:</u> In this project we focused on plant N-acetylglucosaminyltransferase I (GnTI or MGAT1) which is the key enzyme for the initiation of complex N-glycan formation in all higher eukaryotes. We have shown previously that the single transmembrane domain of GnTI is a critical determinant for proper localization within the Golgi apparatus. The main objective of this project was the identification of a specific protein (or protein complex) that interacts with a distinct sequence motif present in the GnTI transmembrane domain and is required for GnTI localization in the *cis*/medial Golgi of plants. Since GnTI interacts with other *cis*/medial Golgi-resident N-glycan processing enzymes, the proposed GnTI-receptor might represent a master regulator for the retrograde transport of *cis*/medial Golgi resident enzyme complexes in plants.

<u>2. Methods used:</u> We purified GnTI from the model plant *Arabidopsis thaliana* and used a mass spectrometry-based proteomics approach to identify GnTI interacting proteins. Two of the identified proteins were characterized further by expression in plants and subcellular localization studies.

<u>3. Results:</u> The two proteins reside in the ER and Golgi, respectively, and the interaction with GnTI could be confirmed for both proteins by co-immunoprecipitation experiments. The analysis of *Arabidopsis thaliana* knockout as well as overexpression lines will reveal whether these so far uncharacterized proteins indeed play a role in proper localization of GnTI and subsequent processing of N-glycans.