In mammalian cells proteins and lipids belong to the major groups of macromolecules. Both may undergo modifications. Glycosylation is one from the most common modifications of proteins and lipids present in eukaryotic cells. Glycosylation of proteins may change the structure and function of the macromolecule. It may change polarity of the protein, stabilize its 3D structure and protects against proteolysis. The cell glycoconjugates (glycoproteins, glycolipids and proteoglycans) may form contact zones between cells in animal or human tissues. Many important proteins contain sugar part, including antibodies, receptors and enzymes. In the majority of eukaryotic cells, glycosylation belongs to the most frequent posttranslational modifications of macromolecules. In many mammalian cells and tissues the majority of proteins and a substantial part of lipids is glycosylated. Cellular glycoconjugates play a variety of fundamental roles in the growth and development of eukaryotes, as well as in the cell surface recognition of hosts by pathogens, therefore in our opinion advanced knowledge of glycosylation mechanisms is of crucial importance.

The glycan moiety is synthesized with the involvement of glycosyltransferases and activated sugars – nucleotide sugars. Nucleotide sugars are synthesized in the cytosol. To be available for glycosyltransferases, they must be transported into the endoplasmic reticulum or Golgi apparatus, cellular structures built from membrane vesicles. This function is played by nucleotide sugar transporters (NSTs), which are hydrophobic proteins with a molecular weight of 30-45 kDa. All known proteins belong to the SLC35A-F family.

For a long time, it was accepted that SLC35A3 is the major transporter UDP-*N*-acetylglucosamine (UDP-GlcNAc), one of the most important and common precursor (substrate) for glycosylation. However, our recent studies show that after knock-out of SLC35A3, the glycoconjugate structures are not significantly affected, GlcNAc was still present in *N*- and *O*-glycans, although some cell-specific differences in oligosaccharide structure could be detected. Also transport of the nucleotide sugar, decreased in some cell lines (but not all of them), still remains relatively high.

The aim of this project is to decipher the routes of UDP-GlcNAc delivery from the cytosol to the lumen of the Golgi apparatus, either coordinated by or independent from the SLC35A3 activity. The project includes searching for other protein candidates responsible for transport of UDP-GlcNAc. Another approach to recognize such transporters assumes identification and characterization of membrane complexes, formed by transferases utilizing UDP-GlcNAc as substrate for glycan chain synthesis. Given the diversity of glycan structures and the competitive nature of many glycosyltransferases it is remarkable that the cell can synthesize several different oligosaccharides with high precision.. Therefore, we propose that interaction between NSTs and glycosyltransferases and also between NSTs might constitute a general mechanism utilized in the glycosylation process. This project is a logical continuation of studies carried out in our laboratory and extension of our recently published data, but is focused only on UDP-N-acetylglucosamine as a model of turnover and transport of a nucleotide sugar into Golgi apparatus driven by more than one membrane transporter.

We hope that our project will allow understanding of the mechanism of UDP-GlcNAc translocation from cytosol to the lumen of Golgi apparatus by identification of new proteins involved in this transport. We assume that SLC35A3 transporter is not the main protein involved in UDP-GlcNAc delivery into Golgi apparatus as a substrate for cellular glycosylation.