Glycosylation is an irreversible, enzymatic reaction, involving the incorporation of sugar residues into proteins and lipids. This modification plays a crucial role in development and growth of eukaryotic cells. This process occurs inside the Golgi apparatus and the endoplasmic reticulum. Substrates required in glycosylation are sugars activated by the addition of mono- or diphosphonucleotides (nucleotide sugars). It requires a number of proteins to work properly. The most important are the enzymes that catalyze the reaction (glycosyltransferases) and proteins supplying nucleotide sugar molecules to the appropriate organelles (nucleotide sugar transporters). The proteins from the SLC35 family (solute carrier family 35), are grouped into eight subfamilies (from A to H). They are responsible for the delivery of nucleotide sugars. These small, multi transmembrane molecules are located in the membranes of the Golgi apparatus and endoplasmic reticulum. UDP-*N*-acetylglucosamine (UDP-GlcNAc) is one of the most important nucleotide sugar in the glycosylation process. It is synthesized in the cytosol, whereas there are three proteins, which according to the literature, act as transporters of UDP-GlcNAc: SLC35A3 (A3), SLC35B4 (B4) and SLC35D2 (D2). A3 is considered as the main transporter that is why *SLC35A3* gene transcript is present in all human organs, while *SLC35B4* and *SLC35D2* genes are expressed in selected organs only. Interestingly, there are organs in which all three proteins occur simultaneously. The existence of three proteins performing the same function in mammalian cells is the main goal of the presented project. Another type of glycosylation is the addition of *N*-acetylglucosamine (GlcNAc) to proteins in the cytosol by an O-glycosidic linkage (O-GlcNAc). It is a very dynamic modyfication, catalyzed by O-GlcNAc transferase (OGT). It also requires UDP-GlcNAc as a substrate. Many diseases are associated with abnormalities in this process, e.g. Alzheimer's disease. From our preliminary experiments, it appears that the reactions of addition of *N*-acetylglucosamine within the Golgi apparatus and cytosol could be associated. We suspect that the transporters of this nucleotide sugar are responsible for this interplay between here described processes. Therefore, the second goal of this project is to analyze the impact of UDP-GlcNAc transporters on O-GlcNAcylation.

In this project, we plan to use the modern techniques of molecular biology and biochemistry to investigate the role of A3, B4 and D2 proteins in the transport of UDP-*N*-acetylglucosamine to the lumen of the Golgi apparatus and in the O-GlcNAc process. Our models for the experiments would be cell lines that do not have functional A3, B4 and D2 proteins as well as two or three transporters that we will simultaneously generate during this project. In modified cells we will examine various sugar structures.. We will also check how the lack of the transporters affects the localization and attachment of *N*-acetylglucosamine to proteins using sugar labeled with azide. We also plan to study UDP-GlcNAc transport inside the Golgi apparatus in the generated mutants. For this propose we will use radioactively labelled UDP-*N*-acetylglucosamine. To investigate the changes in O-GlcNAcylation we will employ inhibitors of this reaction, It is necessary because O-GlcNAcylation is a highly dynamic process. We will also analyze the impact of overproduction of OGT transferase on the glycosylation process. The results obtained from the modified lines will be compared with the data obtained for wild type cells.

Abnormalities in the delivery and attachment of UDP-GlcNAc in glycosylation and O-GlcNAcylation cause serious illness especially in the skeletal and nervous systems, and may mediate the development of cancer. Understanding the exact role of nucleotide sugar transporters in these processes may in the future become the key to understand these pathologies and creating therapies. In addition, broadening our knowledge about glycosylation will allow us to better understand many biological processes.