Proteins belong to the most important macromolecules in the cell. They play fundamental role in regulation of the metabolism and also build up most of cellular and tissue structures of any living organism. Although the primary structure of polypeptide chain is encoded in DNA, it is often (especially in eukaryotic cells of higher plants and animals) modified after synthesis (translation). There are many known modifications, for example proteolysis, phosphorylation or glycosylation. The latter type occurs first of all in eukaryotic cells and belongs to the most common posttranslational modifications of proteins and lipids, and is especially frequent in cells and tissues rich in proteins synthesized in secretory pathway. In some human tissues/organs (i.e. kidney or liver) most synthesized proteins are glycosylated. Cellular glycoconjugates (glycoproteins and glycolipids) play a variety of fundamental roles in the growth and development of eukaryotes, as well as in the cell surface recognition.

Monosugars are covalently attached to proteins in endoplasmic reticulum (ER) and Golgi apparatus (GA), the cellular organelle build from stacks of cisterns (vesicles). All known glycosylation reactions need active forms of monosugars – nucleotide sugars (NS) as substrates. However, NS are synthesized outside AG or ER and must be transported across the biological membrane of these organelle. This function is played by nucleotide sugar transporters (NSTs) which are hydrophobic proteins with a molecular weight of 30-45 kDa.

Genetic defects of selected NSTs will be investigated in this project. Such defects belong to congenital disorders of glycosylation (CDG). Recent data suggest that they can occur more frequently than one previously expected and can accompany several systemic diseases. So far such genetic syndromes were described for SLC35A2 (UDP-galactose transporter), SLC35A3 (UDP-GlcNAc transporter and SLC35C1 (GDP-fucose transporter). It is interesting that in some cases supplementation of patients diet with respective monosugar could (at least partially) correct phenotypic defects in glycosylation of macromolecules resulting in significant health improvement. However, to date, molecular bases of such therapies are not known. It is important to understand the basic mechanisms of metabolic processes leading to health improvement.

The aim of this project is to execute a cell-based model studies and characterize molecular bases of a therapy involving sugar-supplemented diet. For this purpose, defects found in CDGs caused by mutated sequences encoding selected NSTs will be examined.

All experiments will be performed on several mammalian cell lines with specific genetic manipulation to inactivate genes encoding SLC35A2, SLC35A3 and SLC35C1.

Rare genetic syndromes are usually out of interest of leading pharmaceutical and biotechnology companies. Results gained from this project, which should uncover molecular bases of therapy used to treat patients with CDGs, would be useful for improving of monosugar treatment at least in some types of CDG, caused by functional mutations in NST transporters. In the future it would be also possible to design a diagnostic test using model cells generated in this work. The project may also explain more detailed function of investigated NS transporters, especially their specificity and the way of proteins' co-operation in NS delivering into ER/Golgi lumen.