

Proteins and lipids, belong to the major groups of macromolecules. In living cells they may undergo many unique modifications. One of them, glycosylation, is the most common 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 (NS), the substrates for glycosylation. 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).

Recent scientific data, including ours, suggested that the synthesis of NS, their translocation to the lumen of ER or GA, and glycoconjugate synthesis occurring with the action of glycosyltransferases, may be structurally and functionally connected, and therefore this project aims to discover connections between the mentioned processes. The proposal aims to analyze the regulation of NS synthesis on the cytosolic side, the potential coupling of such syntheses with NS transport into the GA/ER lumen, and the synthesis of glycoconjugates during glycosyltransferases action in GA. We plan to elucidate structural and/or functional connections between these processes. From the practical point of view, we are also interested in investigations of the influence of monosugar supplementation on glycoconjugate syntheses in cells with the knock-out (KO) of genes encoding selected enzymes involved in NS synthesis. This may be potentially important when the therapy of human congenital diseases of glycosylation (CDG) symptoms caused by defects in NSTs is applied. We will focus on galactosylation and fucosylation because both are common to mammalian cells and transporters for UDP-galactose (UDP-Gal) and GDP-fucose (GDP-Fuc), SLC35A2 and SLC35C1, respectively, were discovered. Both substrates are formed in cytosol in quite similar way, where the dominant, *de novo* pathway and an additional, salvage pathway are involved.

Our planned, research tasks include an *in vitro* reconstitution of main and salvage pathways of UDP-Gal and GDP-Fuc biosynthesis, to analyze potential, functional links between them. These enzymes will be also analyzed not directly, using cell lines with silenced genes, which code proteins taking part in NS biosynthesis and transport. Experiments to check potential, close proximity of investigated enzymes will be also performed. Routinely used in our laboratory methods will be applied. For initial screening, classical immunoprecipitation analyses, with tagged variants of proteins expressed in mammalian cell systems and MS detection, and more sophisticated protein-protein interaction experiments, like BioID, FRET-FLIM, *In Situ* Proximity Ligation Assay (PLA), NanoBit. Co-localization of selected proteins will be also investigated using high resolution electron microscopy. Also the global transcriptome analyses will be performed, to show or exclude changes on genetic level.

All foreseen tasks, listed above, should help to answer the following questions:

- How *de novo* and ‘salvage’ pathways of GDP-Fuc and UDP-Gal synthesis are regulated?
- Are these pathways specific for some glycoconjugate synthesis?
- Are other, not mentioned here, proteins involved in the forming of glycocomplexes that take part in galactosylation or fucosylation?
- Are other genes activated or repressed in KO cells?