Nucleotide sugar transporters as organizing centers of glycosylation-related macromolecular complexes

In eukaryotic cells a significant subset of proteins and lipids undergo glycosylation. This process involves a stepwise, enzyme-catalyzed attachment of sugar residues to the appropriate acceptors and among others results in the emergence of glycoproteins and glycolipids. Glycoconjugates play many important roles in multicellular organisms, including participation in a variety of biological recognition events, therefore, alterations of carbohydrate moieties, termed glycans, cause severe metabolic disorders. Sugars are attached to macromolecules with the involvement of specific enzymes, namely glycosyltransferases, inside the endoplasmic reticulum (ER) and Golgi apparatus. Glycosyltransferases utilize nucleotide-activated sugars as substrates. However, these compounds are synthesized mainly in the cytosol, therefore, presence of specialized proteins with transporting activity is required for glycosylation. These proteins, named nucleotide sugar transporters, carry active forms of sugars across organelle membranes making them available for glycosyltransferases. The process of glycosylation is very complex and apart from glycosyltransferases and nucleotide sugar transporters it requires involvement of many other proteins. We believe that glycosylation-related proteins form multicomponent assemblies in the ER and Golgi membranes to enable tight regulation of glycosylation and ensure the efficiency and fidelity of the corresponding reactions. We know that certain nucleotide sugar transporters homooligomerize and interact with other transporters as well as with the functionally related glycosyltransferases. Based on these findings and the specific structure of nucleotide sugar transporters (presence of multiple transmembrane domains enabling "tight" anchorage to the lipid bilayer) we propose that these proteins are organizing centers of glycosylation-related macromolecular complexes. According to our concept, the biological role of nucleotide sugar transporters is not limited to the delivery of substrates for glycosylation reactions but also facilitates glycosyltransferase assembly into larger functional units enabling an efficient and uninterrupted onset of the glycosylation process. We also intend to identify other proteins that may possibly associate with these assemblies, reveal mechanisms that regulate interactions between these two groups of proteins and find out, where these interactions are initiated. We plan to conduct our research using some novel, extremely sensitive techniques based on bioluminescence, which is emission of light by living cells. Such phenomenon can be triggered by expression of a protein fused with the luciferase enzyme. It is also possible to split the luciferase into two fragments, which alone do not display any activity but can reconstitute an active enzyme molecule when brought into close proximity. The main approach we intend to use relies on simultaneous expression of the two proteins suspected of interaction fused with fragments of split luciferase. If these two proteins interact, a functional enzyme is reconstituted form the fragments triggering the emission of light (this requires addition of an appropriate substrate). The presence of bioluminescence within cells can be detected with a dedicated plate reader (a luminometer) but can be also visualized with a specialized imaging system. The results obtained in this project should provide us with some novel data on the mechanisms of glycosyltransferase organization into larger functional units and allow us to assign nucleotide sugar transporters with some novel functions. Our findings may also help in understanding of the symptoms accompanying diseases caused by alterations in glycosylation resulting from mutations within the genes encoding enzymes and transporters we are studying. Moreover, numerous therapeutic proteins are glycosylated. They are obtained using cell lines, which are often subjected to some genetic modifications aimed to trigger targeted changes in the structures of glycans that are attached to the protein to be synthesized. Knowing the reason why nucleotide sugar transporters and glycosyltransferases interact with each other and what other proteins are components of the corresponding complexes we might be able to better modulate the conditions of therapeutic glycoprotein production.