Principal Investigator: Anant K. Menon
Grant Title: Molecular mechanism of glucosylation of the dolichol-linked N-glycan precursor

Abstract

Protein glycosylation is essential for all eukaryotes, from disease-causing protists such as malaria and trypanosomes, to yeast and mammals. Secretory proteins are almost invariably *N*-glycosylated, *O*- and *C*-mannosylated, and/or GPI-anchored as they enter the lumen of the endoplasmic reticulum (ER). Yeast cells that cannot synthesize *N*-glycoproteins or GPI-anchored proteins are inviable, and mice with the same defects die as embryos. Specific defects in glycosylation cause human disease. For example, defective *O*-mannosylation is a major underlying cause of muscular dystrophies, and a GPI anchoring defect in multipotent hematopoietic human stem cells causes paroxysmal nocturnal hemoglobinuria, an acquired hemolytic disease. Congenital Disorders of Glycosylation (CDGs) are a family of severe inherited diseases characterized by predominantly neurological symptoms.

Protein *N*-glycosylation occurs in the *lumen* of the ER. The oligosaccharide moiety from dolichol-diphosphate-oligosaccharide (the 'dolichol-linked *N*-glycan precursor' or 'dolichol-linked oligosaccharide (DLO)') is transferred to specific asparagine residues of secretory proteins as they emerge from the protein translocation channel into the ER lumen. Synthesis of the dolichol-linked *N*-glycan precursor is initiated on the cytoplasmic face of the ER and after seven glycosyltransfer reactions, the resulting glycolipid is flipped across the ER membrane to be further elaborated before participating in the protein *N*-glycosylation reaction. The optimal *N*-glycan precursor oligosaccharide in yeast and mammals contains a terminal cap of three glucose residues that is sourced from the glycolipid glucosyl-phosphoryl dolichol (GPD) in the ER lumen. Paradoxically, this lipid is synthesized on the *cytoplasmic* face of the ER and must be flipped across the ER membrane to provide a source of *lumenal* glucose. As the spontaneous rate of GPD flipping is extremely low, a specific transporter is needed to move GPD across the ER membrane at a physiological rate. **The molecular identity of this important transport protein - herein termed GPD scramblase - is not known** and its discovery remains a major challenge for molecular cell biology. The central role of glucosylation in optimal ER protein *N*-glycosylation was recognized more than 3 decades ago, and its relevance for cellular physiology and human disease is abundantly clear as glucosylation deficiency results in Congenital Disorders of Glycosylation. **Our aim is to identify GPD scramblase** via an unprecedented approach that combines bioinformatics and biochemistry. Our approach is well-suited to the 1-year period of the Mizutani Foundation grant.

Although GPD is important in yeast and humans, not all *N*-glycosylation-competent organisms have glucose in their *N*-glycan precursor. Taking advantage of this fact, we implemented an innovative bioinformatics approach for assignment of protein function. Using this method, termed phylogenetic profiling, we identified a number of polytopic ER membrane proteins as GPD scramblase candidates in yeast. We will test these candidates by *in vivo* approaches, for example by comparing the phenotypes of the corresponding yeast mutants to GPD synthase deficiency, as well as by using reconstitution-based assays of GPD scrambling using previously described radiolabeled GPD analogs. Identifying GPD scramblase is a highly significant overall objective that is expected to open new directions for study of the molecular mechanism of lipid scrambling, reveal new genetic loci associated with CDGs, and illuminate potential targets for treatment of *N*-glycan deficiency.