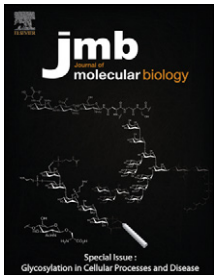
Review



Overview of Nucleotide Sugar Transporter Gene Family Functions Across Multiple Species

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Abstract

Glycoproteins and glycolipids are crucial in a number of cellular processes, such as growth, development, and responses to external cues, among others. Polysaccharides, another class of sugar-containing molecules, also play important structural and signaling roles in the extracellular matrix. The additions of glycans to proteins and lipids, as well as polysaccharide synthesis, are processes that primarily occur in the Golgi apparatus, and the substrates used in this biosynthetic process are nucleotide sugars. These proteins, lipids, and polysaccharides are also modified by the addition of sulfate groups in the Golgi apparatus in a series of reactions where nucleotide sulfate is needed. The required nucleotide sugar substrates are mainly synthesized in the cytosol and transported into the Golgi apparatus by nucleotide sugar transporters (NSTs), which can additionally transport nucleotide sulfate. Due to the critical role of NSTs in eukaryotic organisms, any malfunction of these could change glycan and polysaccharide structures, thus affecting function and altering organism physiology. For example, mutations or deletion on NST genes lead to pathological conditions in humans or alter cell walls in plants. In recent years, many NSTs have been identified and functionally characterized, but several remain unanalyzed. This study examined existing information on functionally characterized NSTs and conducted a phylogenetic analysis of 257 NSTs predicted from nine animal and plant model species, as well as from protists and fungi. From this analysis, relationships between substrate specificity and the primary NST structure can be inferred, thereby advancing understandings of nucleotide sugar gene family functions across multiple species.

Introduction

The addition of glycans to proteins and lipids is a fundamental process for many organisms. Another essential process is the cellular assembly of sugars into polysaccharides, the primary polymeric constituent of the extracellular matrix, especially in plants. Interestingly, plant cell walls account for most of the biomass on earth. These processes evidence the mechanisms developed by eukaryotic cells to enable proper macromolecule formation. In turn, these macromolecules (e.g., proteins, lipids, and polysaccharides) are responsible for a wide range of physiological functions.

Glycoconjugate synthesis primarily occurs in the Golgi apparatus and the endoplasmic reticulum (ER). These organelles contain glycosyltransferases, enzymes capable of transferring sugars to proteins, lipids, and polysaccharides. These macromolecules can also receive additional sulfate groups in the Golgi apparatus and ER through a process mediated by the sulfotransferases contained in these organelles. Glycosyltransferases and sulfotransferases use activated sugars and sulfates as substrates. These substrates include nucleoside diphosphate or monophosphate sugars, referred to as nucleotide sugars, and the nucleotide sulfate 3′-phosphoadenosine 5′-phosphosulfate (PAPS).

While both substrates (i.e., nucleotide sugars and PAPS) are present in the cytosol, these must be transferred into the ER and Golgi apparatus since the catalytic domain of most glycosyltransferases

and sulfotransferases faces the lumen of these organelles. Specifically, the molecular mass (~500–650 Da) and negative charge of phosphate or sulfate groups present in nucleotide sugars mean that these substrates cannot diffuse through membranes. Instead, translocation of these substrates depends on membrane proteins known as nucleotide sugar transporters (NSTs). Proper glycoconjugate synthesis and the addition of sulfate groups onto proteins, lipids, and polysaccharides depend on these transporters, and any alteration in the function of a given NST will disrupt normal organism functioning. In humans, disruptions can lead to varying pathological conditions [1–4]. In plant species, disruptions can alter the formation of the cell wall, which acts as an exoskeleton and contains sugars useful as lignocellulosic biofuels [5].

The first biochemical characterization of NSTs revealed these to be saturable in a K_m range of 1–10 μM, and additional evidence suggests that different transporters import distinct nucleotide sugars [6,7]. Biochemical analyses further showed that nucleotide sugar import concomitantly occurs with the export of a nucleoside monophosphate. Specifically, nucleoside diphosphate is produced upon the transfer of the nucleotide sugar into the acceptor. This diphosphate is then cleaved by a nucleoside diphosphatase into nucleoside monophosphate + P_i [7,8].

Biochemical features of the NSTs were made clearer through the reconstitution of these transporters into liposomes, an experimental approach that led to the development of a purification assay. Subsequent assays confirmed results obtained with Golgi apparatus-derived vesicles, including substrate specificities and that NSTs are low abundance proteins, making the identification of transporter-encoding genes through protein purification and sequencing a difficult task [9–12]. Genes encoding for NSTs were first cloned by complementing mutant cells that biochemically lacked NST activity. This approach led to the identification of genes coding for the UDP-*N*-acetylglucosamine [13,14], CMP-sialic acid (CMP-SA) [15], UDP-galactose [16,17], and GDP-mannose [18,19] transporters. Identification of these genes was important to identifying additional NSTs discovered *via* genetic means [20–25] and sequence homology [26,27].

Indeed, sequence comparisons and assessments of NST-related sequences led to the identification of the human gene coding for the PAPS transporter [28], indicating that nucleotide sugars and nucleotide sulfate are transported by the same class of polytopic proteins. Analyses of the protein sequences predicted from the different cloned genes revealed common features among all NSTs. These transporters possess 6–10 transmembrane domains, range from 45 to 55 kDa in size, and form homodimers, the possible functional unit, in the Golgi apparatus [10–12].

As more NST sequences become available, it becomes possible to examine the primary sequence similarities of NSTs that share substrate specificity. This information is highly relevant when considering that numerous developing genome projects are releasing inaccurate annotations of new genes coding for NSTs. Initial analyses of the UDP-*N*-acetylglucosamine transporters from *Kluyveromyces lactis* and mammals showed that these two transporters were less similar than a group of three NSTs fromin mammals that transport UDP-*N*-acetylglucosamine, UDP-galactose, and CMP-SA [29]. Therefore, substrate specificity predictions based on the primary sequence of an NST might be incorrect [30].

Functional characterizations of NSTs in different species have aided in clarifying the connection between the primary sequence and substrate specificity. In particular, Handford *et al.* [31] performed a phylogenetic analysis of 26 NST sequences from different species and suggested a relationship between substrate specificity and the primary sequence. However, expanding on studies such as the one presented by Handford *et al.* [31] has been difficult due to the limited number of characterized NSTs and limited availability of various nucleotide sugars.

Functional assessment of cloned NSTs

Functionally characterizing cloned NSTs is challenging since the biochemistry of membrane proteins is more complex than that of soluble proteins. Nevertheless, the expression of NST genes in heterologous systems, such as in isolated vesicles of the yeast *Saccharomyces cerevisiae*, permits activity assessments. An advantage of a yeast-based system is that it only transports GDP-mannose and UDP-glucose into the ER and Golgi apparatus, therefore facilitating the detection of transport signals from other substrates. Despite this advantage, a potential drawback of this system is a likely restriction of nucleoside monophosphate, one of the substrates involved in the nucleotide sugar/nucleoside monophosphate exchange. This restriction can result from some nucleotide sugars being unused by the yeast. Consequently, the sugar moieties needed for the release and formation of nucleoside monophosphate would not be released. However, the endogenous transport of UDP-glucose and GDP-mannose suggests the likely existence of UMP and GMP pools in the Golgi lumen, thus allowing for the functional characterization of NSTs in yeast vesicles.

The study of NSTs *via* heterologous systems has revealed that substrate specificity varies from a small to larger number of nucleotide sugars [21,24,25,32,33]. Although informative, the reliance of transport assays on commercially limited, radiolabeled nucleotide sugars curbs the number of testable substrates. This problem is particularly relevant for species with a large