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MINI REVIEW

Transporters of nucleotide sugars, nucleotide sulfate and ATP in the Golgi apparatus membrane: Where next?

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Recently the UDP-GlcNAc, CMP-sialic acid and GDP-mannose transporters from the Golgi apparatus membrane were cloned (Abeijon *et al.*, 1996; Eckhardt *et al.*, 1996; Ma *et al.*, 1997). These transporters, as well as those for other nucleotide sugars, PAPS and ATP, are required for these nucleotide derivatives to reach to Golgi lumen from the cytosol and serve as substrates in glycosylation, sulfation, and phosphorylation of glycoproteins, proteoglycans, and glycolipids. Previously, biochemical and genetic evidence demonstrated that these transporters are highly specific for solute transport (Hirschberg, 1996; Hirschberg and Snider, 1987), are often organelle specific (Hirschberg, 1996), appear to play a regulatory role in the biosynthesis of Golgi lumenal macromolecules (Abeijon *et al.*, 1993; Toma *et al.*, 1996), and use antiporters with the corresponding nucleoside phosphate as the mechanism for concentration of solutes in the Golgi lumen (Hirschberg and Snider, 1987; Milla and Hirschberg, 1989; Waldman and Rudnick, 1990; Abeijon *et al.*, 1993; Berninsone *et al.*, 1994). The aim of this article is not to provide a comprehensive review of this topic, which has been done recently (Hirschberg, 1996), but to highlight some important unanswered questions and new avenues of experimentation of this topic.

Structure of nucleotide sugar, ATP, and PAPS transporters of the Golgi apparatus membrane and their relationship to similar transporters in other membranes

The complete sequencing of the human genome is underway while that of *S.cerevisiae* and *C.elegans* has been completed. This and the ongoing cloning of the above Golgi membrane transporters raises the challenge to determine the relationship of structural homologs of the above transporters to specific functions. Do many of the Golgi membrane transporters have common structural features? Biochemical experiments with Golgi vesicles have suggested that nucleotide sugar transporters have a putative binding motif, determined by the nucleoside base (Capasso and Hirschberg, 1984) and a putative translocation motif, which is determined by the sugar (Capasso and Hirschberg, 1984), that is, GMP is a competitive inhibitor of GDP-fucose transport in mammals (Sommers and Hirschberg, 1982) and GDP-mannose transport in yeast (Abeijon *et al.*, 1989) even though the former nucleotide sugar doesn't cross the yeast Golgi membrane and the latter not the mammalian one.

Transport of all uridine nucleotide sugars is inhibited competitively by UMP while the sugars have no effect (Capasso and Hirschberg, 1984). UMP is the antiporter for all uridine containing nucleotide sugars (Hirschberg and Snider, 1987; Waldman and Rudnick, 1990; Milla *et al.*, 1992); thus, one would expect all these uridine nucleotide sugar transporters to have common structural features facing the lumenal and cytosolic side of the membrane. By analogy to the ATP/ADP transporter of mitochondria (Klingenberg, 1993), one would expect the affinity for the corresponding nucleoside monophosphate of each transporter to be higher in its lumenal recognition domain than in its cytosolic one and the opposite for the nucleotide derivative.

Hydrophobicity plots and different algorithms for the putative orientation of membrane proteins provide only a beginning hypothesis for the topography of such proteins; thus, the number of transmembrane spanning domains and regions, including the amino and carboxy terminus, facing the cytosol or the lumen will need to be determined directly. The quaternary structure of these transporters is important to establish: to what extent is recent evidence showing that the Golgi membrane PAPS transporter is a homodimer (Mandon *et al.*, 1994b), a general feature of these transporters.

Regulation of nucleotide sugar, ATP, and PAPS transport in the Golgi membrane and possible diseases

Recent evidence in mammals and yeast suggests that Golgi membrane transporters play a regulatory role in determining which macromolecules undergo specific posttranslational modifications in the lumen of the Golgi apparatus (Abeijon *et al.*, 1993; Toma *et al.*, 1996). The supply of nucleotides and nucleotide derivatives in the Golgi lumen is limiting under physiological conditions thereby allowing those reactions with low K_m values to take preference over those with higher ones. An open question is whether overexpression of transporter proteins in the membrane of the Golgi apparatus will affect transport activity. Can intrinsic activities of these different transporters be modulated by different effectors including cytosolic and lumenal nucleotides that are known to be competitive inhibitors of nucleotide sugar transport (Capasso and Hirschberg, 1984)? Is the expression of the different transporter proteins subject to transcriptional or translational regulation during different physiological conditions and development?

Detailed functional studies of these transporters will require high microgram amounts for reconstitution into liposomes. These proteoliposomes should then be useful in electrophysiological studies analogous to recent ones with CFTR (Bear *et al.*, 1992) to address what nucleotide and phosphate species cross the membrane, what their charges are, and to study the possible existence of cotransporters coupled to the antiporters. A combination of genetics and overexpression of wild-type and

mutant transporter proteins followed by reconstitution into liposomes should allow determination of structural motifs required for membrane insertion, nucleotide recognition, and specific sugar translocation.

Studies of the yeast GDPase, which plays a pivotal role in the antiport mechanism for GDP-mannose entry into the Golgi lumen (Abeijon *et al.*, 1993; Berninsone *et al.*, 1994), have shown that the specificity of this enzyme can be altered depending on whether Ca^{+2} or Mn^{+2} are added to the reaction (Abeijon *et al.*, 1993). To what extent may this be another regulatory mechanism of nucleotide sugar transport in mammals which use uridine and guanosine nucleotide sugars?

Genetic diseases affect transport of sugars and amino acids in membranes of lysosomes (Gahl *et al.*, 1982; Rosenblatt *et al.*, 1985; Mancini *et al.*, 1989; Tietze *et al.*, 1989). Are there diseases related to the above Golgi membrane transporters? One would expect mammalian homozygotes in some Golgi transporter mutations to be lethal, as many transporters are highly specific and gene disruptions of glycosyltransferases acting downstream from these transporters were found to be so, that is, N-acetylglucosaminyltransferase I (Ioffe and Stanley, 1994; Metzler *et al.*, 1994). Nevertheless, the possibility exists that during pathological conditions, where some of these transporter activities are absent or diminished, other transporters may partially compensate for lost function, that is, the UDP-GlcNAc transporter may have some affinity, although greatly diminished, for UDP-GalNAc transport.

A combination of genetics, gene disruption, and RNA antisense technology should be applied to determine the possible role of these transporters during development and differentiation.

Targeting and subcellular distribution

What structural features determine that these transporters become localized in the Golgi apparatus and/or the endoplasmic reticulum and not another organelle? To what extent will transporters for the same solute, which are localized in different organelles, differ in structure? ATP transporters occur in mitochondria (Klingenberg, 1992), the endoplasmic reticulum (Clairmont *et al.*, 1992; Mayinger and Meyer, 1993; Mayinger *et al.*, 1995), and the Golgi apparatus (Capasso *et al.*, 1989): all of them should have recognition features for ATP and the putative antiporters ADP or AMP, as well as specific organellar targeting features. Elucidations of them will be of primary importance.

The sub-Golgi distribution of the different transporters relative to each other and to transferases that use the same nucleotide sugar as substrate will be of importance. Will there be polarization in the Golgi apparatus of these transporters in the same general manner as glycosyltransferases in some cells? Will there be Golgi apparatuses in which there is major overlapping of these different proteins? In the case of the yeast Golgi apparatus, the possibility exists that these transporters are not polarized because in many instances, this organelle consists of only one cisternae (Preuss *et al.*, 1992). Nevertheless, different cisternae may be enriched in individual transporters. Clearly, all transporters could colocalize and still allow oligosaccharide chain specificity to proceed normally as a result of substrate specificities of glycosyltransferases. Within the Golgi membrane, do the transporters and the transferases exist as structural or functional complexes? Radiation inactivation studies suggest that the PAPS transporter is not in a *functional* complex with any corresponding sulfotransferases (Mandon *et al.*, 1994a,b) and that galactosyl and sialyltransferases are not in a functional complex with the transporters (Fleischer *et al.*, 1993). Is this a general observation?

Undoubtedly, studies will address the Golgi targeting features of these transporters. These approaches will consist of either mutagenizing different amino acids of the transmembrane and adjacent regions of these proteins or attaching such regions to plasma membrane proteins and studying their subcellular localization (Machamer, 1993; Gleeson *et al.*, 1994; Colley, in press). These studies with multitransmembrane proteins of the Golgi membrane (Rudolph *et al.*, 1989; Swift and Machamer, 1991; Antebi and Fink, 1992) and the above nucleotide sugar transporters, by analogy to glycosyltransferases, may not always yield interpretable results. The extent to which constructs expressed in one cell line or organism will localize to the same organelle in another cell line or organism must be determined. Do transporters recycle between the Golgi and the ER? Some, as in *S.cerevisiae* (Abeijon *et al.*, 1996), have a KKXX signal (Jackson *et al.*, 1990; Townsley and Pelham, 1994; Schroeder *et al.*, 1995), while some in *K.lactis* (Abeijon *et al.*, 1996) do not. Is this of relevance?

In summary, although much has been learned about Golgi transport of nucleotide sugars, PAPS, and ATP during the past years we are clearly at the dawn of a new era. This will, hopefully, bring more investigators into this field and thereby allow a faster pace in obtaining answers to the above important questions.

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