



Gateway to the Golgi: molecular mechanisms of nucleotide sugar transporters

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The Golgi apparatus plays a central role in the secretory pathway as a hub for posttranslational modification, protein sorting and quality control. To date, there is little structural or biochemical information concerning the function of transporters that reside within this organelle. The SLC35 family of nucleotide sugar transporters link the synthesis of activated sugar molecules and sulfate in the cytoplasm, with the luminal transferases that catalyse their attachment to proteins and lipids during glycosylation and sulfation. A recent crystal structure of the GDP-mannose transporter has revealed key sequence motifs that direct ligand recognition and transport. Further biochemical studies unexpectedly found a requirement for short chain lipids in activating the transporter, suggesting a possible route for transport regulation within the Golgi.

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Introduction

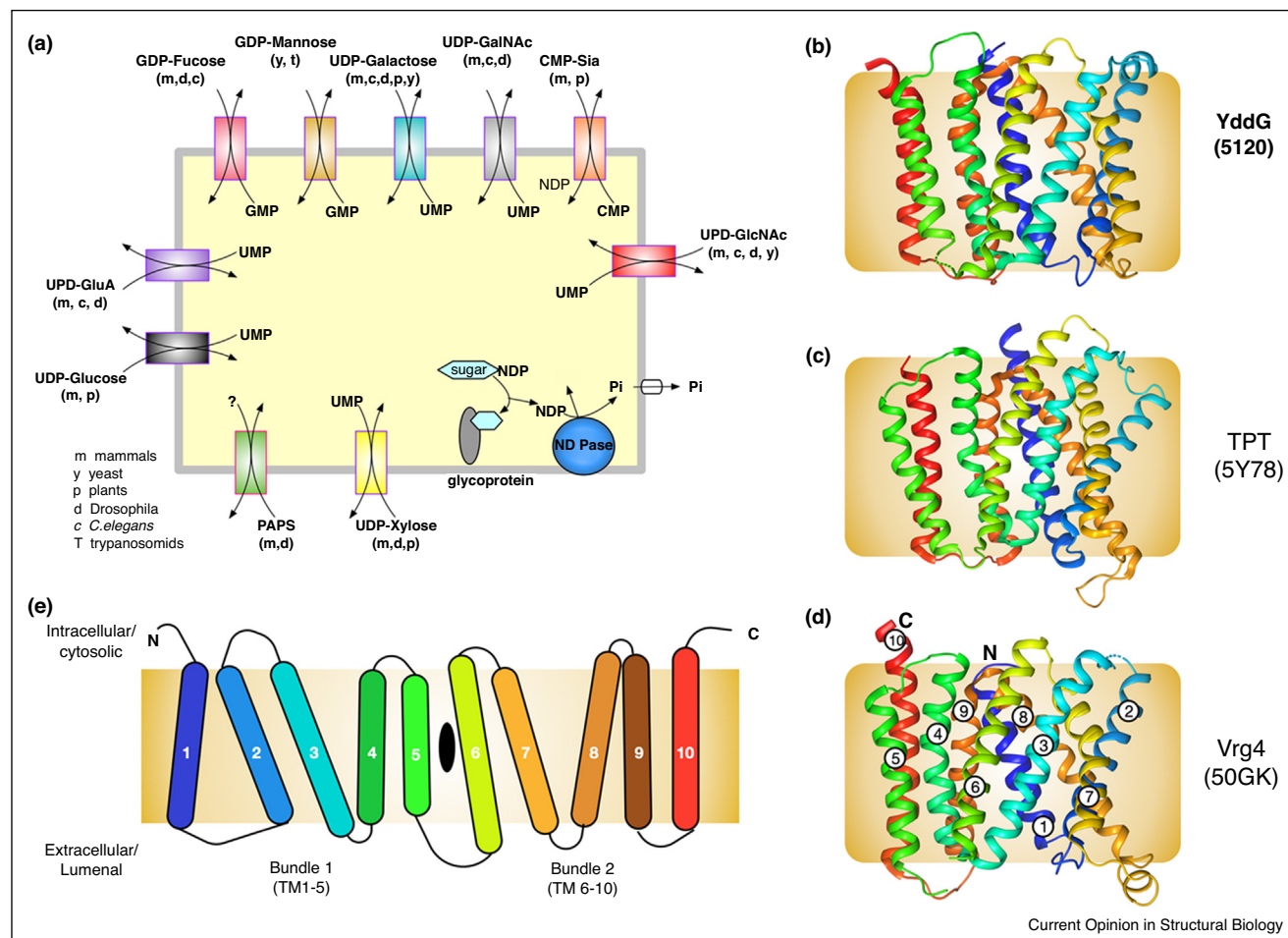
Members of the Solute Carrier 35 family are responsible for the transport of nucleotide sugars into the Golgi apparatus (Golgi) or Endoplasmic Reticulum (ER) and form an essential link between the synthesis of nucleotide sugars and glycosylation in eukaryotic cells [1–3]. Glycosylation within eukaryotes is compartmentalised to maintain tight regulation and increase efficiency and occurs within the lumens of the ER and Golgi, where the glycosyltransferase enzymes reside [4,5]. Nucleotide sugar transporters (NSTs) are required to supply glycosyltransferases with activated nucleotide sugars, which are necessary for glycosylation and are synthesised in either the cytosol or nucleus. NSTs are highly conserved from simple eukaryotes through to fungi, parasites, plants and mammals [6,7*] with 31 members

currently identified within humans. These can be subdivided into seven subfamilies A–G, with at least one member having been identified for each nucleotide sugar [8*]. NSTs have been shown to be obligate antiporters [9**], moving nucleotide sugars into the ER or Golgi lumen in exchange for their cognate nucleoside monophosphate in an electroneutral exchange [10] (Figure 1a). While most NSTs are responsible for nucleotide sugar transport, a small number also recognise activated sulphate (adenosine 3'-phosphate 5'-phosphosulfate, PAPS), which is required for sulphating proteins and the synthesis of heparan sulphate [9**]. In addition, there are several orphan SLC35 transporters, some of which are localised to the plasma membrane, where their role in cell physiology is currently unknown. However, one member, SLC35F2 has been shown to play an important role in the transport of an anticancer drug, YM-155, suggesting a potentially far broader role for these transporters in human health and disease [11**]. Indeed, NSTs are amongst only a small number of transporters implicated in Mendelian diseases, such as Leukocyte adhesion deficiency II [12,13] and the Congenital Disorder of Glycosylation II_f, resulting in developmental abnormalities [14]. In addition, the transport of nucleotide sugars is particularly important for virulence within pathogenic fungi and trypanosomatid parasites [15–17,18*]. These organisms contain a cell wall or surface glycocalyx predominantly formed of glycomannosylated conjugates that form a protective coat against the human immune system [19,20]. Recently the first crystal structure of an NST, the GDP-mannose transporter from *Saccharomyces cerevisiae*, Vrg4 [21], was reported [22**], revealing important insights not only into the architecture and substrate selectivity, but also the important role that lipids play in regulating transport across the Golgi membrane. Here, we discuss these advances and the impact these new results have on our understanding of nucleotide sugar transport across the Golgi membrane.

Architecture of the SLC35 family fold

The crystal structure of Vrg4 revealed the presence of 10 transmembrane (TM) alpha helices, confirming previous epitope mapping studies on the mammalian CMP-sialic acid transporter [23]. Architecturally this fold is distinct from the LeuT/APC superfamily, which also contain 10 TMs [24] and belongs to the drug/metabolite exporter (DME) superfamily [25]. To date there are three crystal structures of DME family members; the amino acid exporter YddG from the bacterium

Figure 1



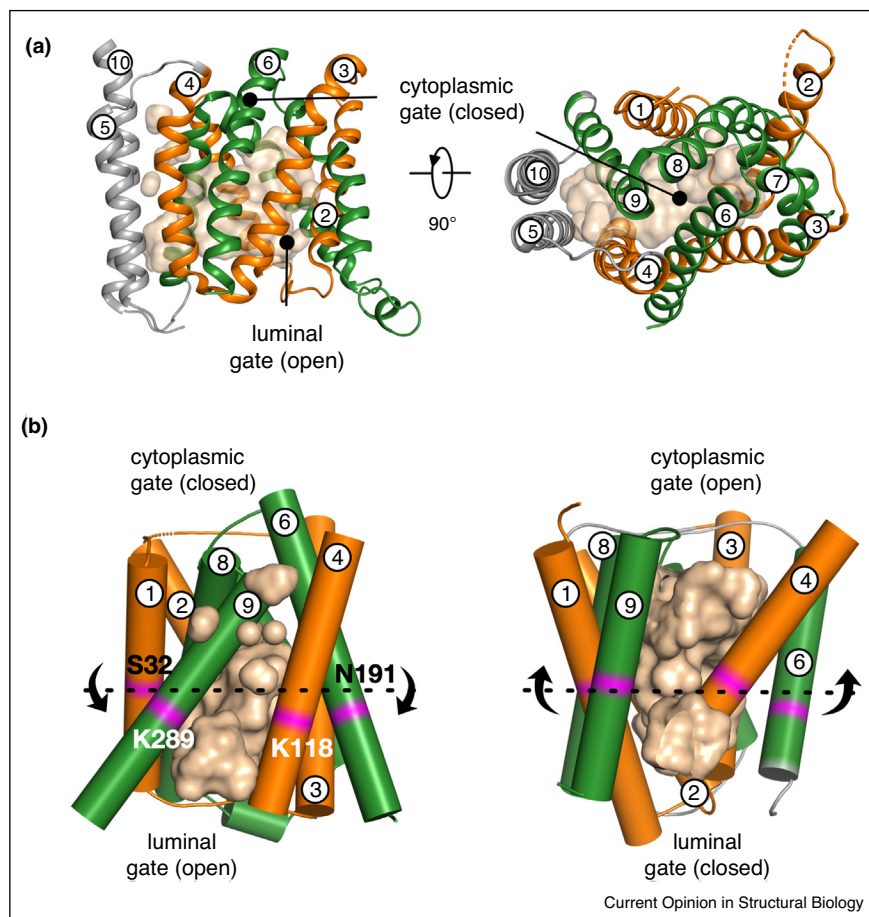
Crystal structure of GDP-mannose transporter reveals structural similarity to the drug/metabolite exporter superfamily. **(a)** Transport activities of representative SLC35 family members, showing the nucleotide sugars used for glycosylation in different species (adapted from Ref. [54]). Also shown is the mechanism of glycosylation of a protein and the fate of the NDP moiety following sugar transfer. **(b–d)** Crystal structures of DME family members highlighting the structural similarity, with TM helices coloured blue to red from the N-terminus. The PDB codes for each structure are shown. **(e)** Topology of the DME superfamily, with TM helices coloured as in b.

Starkeya novella [26^{••}] (Figure 1b), the triose-phosphate/phosphate antiporter TPT from the thermophilic red alga *Galdieria sulphuraria* [27] (Figure 1c) and the GDP-mannose from *S. cerevisiae*, Vrg4 [22^{••}] (Figure 1d). The topology of the DME family is composed of two five TM bundles, related by a pseudo twofold symmetry axis running parallel to the membrane (Figure 1e). Each bundle consists of a pair of two helices and an extra helix. The extra helix from each bundle come together to form part of a dimer interface, discussed further below. All three DME structures share this overall fold despite the lack of any sequence or functional similarity, demonstrating that this topology is likely to be conserved across the wider DME superfamily [25].

In order to shuttle ligands across the membrane, solute carriers use a general alternating access mechanism,

whereby a centrally located binding site alternates between either side of the membrane [28]. Within the DME fold two gates can be identified that open and close during transport, which facilitate access to a central substrate binding site [22^{••}, 29^{••}]. The gate comprised of TMs 6–7, which pack against TMs 8–9 seal the substrate binding site from the cytoplasm. On the opposite side of the membrane, TMs 1–2 and TMs 3–4 form the opposing gate, controlling access to the outside of the cell or the ER and Golgi lumen (Figure 2a). In order to maintain strict coupling of transport to ligand binding, specific interactions between the gating helices and the ligand have to be formed and broken to facilitate sequential opening and closing of these gates. Structural alignments between the outward facing crystal structure and inward-facing model of Vrg4 [22^{••}], combined with molecular dynamics

Figure 2



Alternating access mechanism within the SLC35 nucleotide sugar transporter family. **(a)** Crystal structure of Vrg4 showing the cytoplasmic gating helices, TMs 6–7 and 8–9 (green), which occlude access to the central binding site cavity (wheat surface). The luminal gate is open, and constructed from TMs 1–2 and 3–4 (orange). TMs 5 and 10, which are not involved in gating are shown in grey. **(b)** Movement of gating helices predicted from comparison of the crystal structure (left) with the repeat swapped model of the cytoplasmic facing state (right). Indicated in magenta are the conserved side chains that the transporter pivots around following ligand binding within the central cavity.

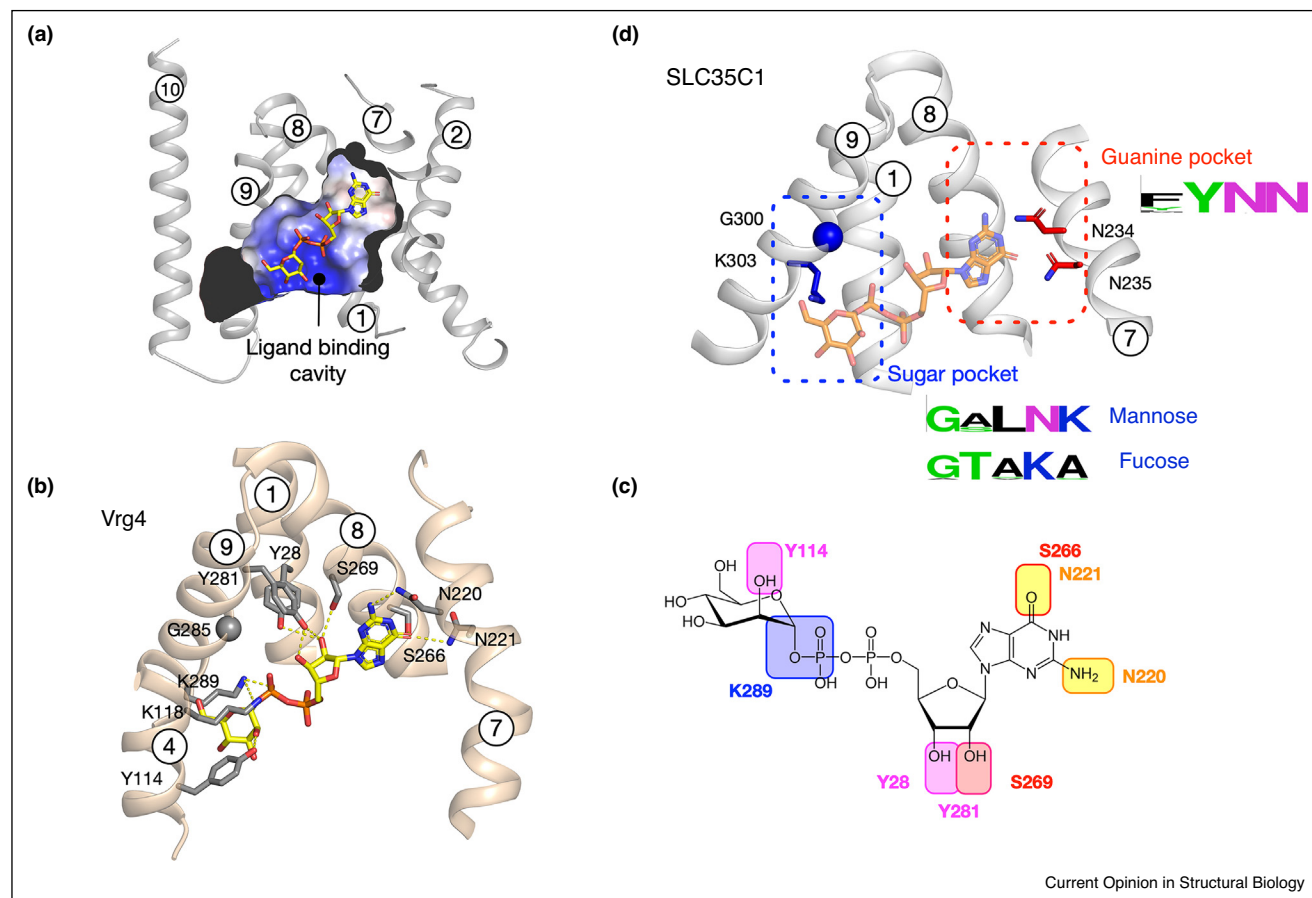
simulations of the algal TPT protein [29^{••}] have provided two complementary mechanistic models for alternating access transport within the DME. Specifically, within Vrg4 pivot points were identified at residues Ser32 (TM1), Lys118 (TM4), Asn191 (TM6), and Lys289 (TM9), which would allow for alternating access transport (Figure 2b). Interestingly, equivalent positions within the helices in TPT were also identified as being important for coupling ligand binding to transport, suggesting a conserved transport mechanism within the wider DME superfamily.

Substrate selectivity within the SLC35 family

The crystal structure of Vrg4 was determined in the luminal facing state in complex with its nucleotide-sugar ligand, GDP-mannose. The nucleotide sugar binds in an extended conformation within the central ligand binding site (Figure 3a). A notable feature of the structure was the

position of the ligand, with both the nucleotide and sugar moieties being accommodated within separate binding pockets at either end of the transporter. The presence of separate pockets for nucleotide and sugar recognition explains how this family distinguishes between different substrates. The guanine ring of GDP-mannose is seen interacting with two asparagine side chains within a conserved FYNN motif, located on TM7 (Figure 3b). It is likely the antiported ligand, guanosine monophosphate (GMP), interacts with the same pocket, but this remains to be experimentally determined. In contrast, the beta-phosphate and glycosidic bond oxygen of the mannose group interacts with the conserved lysine, Lys289, on TM9, which forms part of the previously identified GALNK²⁸⁹ motif [30]. This interaction likely couples GDP-mannose binding to conformational rearrangements that result in transport, as TM9 is one of the gating helices. The ribose group in contrast does not appear

Figure 3



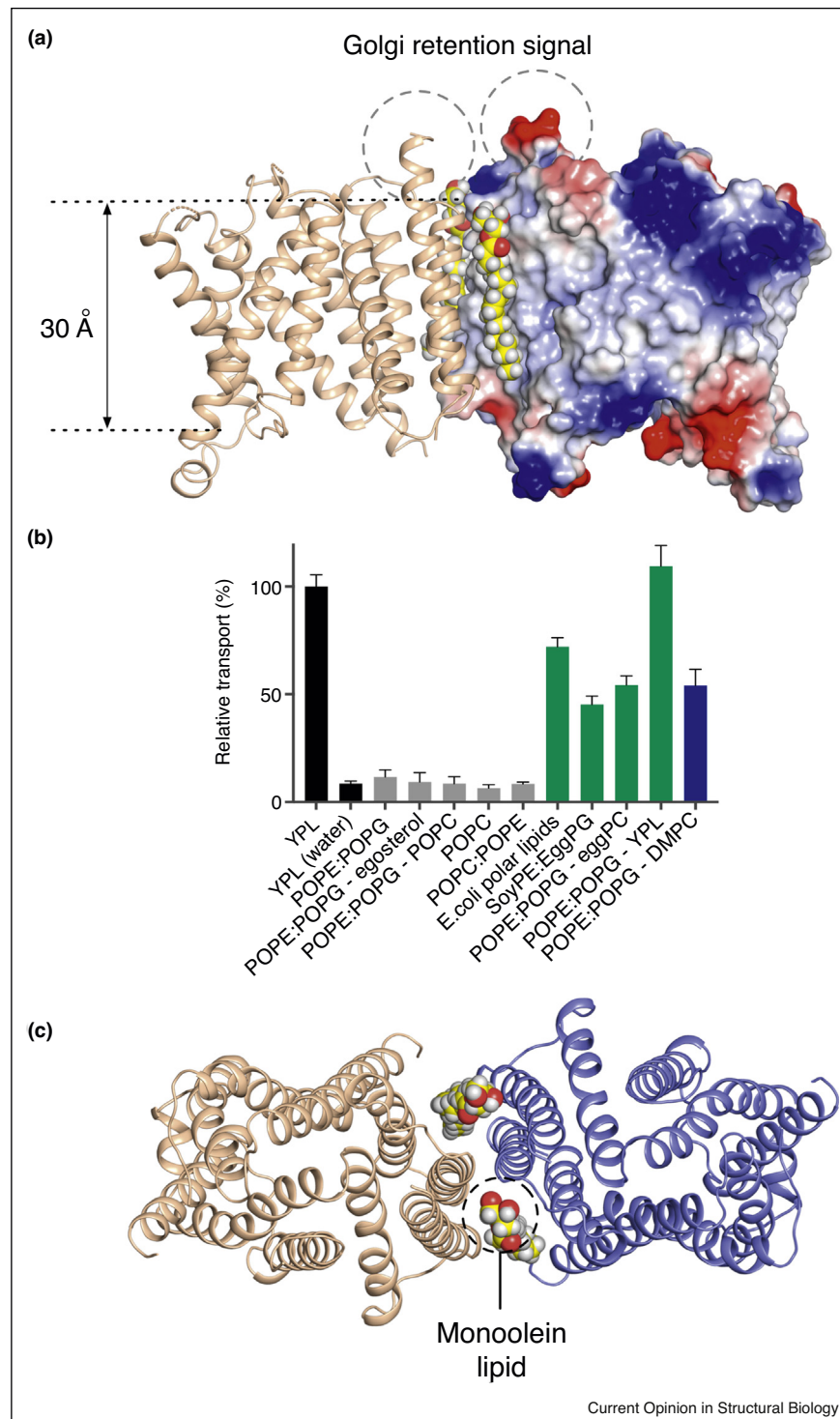
Nucleotide sugar recognition within the SLC35 family. **(a)** Crystal structure of Vrg4 in complex with GDP-mannose revealed specificity pockets for the nucleotide and sugar moieties of the ligands. The central binding cavity is coloured according to surface electrostatics. **(b)** Close up view of the ligand binding site, showing the hydrogen bond interactions (dashed lines) to GDP-mannose. **(c)** Schematic representation of the interactions shown in (b). **(d)** Homology model of the human GDP-fucose transporter (SLC35C1) built using the Vrg4 structure. The conserved guanine and sugar binding pockets are indicated by dashed boxes. The consensus motifs for mannose (GALNK), fucose (GTAKA) and guanine recognition (FYNN) are shown.

to be recognised by a specific sequence motif, instead making interactions to conserved side chains across several gating helices, Y28 (TM1), S269 (TM8) and Y281 (TM9) (Figure 3c).

In humans, there are nine nucleotide sugars which are used for glycosylation [31]. CMP-sialic acid and GDP-fucose are the only ones to utilise cytosine and guanine nucleotides, while the remaining seven sugars are all conjugated to uridine [32]. At present, it is not possible to unambiguously assign specificity to the SLC35 family using sequence-based methods alone, as the exact motifs required for ligand recognition remain obscure and sequence identity is not correlated with substrate specificity [8^{*}]. Bioinformatic analyses, however, show that GDP-fucose and GDP-mannose transporters from various species all share the FYNN motif on TM7

(Figure 3d), suggesting this is a conserved motif in guanine recognition. A previous study showed that substituting TM7 was enough to switch substrate specificity from UDP-galactose to CMP-sialic acid supporting the importance of this helix in nucleobase specificity [33,34]. A similar correlation between sugar specificity and side chains within the binding site was also observed with respect to the GALNK motif on TM9. This motif is conserved within GDP-mannose transporters, whereas in GDP-fucose transporters this region on TM9 is replaced by an equally well conserved GTAKA motif [22^{**}]. Given the importance of these two motifs in the recognition of GDP-mannose in Vrg4 and the high degree of correlation between these motifs and different substrate specificities, we propose these motifs form the basis for further sequence-based annotation within the SLC35 family.

Figure 4



Lipid regulation and interactions in Vrg4. **(a)** Crystal structure of dimeric Vrg4 highlighting the length of the hydrophobic belt. One monomer is displayed in an electrostatic surface representation. Monoolein lipids are shown as spheres. The Golgi retention signal in each monomer, located at the C-terminal end of TM10 is highlighted. **(b)** Vrg4 requires short-chain-length lipids (DMPC) to function, suggesting a role for membrane thickness in regulation of transport activity. YPL, Yeast Polar Lipids. **(c)** View of (a) rotated 90 degrees, highlighting the two monoolein lipid molecules at the dimer interface.

Regulation of SLC35 transport

The Golgi membrane is known to have a different lipid composition in comparison to the plasma membrane, which combined with a lower concentration of cholesterol results in a thinner bilayer [35,36]. Previous bioinformatic and thermodynamic studies proposed that membrane proteins that reside within the Golgi have shorter transmembrane helices, and this property may contribute to their differential location within the secretory pathway [37,38]. The crystal structure of Vrg4 supports this hypothesis (Figure 4a) as the hydrophobic region of the protein is markedly shorter than observed for plasma membrane localised proteins [22^{**}]. However, it has been unclear to what extent, if any, hydrophobic matching plays on regulating proteins within the secretory pathway. Previous studies reconstituting nucleotide sugar transport activity have used ER or Golgi lipids preparations [39^{*},40], where one study highlighted the importance of Golgi lipids for UDP-xylose transport [41]. We, therefore, investigated the transport function of Vrg4, using a reconstituted system with both natural lipid extracts and synthetic lipids preparations of defined chain length (Figure 4b). Vrg4 activity was observed in the natural lipid extracts which contain a variety of both lipid headgroup and acyl chain length. However, Vrg4 was unable to transport in liposomes comprising pure 1-palmitoyl-2-oleoyl phosphatidyl ethanolamine (POPE):1-palmitoyl-2-oleoyl phosphatidyl glycerol (POPG) lipids, which contain acyl chain lengths of 18:0 and 16:1, unless these were spiked with the short chain lipid DMPC, which in contrast, contains acyl chains of 14:0. This result demonstrates that hydrophobic matching is likely to play an important regulatory role for transporter function in the secretory pathway.

There are a number of mechanisms which allow for the retention and retrieval of proteins within the secretory pathway [42]. COPI vesicle coat complexes are responsible for retrieving escaped ER and Golgi proteins based on a di-lysine retrieval sequence [43]. The kin hypothesis, in contrast, posits that Golgi resident membrane proteins form higher order oligomers that regulate their interaction with transport vesicles [44,45]. NSTs involved in the transport of UDP-GalNAc, GDP-fucose, PAPS and GDP-mannose are reported to form homo-oligomers ranging from dimers to hexamers [46–49] although the oligomeric state of a functional NST has not yet been conclusively determined. Interestingly, in the crystal structure, Vrg4 forms a compact dimer in a physiological arrangement, that is, both monomers facing the same way in the membrane (Figure 4a). The dimer interface is formed between TM5 and 10 and contains two well-ordered lipid molecules, which contribute ~60% to the total buried surface area of 1514 Å² (Figure 4c). This arrangement places two di-lysine Golgi retrieval sequences located on TM10 and shown to be functionally important for Vrg4 localisation [50], in close proximity (Figure 4a). This structural arrangement may increase the

avidity of interaction with the COPI coatomer complex and point to a broader role for lipid mediated dimerization in regulating the localisation of NSTs within the cell.

SLC35 transporters in human health and disease

There are two congenital disorders of glycosylation that have been mapped to defects in nucleotide sugar transport [51]. Congenital Disorder of Glycosylation II_f (CDG-II_f) is caused through the absence of the CMP-sialic acid transporter due to a premature stop codon in the gene for this protein [14]. Leukocyte adhesion deficiency II (LADII), however, is caused through point mutations within the GDP-fucose transporter [12,13], and results in a complete lack of fucosylated glycoconjugates [13]. Genetic mapping identified two separate mutations in these patients, Arg147Cys and Thr308Arg [13,52]. Within the context of the Vrg4 crystal structure, Thr308 is located on the same helix as the GTAKA motif and packs against TM10, which as discussed above forms a potential dimer interface. Whereas, Arg147 is equivalent to Lys118 (TM4), which sits close to the sugar binding site and whose mutation to alanine abolishes activity. However, a conservative arginine substitution at this position in Vrg4 supports function, demonstrating the importance of a positive charge in this part of the binding site.

It is well established that pathogenic fungi, such as *Candida albicans*, *Aspergillus fumigatus* and *Cryptococcus neoformans*, require GDP-mannose for virulence [15,16]. This is in contrast to mammalian glycosylation pathways, which do not use GDP-mannose, instead using UDP-mannose [32]. The discovery of a specificity pocket for the sugar group within the SLC35 transporter family also presents new opportunities for the development of novel antifungal medicines [53]. It should now be possible to investigate the extent to which this pocket can be targeted for the generation of new antifungal medications that would selectively inhibit a GDP-mannose transporter over the human GDP-fucose system.

Concluding remarks

Over 30 years of research into nucleotide sugar transport has revealed the identity of the genes coding for the transporters responsible for this activity, their mechanism of action and linked them to several developmental disorders in both humans and cattle [54^{*}]. However, several important questions remain. Although 31 SLC35 family genes have been annotated in the human genome, only 9 have been ascribed to a particular function, with the vast majority remaining orphan transporters. Annotating these orphan transporters is going to be important to fully understand the role of SLC35 transporters in both glycosylation and also wider metabolic pathways. A particularly relevant question is the molecular basis for multisubstrate specificity as observed in several NSTs [55,56] as this will help to further

understand the link between substrate specificity and transport. The crystal structure of Vrg4 and associated identification of specificity pockets for nucleotide and sugar moieties provide a starting point in this endeavour.

The annular lipid environment is emerging as one of the most important elements that regulates both stability and function of transporters [57]. However, it is currently unclear to what extent intracellular transport is regulated by a changing lipid environment, as in the secretory pathway. The role of lipids in regulating transport in Vrg4 is an exciting new development in this regard. We propose that this observation is likely to have broader implications for understanding the regulation of transport across organellar membranes. Following up this observation to understand the mechanism by which lipids regulate nucleotide sugar transport is, therefore, a key question for future research.

Conflict of interest statement

Nothing declared.

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