GDP-Fucose Transporter 1 (SLC35C1)

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Hans Bakker, Angel Ashikov, Francoise H. Routier, and Rita Gerardy-Schahn

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Introduction

GDP-fucose is used by fucosyltransferases in both the Golgi and endoplasmic reticulum (ER). As it is synthesized in the cytoplasm, transport into the lumen of the secretory pathway organelles is required. The first indication that GDP-fucose, like other nucleotide sugars, is transported by a carrier-mediated process was obtained by Sommers and Hirschberg (1982). The gene encoding the GDP-fucose transporter was identified independently by two groups using complementation cloning in patient-derived cells (Lübke et al. 2001; Lühn et al. 2001). These patients were suffering from leukocyte adhesion deficiency II (LAD II), also known as congenital disorder of glycosylation (CDG) type IIc. LAD II patients were

H. Bakker (✉) • A. Ashikov • F.H. Routier • R. Gerardy-Schahn
Institute for Cellular Chemistry, Hannover Medical School, Hannover, Germany
e-mail: bakker.hans@mh-hannover.de; ashikov.angel@mh-hannover.de; routier.francoise@mh-hannover.de; gerardy-schahn.rita@mh-hannover.de

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originally described as having a deficiency of the fucosylated glycan Sialyl-Lewis X (Etzioni et al. 1992; Etzioni 1994; Phillips et al. 1995). The lack of fucose likely prevents interaction of the endothelial adhesion molecules E- and P-selectin with glycoproteins present on leukocytes. The latter are then unable to migrate through the endothelial layer to reach the sites of inflammation (Wild et al. 2002). In addition to the immunological problems, LAD II patients present characteristic symptoms of CDG patients such as severe mental and growth retardation (Etzioni 2010).

Databanks

GDP-fucose transporter (SLC35C1)

GDP-fucose transporter 1 (SLC35C1)

Species	Gene symbol	mRNA	UniProt ID	Gene ID
Homo sapiens	*SLC35C1*	NM_018389	Q96A29	55343
Mus musculus	*Slc35c1*	NM_211358	Q8BLX4	228368
Rattus norvegicus	*Slc35c1*	NM_001107748	F1LYZ2	311204
Danio rerio	*slc35c1*	NM_001008590	Q5PR94	494047
Drosophila melanogaster	*nac* (*Gfr*)	NM_141525	Q9VHT4	40981
C. elegans	*nstp-10*	NM_073066	Q968A5	179342

Name and History

The GDP-fucose transporter was cloned by complementation cloning in LAD II patient-derived cells (Lübke et al. 2001; Lühn et al. 2001). These patients had been shown to have reduced import of GDP-fucose transport into the Golgi (Lübke et al. 1999). All nucleotide sugar transporters obtained a systematic gene symbol and belong to the solute carrier 35 family (SLC35). The GDP-fucose transporter has the official HUGO Gene Nomenclature Committee (HGNC) (Seal et al. 2011) gene symbol SLC35C1 (Ishida and Kawakita 2004). The SLC35 family now has 30 members and is subdivided into subfamilies SLC35A – G based on phylogeny.

Structure

All nucleotide sugar transporters are membrane-spanning proteins of 300–400 amino acids. The hydrophobicity profiles predict eight to ten transmembrane-spanning domains (TMDs). Human SLC35C1 consists of 364 amino acids with eight membrane domains predicted and two hydrophobic segments that do not

reach the threshold to be predicted as TMDs (http://www.cbs.dtu.dk/services/TMHMM). However, based on the experimentally determined membrane topology of the mouse CMP-sialic acid transporter (SLC35A1) (Eckhardt et al. 1999), it is assumed that SLC35C1 has ten TMDs with the N- and C-terminus facing the cytoplasmic side of the Golgi membrane. The GDP-fucose transporters from *C. elegans* (Lühn et al. 2001) and *Drosophila* (Lühn et al. 2004; Geisler et al. 2012) have the same predicted topology.

Within the SLC35 family, SLC35C1 is most related to SLC35C2, a putative nucleotide sugar transporter with unclear function (Chen et al. 2005; Lu et al. 2010). Transporters of different GDP-activated sugars appear evolutionary related. Yeast and fungi express a GDP-mannose transporter (VRG4) that is closely related to animal GDP-fucose transporters (Dean et al. 1997; Engel et al. 2012). However, these organisms do not synthesize or use GDP-fucose. Animals, in turn, lack GDP-mannose transport. In the protozoan parasite *Leishmania*, a transporter (LPG2) has been identified that is able to transport GDP-fucose, GDP-mannose, and GDP-arabinose (Hong et al. 2000).

Activity Assay and Substrate Specificity

Nucleotide sugar transport can be conveniently measured by an in vitro assay using radiolabelled nucleotide sugar and isolated sealed vesicles. GDP-fucose transport activity was first demonstrated using Golgi-enriched vesicles isolated from rat liver. The vesicles were first incubated with radiolabelled GDP-fucose to allow internalization of the nucleotide sugar and then separated from unincorporated GDP-fucose by ultracentrifugation. Transport was then determined from the radioactivity associated with the vesicles (Sommers and Hirschberg 1982). This method described in detail by Perez and Hirschberg (1987) has also been used to demonstrate that GDP-fucose transport was absent from fibroblasts derived from LAD II patients (Lübke et al. 1999). Vesicles isolated from *Saccharomyces cerevisiae* recombinantly expressing the transporter of interest are often used for in vitro assay (Muraoka et al. 2007). However, in the case of GDP-fucose, a background transport likely due to the very active GDP-mannose transporter can be measured.

An alternative way to determine GDP-fucose transport activity of unknown transporters is complementation of GDP-fucose transport-deficient cell lines. Fibroblasts from LAD II patients and detection of fucosylated glycans by Aleuria aurantia lectin (AAL) were originally used to clone the human GDP transporter (Lübke et al. 2001; Lühn et al. 2001) and also enabled the characterization of the *Drosophila* GDP-fucose transporter (Lühn et al. 2004). Alternatively, an SLC35C1-deficient CHO cell line, recently described, could be used for expression of unknown transporters and has been used to determine the impact of mutations in SLC35C1 on transport activity (Zhang et al. 2012). This cell line was generated by

knocking out the GDP-fucose transporter using zinc-finger nuclease technology to produce unfucosylated recombinant proteins for biotechnological applications (Haryadi et al. 2013).

Preparations

Puglielli and Hirschberg have described the purification and reconstitution in liposomes of the rat liver GDP-fucose transporter (Puglielli and Hirschberg 1999). In a first purification step, a Golgi-enriched vesicle fraction, prepared from 250 g of rat livers, was treated with sequentially higher Triton X-100 concentrations. Most GDP-fucose transport activity was extracted with the highest concentration of 1.5 %. The extract was further purified using DEAE and Blue Sepharose columns and a glycerol gradient. Purification was followed by reconstitution of aliquots into proteoliposomes and measuring transport activity by a method using anion exchange to separate proteoliposomes from free GDP-fucose (Mayinger and Meyer 1993). Overall GDP-fucose transport activity was enriched by a factor 15,000, with a yield of 37 %. Photoaffinity labelling with a GDP-fucose analog identified a 39 kDa protein by SDS-PAGE.

Biological Aspects

Interestingly, although Golgi fucosylation was strongly impaired in SLC35C1-deficient patients and knockout mouse, fucosylation in the ER was not extensively affected. Two fucosyltransferases using GDP-fucose act in the ER (Fig. 124.1). These are protein *O*-fucosyltransferase 1 (POFUT1) and protein O-fucosyltransferase 2 (POFUT2) that act on EGF-like repeats of Notch and thrombospondin type 1 repeats, respectively (Luo et al. 2006). However, the severe Notch phenotype resulting in death at mid-gestation observed in POFUT1-deficient mice (Shi and Stanley 2003) is not mimicked in the *Slc35c1* minus mouse (Hellbusch et al. 2007). Moreover, a GDP-fucose transporter mutant of *Drosophila* was affected in Notch signalling but was viable (Ishikawa et al. 2005), suggesting a reduction of Notch fucosylation rather than a complete lack of fucosylation, which would be lethal (Okajima and Irvine 2002). Similarly, the ability to improve fucosylation in patients and cells by fucose supply suggests the existence of an alternative GDP-fucose transport pathway into the ER/Golgi (Marquardt et al. 1999; Sturla et al. 2001; Hidalgo et al. 2003; Helmus et al. 2006; Hellbusch et al. 2007). It may be that the Golgi and ER membranes are inherently leaky for nucleotide sugars and that significant GDP-fucose transport takes place if the cytoplasmic level of GDP-fucose is elevated. This might especially be true for the ER, even at normal GDP-fucose level, as it has been described that the ER membrane is more permeable than other membranes for small charged molecules (Le Gall et al. 2004). If this process is facilitated by other members of the SLC35 family is not known. In mammals, no other transporter with clear GDP-fucose

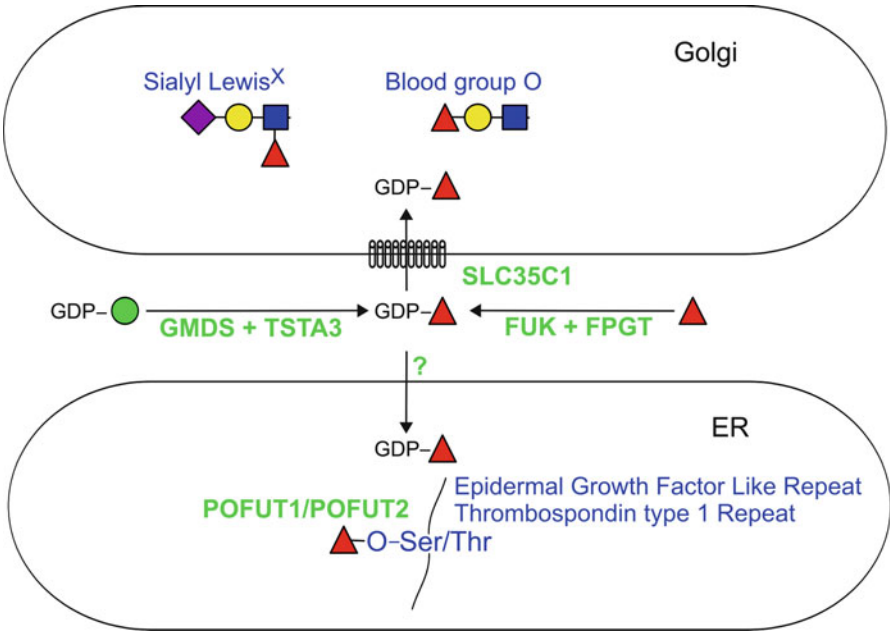

Fig. 124.1 SLC35C1 supplies the fucosyltransferases of the Golgi apparatus with substrate. A variety of fucosylated structures are made within the Golgi by 11 different fucosyltransferases, for example, Sialyl-Lewis X and structures of the ABO blood group system. How the protein *O*-fucosyltransferases of the ER are supplied with GDP-fucose is not resolved

transport activity has been identified, but a *Drosophila* homolog of SLC35B4 was shown to transport a variety of nucleotide sugars including GDP-fucose (Ishikawa et al. 2010). The *C. elegans* ortholog of this transporter was also shown to complement LAD II fibroblasts to some extent in the initial complementation screen for *C. elegans* SLC35 family members (Lühn et al. 2001). A mammalian candidate for a second GDP-fucose transporter is SLC35C2. This protein was initially identified as a negative regulator of fucosylation in a screen for genes that reduced fucosylation in the gain-of-function CHO cell mutant LEC11B (Chen et al. 2005). SLC35C2 thus reduced fucosylation, at least in this background. This transporter was, however, shown to enhance the fucosylation of Notch in the ER (Lu et al. 2010).

Knockout Mouse and Transgenic Mice

A mouse model of leukocyte adhesion deficiency II or congenital disorder of glycosylation IIc (CDG-IIc) has been described (Hellbusch et al. 2007). The mouse mimics the observed phenotype of LAD II patients. Lectin binding studies showed lack of fucosylated glycans in SLC35C1-deficient mice. Supplying cell with fucose resulted in partial restoration of fucosylation, suggesting the existence

of an additional GDP-fucose import machinery, only able to supply the Golgi with sufficient GDP-fucose at high cytoplasmic levels. The mice could be used for experiments that were not possible in patients. Intravital microscopy showed that selectin-dependent rolling of leukocytes was strongly impaired in the knockout mouse (Yakubenia et al. 2008). It was, however, shown that lymphocyte homing to the spleen was normal in SLC35C1-negative mice.

Human Disease

SLC35C1 mutations are the cause of leukocyte adhesion deficiency II, after the recognition as a glycosylation defect also described as congenital disorder of glycosylation IIc (CDG-IIc). LAD II is a rare inherited human disease with less than ten reported patients (Hanna and Etzioni 2012). Patients have severe mental and growth retardation but are more specifically characterized by a mobilization defect of leukocytes to sites of inflammation, resulting in recurrent bacterial infections (Etzioni et al. 1992; Etzioni 1994). Leukocytes fail to interact with the blood-vessel wall and are unable to penetrate into the surrounding tissue. The initial interaction of leukocytes with endothelial cells is mediated by E-selectin and P-selectin, expressed on the endothelial cells. These cell surface receptors interact with the carbohydrate structure Sialyl-Lewis X (NeuAcα2,3Galβ1,4(Fucα1,3)GlcNAc) on leukocytes (Foxall et al. 1992). The suspect of a carbohydrate disorder was strengthened by the fact that LAD II patients had the rare Bombay blood group, in which the Fucα1,2Gal structure (blood group O) is missing (Etzioni et al. 1992). The absence of reaction of neutrophils with the Sialyl-Lewis X antibody then suggested a general defect in the generation of fucosylated glycans. Although a defect in GDP-fucose biosynthesis was initially hypothesized (Sturla et al. 1998; Karsan et al. 1998; Becker and Lowe 1999; Körner et al. 1999), it was shown that LAD II fibroblasts had reduced transport of GDP-fucose into the Golgi lumen (Lübke et al. 1999). The cloning of the transporter then confirmed that the LAD II patient under investigation had a point mutation in SLC35C2 (Lübke et al. 2001; Lühn et al. 2001). In another patient, the expressed transporter was truncated (Helmus et al. 2006). Distinct mutations in the GDP-fucose transporter could, however, not be linked to different responsiveness of patients to oral fucose therapy (Marquardt et al. 1999; Sturla et al. 2001; Hidalgo et al. 2003; Helmus et al. 2006).

Further Perspectives

It is rather clear how lack of fucosylation results in leukocyte adhesion deficiency. It is, however, still unclear what causes the other phenotypes in LAD II patients and SLC35C1 knockout mice. Another issue still to be solved is the mechanism by which cells can still metabolize GDP-fucose in the absence of a functional GDP-fucose transporter and how ER fucosyltransferases are differently supplied with GDP-fucose.

Cross-References

- ▶ Fucokinase (FUK)
- ▶ Fucose-1-Phosphate Guanylyltransferase (FPGT)
- ▶ Fucosyltransferases 12, 13: Protein *O*-Fucosyltransferases 1 and 2 (POFUT1, POFUT2)
- ▶ GDP-Mannose Pyrophosphorylase A,B (GMPPA,B)
- ▶ Tissue Specific Transplantation Antigen P35B (= GDP-4-keto-6-D-Deoxymannose Epimerase-Reductase) (TSTA3)
- ▶ UDP-Xylose and UDP-*N*-Acetylglucosamine Transporter (SLC35B4)

Further Reading

Lübke et al. (2001), Lühn et al. (2001): The original papers describing the cloning of the GDP-fucose transporter.

Hanna and Etzioni (2012): A recent review from the discoverer of LAD II patients.

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