GLUT8, the enigmatic intracellular hexose transporter

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GLUT8, the enigmatic intracellular hexose transporter. Am J Physiol Endocrinol Metab 296: E614–E618, 2009. First published January 27, 2009; doi:10.1152/ajpendo.91019.2008.—GLUT8 is a class III sugar transporter predominantly expressed in testis and brain. In contrast to the class I and II transporters, hydrophobicity plots predict a short extracellular loop between transmembrane domain (TM)1 and TM2 and a long extracellular loop between TM9 and TM10 that contains the only N-glycosylation site. In vitro translated GLUT8 migrates as a 35-kDa protein that is glycosylated in the presence of microsomal membranes. In heterologous expression systems, glucose transport activity (Km of 2 mM) was inhibited by fructose and galactose. The transporter carries an NH2-terminal endosomal/lysosomal targeting motif ([DE]XXXL[LI]). Accordingly, constitutive GLUT8 has been found to be associated with endosomes and lysosomes but also with membranes of the endoplasmic reticulum. A similar distribution was detected after overexpression of wild-type or tagged GLUT8 in different cell systems. In these cells, none of the conventional signals tested induced a translocation of GLUT8 to the plasma membrane. Therefore, GLUT8 appears to catalyze transport of sugars or sugar derivatives through intracellular membranes. Scl2a8 knockout mice were viable, developed normally, and showed mild alterations in brain (increased proliferation of neuronal cells in dentate gyrus of the hippocampus, hyperactivity), heart (impaired transmission of electrical wave through the atrium), and sperm cells (reduced number of motile sperm cells associated with reduced mitochondrial membrane potential and ATP levels in sperm). The links between molecular function, cellular localization and phenotype of the knockout mouse is unclear and remains to be determined.

FACILITATED DIFFUSION OF SUGARS into cells is catalyzed by membrane proteins of the GLUT family (GLUT1–12, GLUT14, and HMIT; see Refs. 34, 36, 47). GLUT proteins share common structural characteristics such as 12-transmembrane helices, an N-linked glycosylation site, intracellular NH2 and COOH termini, and several conserved residues and motifs designated “sugar transporter signatures” (19). Based on a sequence comparison, the GLUT family is divided into three classes (19). GLUT proteins differ in their substrate specificity, kinetics of transport, tissue distribution, and cellular localization. Most transporters, with the exception of GLUT5 (7) and HMIT (43), transport more than one sugar. For detailed information on substrate specificity and the tissue distribution of the different transporters, the reader is referred to other reviews (19, 34, 47). This review is part of a series of reviews recently published in this journal on GLUT proteins, namely GLUT3 (40), GLUT5 (12), and GLUT7 (9).

Cloning, Structure, and Functional Characterization of GLUT8

Efforts that led to the identification of GLUT8 were driven by findings from the GLUT4 knockout mouse, suggesting that a second insulin-regulated glucose transporter might exist (20). An in silico search of the databases for expressed sequence tags (EST) homologous to the GLUT4 sequence led to the discovery and subsequent cloning of GLUT8 by the group of B. Thorens (16) and by our group (11). The mouse gene (Scl2a8) spans ~9 kb and consists of 10 exons; its organization is highly similar to that of the Scl2a6 gene [GLUT6 (33)]. GLUT8 mRNA is expressed predominantly in testis; smaller amounts were detected in most other tissues, including brain (cerebellum, brain stem, hippocampus, and hypothalamus), spleen, and liver and also in insulin-sensitive tissues such as skeletal muscle, heart, and adipose tissue.

The amino acid sequence of GLUT8 (29.4% sequence identity with GLUT1) exhibits all elements and motifs (sugar transporter signatures) that are characteristic of the GLUT family, e.g., 12 membrane-spanning helices, the GRR/K motifs in loops 2 and 8, glutamate and arginine residues in loops 4 and 10, the PESPR, the PETKG motifs after helices 6 and 12, respectively, and several other highly conserved residues (11, 34). GLUT8 is a member of the class III transporters, which also comprise GLUT6, GLUT10, GLUT12, and HMIT. As predicted by hydrophobicity plots, this class is characterized by a larger and presumably glycosylated extracellular loop 9 (19). The molecular mass of human GLUT8 is 51.5 kDa. In vitro translation products of GLUT8 mRNA migrate at an apparent molecular mass of 35–38 kDa (16, 48); when isolated from cells transfected with GLUT8 cDNA or from lysates of mouse testis, the apparent molecular mass is 37 or 50 kDa (Refs. 14 and 16, respectively).
High-affinity glucose transport activity ($K_m$ 2 mM) was demonstrated in *Xenopus* oocytes after injection of GLUT8 mRNA (16) and with recombinant GLUT8 generated in COS-7 cells in a system of reconstituted membranes (11). GLUT8 transport activity was specifically inhibited by fructose and galactose, indicating that GLUT8 might be a multifunctional sugar transporter (16).

**Cellular Localization of GLUT8**

GLUT8 carries a NH$_2$-terminal dileucine motif that directs the protein to an intracellular localization; mutation of the leucine residues to alanine targets the transporter to the plasma membrane (16, 22, 36). A similar dileucine motif is present in the COOH terminus of GLUT4, which is located in an intracellular compartment and translocates to the cell surface in response to insulin (32, 38). Thus, an intensive search for stimuli of a translocation of GLUT8 to the plasma membrane has been performed and was unequivocally demonstrated for two other class III transporters. HMIT translocates in response to cell depolarization, activation of protein kinase C, or increased intracellular calcium concentrations (43), and GLUT12 is recruited to the cell surface in response to high glucose and serum concentrations (45). Insulin-stimulated translocation of endogenous GLUT8 to the plasma membrane of blastocysts has been described (8), but this finding could not be reproduced in primary adipocytes, COS-7 cells, or insulin-responsive neuroblastoma cells (N2A) transfected with GLUT8 cDNA (22, 39). In addition, agents causing membrane depolarization, activation of protein kinase A or C, activation or inhibition of tyrosine kinase-linked signaling pathways, glucose deprivation, AMP-activated protein kinase stimulation, and osmotic shock failed to stimulate translocation of GLUT8 to the cell surface in PC-12 cells (44). Furthermore, transfection of PC-12 cells expressing a myc-tagged GLUT8 with a dominant negative dynamin mutant failed to provide evidence for constitutive cycling of the transporter between intracellular compartments and the cell surface (44). Thus, the available evidence indicates that GLUT8 is primarily, if not exclusively, associated with intracellular membranes.

Contradictory data have been reported as to the site of the intracellular localization of GLUT8 in different cells. Data obtained from brain sections with immunogold electron microscopy have suggested that GLUT8 is associated with the endoplasmic reticulum and intracellular membranes. Fractionation of hippocampal neurons and Western blot analysis revealed that GLUT8 is associated with microsomal membranes. Interestingly, peripheral glucose administration produced an increase in GLUT8 levels in a high-density microsomal fraction, whereas it decreased GLUT8 levels in low-density microsomes (27). The authors concluded that peripheral glucose administration induces a translocation of GLUT8 to the rough endoplasmic reticulum in the rat hippocampus and speculated that GLUT8 might catalyze transport of glucose that is produced during glycosylation of proteins in the rough endoplasmic reticulum out of the organelle into the cytosol (Fig. 1). However, it is also conceivable that GLUT8 is required for sugar transport into the endoplasmic reticulum to allow appropriate glycosylation of proteins.

The subcellular localization of GLUT8 was thoroughly investigated by immunohistochemistry and confocal immunofluorescence microscopy as well as by subcellular fractionation of homogenized PC-12 cells expressing a myc-tagged GLUT8 (44). These techniques detected GLUT8 in a perinuclear compartment, partially colocalized with a marker of the endoplasmic reticulum (calreticulin), but not with markers for the trans-Golgi network (TGN38), early endosomes, lysosomes (LAMP1), and synaptic-like vesicles (44).

Alignment of the GLUT8 sequence from different species revealed that the dileucine motif is part of a highly conserved late endosomal/lysosomal-targeting motif [DE]XXXL[LI] (1). This motif mediates targeting of proteins to endosomes, lysosomes and lysosome-related organelles by interaction with an...
adaptor protein [AP1–4 (6)]. Actually, interaction of the dileucine motif with subunits of AP1 and AP2 was demonstrated by two-hybrid and GST pull-down assays (36). AP1 mediates sorting from the trans-Golgi network (TGN) to endosomal compartments and between early and recycling endosomes, whereas AP2 participates in the dynamin-dependent, clathrin-mediated endocytosis from the plasma membrane (17). Mutation of the dileucine motif for alanines or the siRNA-mediated inhibition of AP2 expression resulted in an accumulation of GLUT8 at the cell surface, demonstrating that recruitment of GLUT8 to the endocytic machinery occurs via direct interaction of the dileucine motif with AP2 (36). Furthermore, there is a striking similarity of the EXXXLL motif of GLUT8 with the EXXXLI motif of the lysosomal protein LIMPII. Consequently, exchange of the glutamate for arginine disrupted the motif and targeted GLUT8 to the plasma membrane (1). However, GLUT8 lacks a pair of arginines that appear required for the correct sorting of LIMPII (31, 32). Exchange of the glutamate for arginine in EXXXLI disrupted the lysosomal sorting motif in GLUT8. It should be noted that this mutation generated a motif similar to that of the COOH-terminal dileucine motif in GLUT4 (1, 32). Subcellular localization was also investigated by colocalization experiments and electron micrographs as well as by subcellular fractionation and Western blotting. In HEK 293 and Chinese hamster ovary (CHO) cells stably expressing GLUT8, the protein colocalized with the late endosomal markers Rab7, Rab9, Syntaxin 8, and the lysosomal marker LAMP1 (Table 1). Furthermore, GLUT8 labeled with immunogold was detected by electron microscopy in lysosomes but not in the endoplasmic reticulum or Golgi apparatus (1). These results were confirmed by analysis of total membrane extracts from cells stably expressing GLUT8; its subcellular distribution corresponded to that of markers of late endosomes (Rab9, Syntaxin 8) but not with that of early endosomes (EEA1), Golgi (p58), or endoplasmic reticulum (calnexin). Separation of early (EEA1) from late endosomes (Rab9) revealed that GLUT8 was present in late but not in early endosomes. Therefore, the authors of the study suggested that GLUT8 might mediate transport of sugars originating from lysosomal degradation of glycoproteins across the late endosomal/lysosomal membrane (1).

In testis, association of GLUT8 with the acrosomal membrane of mature spermatozoa has been reported (37), whereas a second study detected GLUT8 in the acrosome, in the mid- and end piece of spermatozoa as well as in Leydig cells (21). Interestingly, the acrosome shows similarities to lysosomes by the presence of a variety of lysosomal proteins as well as the low pH (28, 41). For example, both the acrosome reaction and the exocytosis of secretory vesicles are triggered by calcium, and calcium-dependent exocytosis has also been shown for lysosomes in nonsecretory cells (17).

Most studies of the subcellular localization of GLUT8 have used heterologous expression systems. It can be argued that, in these systems, specific chaperones might be absent or limited, resulting in mistargeting of GLUT8 to default pathways. However, in a few studies, the subcellular localization of constitutive GLUT8 was investigated in brain and testis, the latter with tissue from Slc2a8 knockout mice as a control for nonspecific binding of the antiserum. These experiments demonstrated a specific immunoreactivity of GLUT8 in intracellular compartments of spermatides (13). In addition, constitutive GLUT8 was colocalized with the lysosomal marker Lamp1 in mouse testis (Table 1; Schmidt S, Gawlik V, Augustin R, Joost HG, and Schürmann A, unpublished data). Thus, there is, in part, conflicting evidence for a localization of GLUT8 in both lysosomes and endoplasmic reticulum. It should be noted that the data favoring a lysosomal localization are supported by the presence of a corresponding targeting motif in the GLUT8 sequence.

**Phenotype of the Slc2a8 Knockout Mouse: Growth and Glucose Homeostasis**

Slc2a8 knockout mice generated independently in two different laboratories were viable with normal growth and no apparent abnormality (13, 23). This finding was unexpected,

Table 1. Subcellular localization of GLUT8

<table>
<thead>
<tr>
<th>GLUT8</th>
<th>Detection Method</th>
<th>Subcellular Localization (Marker)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constitutive GLUT8 (brain)</td>
<td>Immunogold EM</td>
<td>ER</td>
<td>(27)</td>
</tr>
<tr>
<td>Constitutive GLUT8 (brain)</td>
<td>Subcellular fractionation and Western blotting</td>
<td>High-density and low-density microsomes</td>
<td>(27)</td>
</tr>
<tr>
<td>Transfected GLUT8-myc (PC-12 cells)</td>
<td>Immunocytochemistry</td>
<td>TGN (TG38), lysosomes (LAMP1), ER (calreticulin)</td>
<td>(44)</td>
</tr>
<tr>
<td>Transfected GLUT8-myc (PC-12 cells)</td>
<td>Subcellular fractionation and Western blotting</td>
<td>ER (calreticulin)</td>
<td>(44)</td>
</tr>
<tr>
<td>Transfected wild-type GLUT8 (HEK293 and CHO cells)</td>
<td>Immunocytochemistry</td>
<td>Late endosomes (Rab7, Rab9, syntaxin 8), lysosomes (LAMP1)</td>
<td>(1)</td>
</tr>
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<td>Transfected wild-type GLUT8 (HEK293 and CHO cells)</td>
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</tr>
<tr>
<td>Constitutive GLUT8 (blastocysts)</td>
<td>Immunocytochemistry</td>
<td>Plasma membrane after insulin stimulation</td>
<td>(8)</td>
</tr>
<tr>
<td>Constitutive GLUT8 (testis)</td>
<td>Immunocytochemistry</td>
<td>Lysosomes (LAMP1)</td>
<td>Schmidt, Gawlik, Augustin, Joost, Schürmann (unpublished data)</td>
</tr>
</tbody>
</table>

ER, endoplasmic reticulum; EM electron microscopy; TGN, trans-Golgi network.
since it had been reported earlier (26) that suppression of GLUT8 expression by antisense mRNA in preimplantation embryos caused increased apoptosis of blastocysts. One possible explanation for the survival of the Slc2a8 knockout blastocysts might be compensation by another glucose transporter. However, in brain and testis of postnatal mice, no altered expression of another GLUT protein was detected (13, 35). In addition, so far, the deletion of several other GLUT proteins (GLUT1, -2, -3, -4) did not result in embryonic death in the blastocyst state, indicating that the zygote develops independently of the substrate glucose.

Slc2a8 knockout mice exhibited no differences in body weight development or fat mass accumulation (13, 23), although GLUT8 expression increases markedly during fat cell differentiation (33). In addition, Slc2a8 knockout mice showed normal serum triglyceride and free fatty acid levels. No differences in plasma glucose or insulin levels between fed wild-type and knockout mice were observed, indicating that GLUT8 does not play a significant role for maintenance of whole body glucose homeostasis (13, 23). A slightly impaired transmission of the electrical wave through the atrium leading to a reduction in P-wave was identified by Membrez et al. (23) without alterations in heart size and morphology.

Phenotype of the Slc2a8 Knockout Mouse: Spermatozoa Function

Interestingly, both laboratories characterizing the phenotype of Slc2a8 knockout mice noticed a statistically significant reduction of homozygous knockout animals from the expected 25% to 17 (23) or 22% (13). We therefore performed a detailed analysis of Slc2a8−/− spermatozoa (13) and observed a significant reduction of the number of motile sperm cells by ∼50% compared with that of wild-type spermatozoa. In contrast, both sperm count and histopathology of testis sections were not altered compared with controls. Electron microscopy revealed normal development of acrosome and surrounding membranes in early and late spermatides of wild-type mice, but a lack of condensation of mitochondria in some late spermatides of knockout animals. According to this observation, the mitochondrial membrane potential was significantly reduced by more than 20%, and ATP levels in knockout sperm cells were reduced by 50% (13). The link between GLUT8 and the mitochondrial potential remains unclear. Since glucose uptake into spermatozoa of Slc2a8 knockout mice was normal, we speculated that GLUT8 mediates the transport of sugar metabolites between the cytosol and intracellular organelles, as shown in Fig. 1, and that these sugars are required for energy production (13).

Phenotype of the Slc2a8 Knockout Mouse: Behavioral Traits

Since GLUT8 mRNA as well as protein was detected in numerous brain regions [cortex, cerebellum, paraventricular hypothalamic nucleus, amygdala, supraoptic nucleus, dentate gyrus of hippocampus (11, 16, 27, 29, 30)], brain morphology, neuronal functions, and behavioral phenotype of GLUT8 knockout mice were studied extensively.

In the absence of GLUT8, cell proliferation was increased in the dentate gyrus of the hippocampus of 12-wk-old mice; 50% more bromodeoxyuridine (BrdU)-positive cells were detected one day after injection of BrdU. Since Slc2a8 knockout mice did not show morphological alteration in the hippocampus compared with controls (23), it remains to be tested whether the rate of apoptosis is elevated in GLUT8 knockout neurons. Contrary to the expectations, no significant differences in memory acquisition and retention (Morris water maze or homing board task) were observed between control and knockout mice (23).

In addition to the hippocampus, GLUT8 is expressed in brain regions that are also known to play a role in learning and memory (5, 25) as well as in behavioral traits such as exploration (15), fear and anxiety (3, 4, 10), and hyperactivity (2). Therefore, our group studied a wide range of behavioral characteristics with the modified hole board (mHB) test (35). In this test, Slc2a8 knockout mice were hyperactive, as indicated by an increase in mean velocity and total distance traveled. Hyperactivity was confirmed in the home cage by monitoring both locomotor and voluntary wheel running activity (35). Moreover, increased defecation and reduced latency of the first defecation of Slc2a8 knockout mice was detected by the mHB test, was interpreted as increased arousal. We concluded that the increased activity and arousal was due to metabolic alterations in neurons expressing GLUT8, possibly as a consequence of alterations in the intracellular transport of so far undefined metabolites.

Summary and Future Perspectives

A large body of evidence indicates that GLUT8 is an intracellular hexose transporter, which was detected in various intracellular compartments, the rough endoplasmic reticulum, trans-Golgi, late endosomes, and lysosomes (Table 1). With the exception of one study performed in blastocysts (8), none of the conventional signals triggering translocation of proteins to the plasma membrane has been shown to stimulate a subcellular redistribution of GLUT8. Thus, the present data suggest that GLUT8 is a constitutively intracellular transporter potentially catalyzing transport of sugars or sugar derivatives into or out of intracellular organelles. Despite some controversy, the most plausible intracellular localization is its association with lysosomes, which has been demonstrated in heterologous expression systems as well as in cells constitutively expressing GLUT8. In addition, the lysosomal localization of GLUT8 is consistent with its characteristic NH2-terminal [DE]XXXL[LI] targeting motif. Disruption of the Slc2a8 gene produced subtle modifications of behavioral traits and impairment of the transmission of electrical wave through the atrium and affected sperm function. The latter is due to a reduction in cellular ATP levels; the exact mechanism of this effect remains to be determined. The moderate phenotype of the Slc2a8−/− mouse is surprising but could be due to the absence of an appropriate challenge. Future studies will also have to provide direct functional evidence for a role of GLUT8 in sugar transport through the lysosomal or endoplasmic reticulum membranes and will have to identify other substrate(s) that are transported by the presumably multifunctional GLUT8. Finally, as GLUT8 is a prototypical member of the class III sugar transporters, progress can be expected from the investigation of other members of this subfamily.

GRANTS

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References


