Characterization of human glucose transporter (GLUT) 11 (encoded by *SLC2A11*), a novel sugar-transport facilitator specifically expressed in heart and skeletal muscle

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Human GLUT11 (encoded by the solute carrier 2A11 gene, *SLC2A11*) is a novel sugar transporter which exhibits significant sequence similarity with the members of the GLUT family. The amino acid sequence deduced from its cDNAs predicts 12 putative membrane-spanning helices and all the motifs (sugar-transporter signatures) that have previously been shown to be essential for sugar-transport activity. The closest relative of GLUT11 is the fructose transporter GLUT5 (sharing 41.7% amino acid identity with GLUT11). The human GLUT11 gene (*SLC2A11*) consists of 12 exons and is located on chromosome 22q11.2. In human tissues, a 7.2 kb transcript of GLUT11 was detected exclusively in heart and skeletal muscle. Transfection of COS-7 cells with GLUT11 cDNA significantly increased the glucose-transport activity reconstituted from membrane extracts as well as the specific binding of the sugar-transporter ligand cytochalasin B. In contrast to that of GLUT4, the glucose-transport activity of GLUT11 was markedly inhibited by fructose. It is concluded that GLUT11 is a novel, muscle-specific transport facilitator that is a member of the extended GLUT family of sugar/polyol-transport facilitators.

Key words: cytochalasin B binding, reconstituted glucose-transport activity, *SLC2A* gene, solute-carrier family 2A, sugar-transporter signature.

INTRODUCTION

Hexose transport into mammalian cells is catalysed by a small family of 45–55 kDa membrane proteins (GLUT1–GLUT5) that belong to the larger family of transport facilitators [1–3]. The GLUT isoforms differ in their expression in different tissues, in their kinetic characteristics and in their substrate specificity [4]. Recently, four novel members of the GLUT family have been identified in searches of the expressed sequence tag (EST) databases for sequences harbouring the so-called 'sugar-transporter signatures'. GLUT8 was independently cloned by two groups [5,6] and was shown to have glucose-transport activity in two different expression systems. GLUT8 mRNA was found in most tissues, with highest levels in testis; the testicular expression appeared to be dependent on the presence of gonadotropins. It has also been shown by Carayannopoulos et al. [7] that GLUT8 is involved in insulin-stimulated glucose uptake in the blastocyst. GLUT6, (coded for by *SLC2A6*, previously designated GLUT9) was predominantly detected in brain and peripheral leucocytes; expression of its cDNA in COS-7 cells gave rise to a reconstitutable glucose-transport activity in membrane preparations [8]. A third glucose-transporter-like sequence was identified independently by two groups and was designated GLUTX [8a] and GLUT9 [9]. Recently, McVie-Wylie et al. [10] introduced GLUT10, a close relative to GLUT8 and GLUT6, which is predominantly expressed in liver and pancreas. Here we describe the identification and characterization of an additional novel sugar transporter, GLUT11, with glucose-transport activity and striking tissue-specific gene expression.

EXPERIMENTAL

RNA preparation and PCR cloning

Total RNA was prepared by homogenization in guanidine thiocyanate and centrifugation through CsCl. The lysates were layered on a CsCl cushion (5.88 M) and centrifuged at 150000 ***g*** (SW40 rotor) for 22 h at 20 °C. Pelleted RNA was dissolved with 300 ml of sodium acetate/Tris buffer, and was neutralized by addition of 50 ml of 2 M potassium acetate (pH 5.5). PCR-generated DNA fragments were isolated and subcloned into pUC18 with the Sureclone® kit (Pharmacia, Freiburg, Germany).

Sequencing

All cDNA clones and PCR products were sequenced in both directions by the method of Sanger (Thermo sequenase sequencing kit; Amersham Life Science, Little Chalfont, Bucks, U.K.).

Northern-blot analysis

Blots generated with RNA from different human tissues were purchased from Clontech Laboratories (Palo Alto, CA, U.S.A.). Probes derived from the GLUT11 cDNA (GenBank accession no. AJ271290; positions 450–1191; identity with other GLUTs is below 60%) were generated with the Klenow fragment of DNA polymerase I and [α-^{32}P]dCTP by random oligonucleotide priming. The nylon membranes were hybridized at 42 °C, and washed twice at 55 °C with 0.12 M NaCl/0.012 M sodium citrate/0.1% SDS.

Abbreviation used: EST, expressed sequence tag.

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The nucleotide sequences for human GLUT11 have been deposited in the EMBL database under the accession number AJ271290.

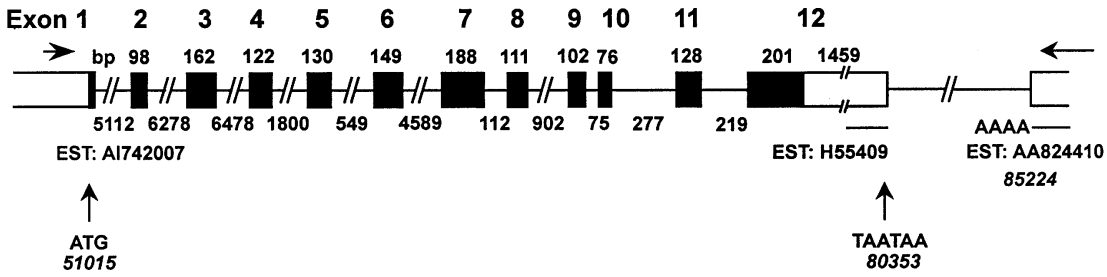

Figure 1 Genomic organization of the GLUT11 gene

Exons are shown as white and black bars. ESTs corresponding with the presumed exon1 or mapping polyadenylation sites and the adjacent gene are given as accession numbers. Italic numbers depict the positions in the genomic clone KB1125A3.

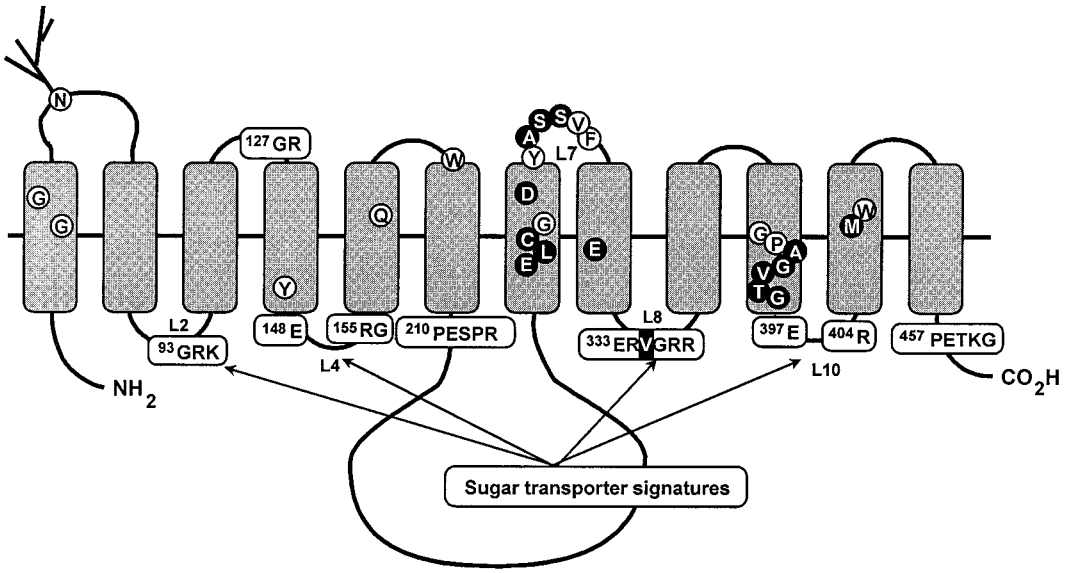

Figure 2 Putative membrane topology of GLUT11 and sequences of its sugar-transporter signatures

The model is based on structural predictions obtained with the HELIXMEM program, and is drawn according to that introduced by Mueckler et al. [1]. Residues of GLUT11 that are conserved in the family of mammalian glucose transporters (determined by sequence comparisons of GLUT1–4) are highlighted. Black backgrounds mark substitutions of these residues within the GLUT11 sequence. The Figure depicts a presumed glycosylation site in loop 1.

Preparation of membrane fractions from human heart

Atrium, septum and left and right ventricle of human heart were homogenized in TES (containing 20 mM Tris, 1 mM EDTA and 255 mM sucrose, pH 7.4). Homogenate was centrifuged at 600 ***g*** for 10 min and the resulting supernatant was centrifuged at 45000 ***g*** for 1 h. The resulting pellet contained plasma membranes and high-density microsomes. The second supernatant was centrifuged at 200000 ***g*** for 75 min and yielded a pellet consisting of the low-density microsomes.

Expression of GLUT11 in COS-7 cells

A fragment of the GLUT11 cDNA comprising the full reading frame was amplified by PCR, and was subcloned into the mammalian expression vector pCMV, which harbours a simian virus 40 origin and a cytomegalovirus (CMV) promoter. COS-7 cells were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.) and were grown in Dulbecco's modified Eagle's medium (pH 7.4) containing 10% fetal calf serum, 20 mM Hepes and 2 mM glutamine. Transfection of COS-7 cells with pCMV-GLUT11 was performed with calcium phosphate/DNA co-precipitates, and membrane fractions were isolated as described previously [11]. The glucose-transporter protein was found exclusively in the plasma membrane (13000 ***g***) and high-density microsomal (45000 ***g***) fractions; these fractions were used for the subsequent assays.

Immunochemical detection of GLUT11

Polyclonal antiserum against a peptide corresponding with the C-terminus of the GLUT11 (QGPTWRSLEVIQSTEL) was raised in rabbits. Proteins separated by SDS/PAGE were transferred on to nitrocellulose and incubated with antiserum at a dilution of 1:2000. Bound immunoglobulin was detected with ^{125}I-protein A.

Assay of cytochalasin B binding

Equilibrium cytochalasin B binding in membranes from transfected cells was assayed by a method established with fat-cell membranes [12] with modifications described in detail elsewhere

[13]. Background resulting from endogenous GLUT1 was determined with membranes from cells transfected with vector alone. For determination of K_q values, Scatchard plots of control and GLUT11 membranes were evaluated graphically by subtraction of background values along radial axes as described previously [14,15].

Reconstitution of glucose-transport activity from membrane fractions

Glucose-transporter protein was solubilized and reconstituted into lecithin liposomes as described previously [16,17]. Initial uptake rates of D-[U-^{14}C]glucose were assayed after 10 s at a substrate concentration of 5 mM. The data were corrected for non-carrier-mediated uptake with tracer L-[1-^{3}H]glucose.

RESULTS

Isolation of the GLUT11 cDNA

To identify unknown glucose-transporter-like sequences, we performed a search of the EST databases with the sequences of the known GLUT isoforms (tBLASTn program). Approx. 200 ESTs were analysed further in separate alignments of amino acid and nucleotide sequences. After exclusion of the known isoforms GLUT1–5 and of sequences lacking residues characteristic for the GLUT family (hexose-transporter signatures), four EST clusters derived from novel transporter-like sequences remained and were designated GLUT8 [6], GLUT6 [8], GLUT11, and the fourth corresponded with a cDNA published as a U.S. patent (GLUTX [8a]). A partial nucleotide sequence of the GLUT11 was obtained by sequencing of an EST clone derived from human placenta (accession no. T52633, IMAGE clone 67188). An additional search of the databases with this sequence revealed that it corresponded with parts of a known genomic sequence (clone KB1125A3). The similarity of the deduced amino acid sequence of GLUT11 with the GLUT family was used to tentatively locate the coding region and its translation start site on the genomic sequence. With the putative N-terminus of the coding region, an additional BLASTn search was performed and led to one EST corresponding with the presumed exon 1 of GLUT11 mRNA (accession no. AI742007, from a subtractive tumour library; IMAGE clone 2367309). Thereafter, a cDNA fragment comprising the full coding region was isolated by reverse transcriptase PCR with RNA from human heart and primer oligonucleotides derived from the putative 5′- and 3′-untranslated regions (upstream primer, 5′-CGGACCTGCCTCTCACGCAATGG-3′; downstream primer, 5′-CTGGCCACCCCTTTGGGACTAGAG-3′). The resulting cDNA sequence (from two independent PCR clones) was 99.9 % identical with the corresponding portions of the genomic sequence.

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                                                  *
GLUT11  -  MRAL.......RRLIQGRILLLTICAAGIGGTFQFGYNLSIINAPTLHIQ  -  43
           |          |  :   :| |     |  | .||:|||.. :|.| | .|
GLUT5   -  MEQQDQSMKEGRLTL...VLALATLIAAFGSSFQYGYNVAAVNSPALLMQ  -  47

                                      *   *     **       *      *
GLUT11  -  EFTNETWQARTGEPLPDHLVLLMWSLIVSLYPLGGLFGALLAGPLAITLG  -  93
           :| |||:  |||| : |  . |:||. ||::| ||  |.|| |||    |
GLUT5   -  QFYNETYYGRTGEFMEDFPLTLLWSVTVSMFPFGGFIGSLLVGPLVNKFG  -  97
           *    *  *          *               *   *   *      *
GLUT11  -  RKKSLLVNNIFVVSAAILFGFSRKAGSFEMIMLGRLLVGVNAGVSMNIQP  - 143
           || .|| |||| :  ||| | || | |||:|.: |||||: |||| |: |
GLUT5   -  RKGALLFNNIFSIVPAILMGCSRVATSFELIIISRLLVGICAGVSSNVVP  - 147
           ** **  *   ***           **   *  **         *  **
GLUT11  -  MYLGESAPKELRGAVAMSSAIFTALGIVMGQVVGLRELLGGPQAWPLLLA  - 193
           ||||| ||| |||| .  :|  .||.. |: ||| ||     ||:||
GLUT5   -  MYLGELAPKNLRGALGVVPQLFITVGILVAQIFGLRNLLANVDGWPILLG  - 197
                  **   *   ***** *             *  * *
GLUT11  -  SCLVPGALQLASLPLLPESPRYLLIDCGDTEACLAALRRLRGSGDLAGEL  - 243
              || |||  ||  |||||||||   |  |   ||. |||   .  |.
GLUT5   -  LTGVPAALQLLLLPFFPESPRYLLIQKKDEAAAKKALQTLRGWDSVDREV  - 247
            *   *              *                *       *
GLUT11  -  EELEEERAACQGCRARRPWELFQHRALRRQVTSLVVLGSAMELCGNDSVY  - 293
            |: :|  | .         .||. |.|| |. |::||    :| | ..:|
GLUT5   -  AEIRQEDEAEKAAGFISVLKLFRMRSLRWQLLSIIVLMGGQQLSGVNAIY  - 297
            *       **       *   * *            *    ***  *
GLUT11  -  AYASSVFRKAGVPEAKIQYAIIGTGSCELLTAVVSCVVIERVGRRVLLIG  - 343
            ||  ::  |||||  :||   |||.  ..     .  |:| .|||.||:
GLUT5   -  YYADQIYLSAGVPEEHVQYVTAGTGAVNVVMTFCAVFVVELLGRRLLLLL  - 348
           *              * *                          **
GLUT11  -  GYSLMTCWGSIFTVALCLQSSFPWTLYLAMACIFAFILSFGIGPAGVTGI  - 393
           |:|:      : | || || .  |  |:.. |: .:::   :||. :  :
GLUT5   -  GFSICLIACCVLTAALALQDTVSWMPYISIVCVISYVIGHALGPSPIPAL  - 397
              * * *  *           *         *               *
GLUT11  -  LATELFDQMARPAACMVCGALMWIMLFLVGLGFPFIMEALSHFLYVPFLG  - 443
           | ||:| | .||.| |.. |:  | ||| |||| | |  : :: |
GLUT5   -  LITEIFLQSSRPSAFMVGGSVHWLSNFTVGLIFPFIQEGLGPYSFIVFAV  - 447
                     ***    *  *
GLUT11  -  VCVCGAIYTGLFLPETKGKTFQEISEELHRLN.....FPRRAQGPTWRSL  - 488
           :|.   ||  | .|||| ||| ||.    ::|     :| : :    : |
GLUT5   -  ICLLTTIYIFLIVPETKAKTFIEINQIFTKMNKVSEVYPEKEE...LKEL  - 494

GLUT11  -  EVIQSTEL  - 496
             : | :
GLUT5   -  PPVTSEQ   - 501
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Figure 3 Alignment of human GLUT11 with its closest relative (GLUT5) in the GLUT family

Residues conserved in GLUT1–5 are marked by asterisks above the sequence. The alignment was performed with the GAP program.

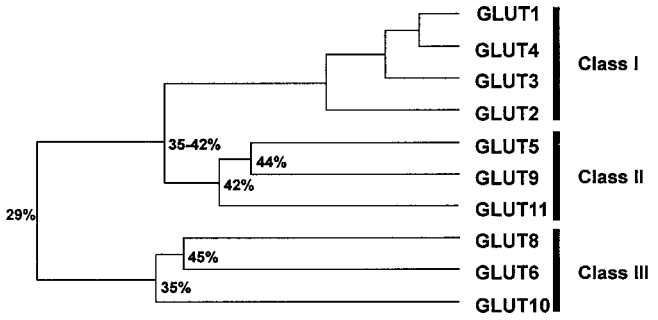

Figure 4 Dendrogram of a multiple alignment of the mammalian GLUT family

The alignment was performed with the CLUSTAL program (open gap cost 10, unit gap cost 10). Numbers at the branches of the tree indicate percentage identity. The GLUT proteins represent the human isoforms. GLUT8 [6] is identical with the independently published GLUTX1 [5]. The numbering of the isoforms follows the numbering of the genes in the *SLC2A* family as defined by the nomenclature committee of the Human Genome Organization. Note that the previously described GLUT9 [8] was changed to GLUT6 (*SLC2A6*).

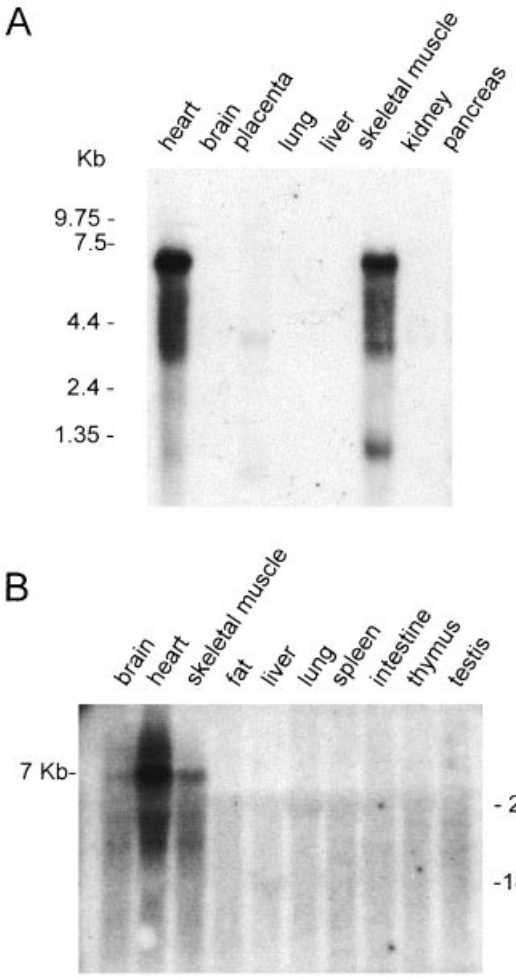

Figure 5 Specific expression of GLUT11 in human and rat heart and skeletal muscle

Northern blots with mRNA from the indicated human (**A**) and rat (**B**) tissues were hybridized as described in the text with human GLUT11 cDNA.

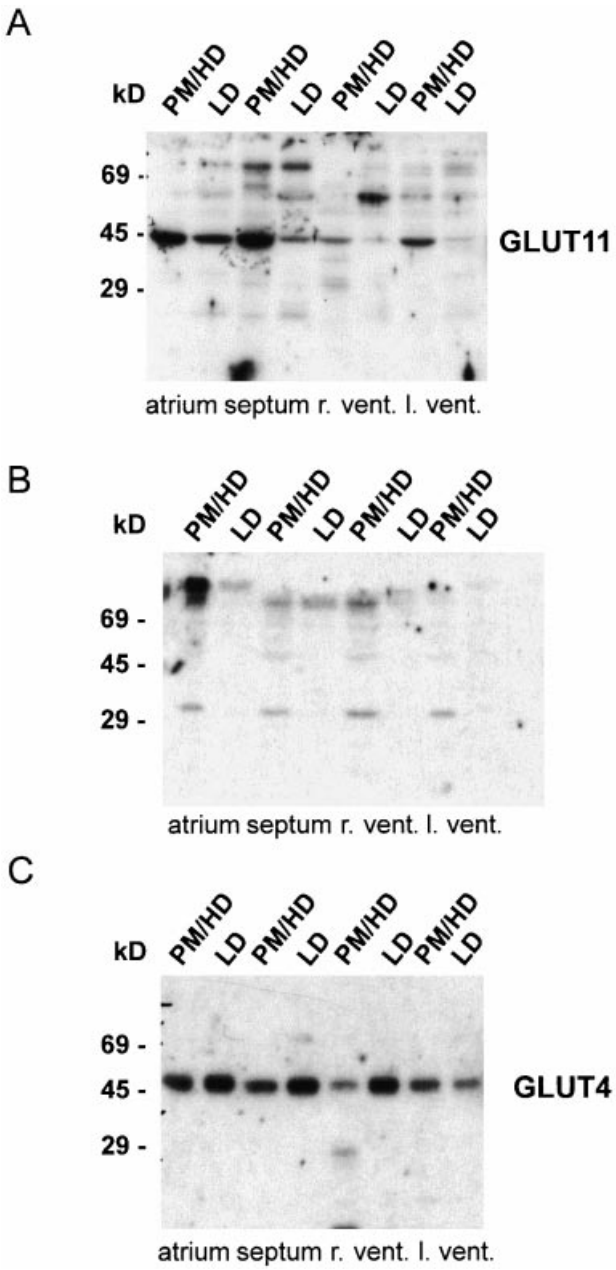

Figure 6 Detection of GLUT11 protein in membrane fractions of human heart

Membrane fractions of atrium, septum and left and right ventricle from human heart were prepared as described, separated by SDS/PAGE and transferred on to nitrocellulose. (**A**) Immunochemical detection of GLUT11 with an antiserum against its C-terminus. (**B**) Immunoassay with pre-immune serum. (**C**) Immunochemical detection of GLUT4 with an antiserum against its C-terminus. PM, plasma membranes; HD, high-density microsomes; LD, low-density microsomes.

Genomic organization of the GLUT11 gene (*SLC2A11*)

The organization of the GLUT11 gene was obtained by comparison of the cDNAs with the genomic sequence (clone KB1125A3). As illustrated in Figure 1, the GLUT11 gene comprises approx. 30 kb and consists of 12 exons. The translation start was assigned to the first AUG of the open reading frame following a stop codon in exon 1. The EST H55409 maps the 3′

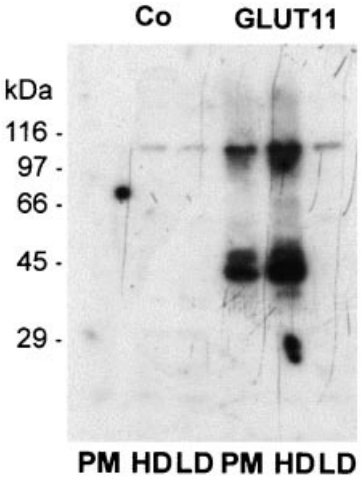

Figure 7 Expression of GLUT11 cDNA in COS-7 cells

COS-7 cells were transfected with blank vector (Co) or human GLUT11 cDNA. Membrane fractions were prepared as described, separated by SDS/PAGE and transferred on to nitrocellulose. GLUT11 was detected immunochemically with antiserum against its C-terminus. PM, plasma membranes; HD, high-density microsomes; LD, low-density microsomes.

end to nt 80353 of the genomic sequence and predicts a 3.4 kb transcript. ESTs derived from a larger 3′-UTR could not be found because of several repetitive sequences in this region. However, the 3′ neighbouring gene is located approx. 5 kb downstream of GLUT11; this distance allows the formation of a larger mRNA (7 kb, see Figure 5 below).

Sequence characteristics of GLUT11

The analysis of the deduced amino acid sequence of the GLUT11 with the HELIXMEM program [18] included in the PC/GENE® software package (IntelliGenetics, Mountain View, CA, U.S.A.) predicts the presence of 12 putative membrane-spanning helices, consistent with the presumed tertiary structure of a transport facilitator [1]. Furthermore, the sequence contains all the motifs which are characteristic of the family of transporter facilitators, in particular motifs corresponding with the PESPR/PETKGR motifs after helices 6 and 12 (Figure 2). Also, motifs and residues that were previously suggested to define the GLUT family (sugar-transporter signatures) are present in GLUT11: the GRR/GRK motifs in loops 2 and 8, glutamate and arginine residues in the intracellular loops 4 and 10, and a tryptophan residue corresponding to Trp-412 in GLUT1. Furthermore, loop 1 harbours the expected glycosylation site. Striking differences from the other members of the GLUT family are three acidic residues (Glu-285, Asp-290 and Glu-321) in the helices 7 and 8, the lack of the QLS motif [19] in helix 7, and the lack of a tryptophan in helix 10 corresponding to Trp-388 in GLUT1.

Individual alignments indicate that the human fructose transporter GLUT5 (Figure 3, 41.9% identical amino acids) and GLUT1 (35% identical amino acids) are the closest relatives of GLUT11. As illustrated in Figure 4, GLUT11 can be assigned to a separate branch of the GLUT family (class II), together with GLUT5 and GLUT9. GLUT8, GLUT6 and GLUT10 are located on a third branch of the family (class III).

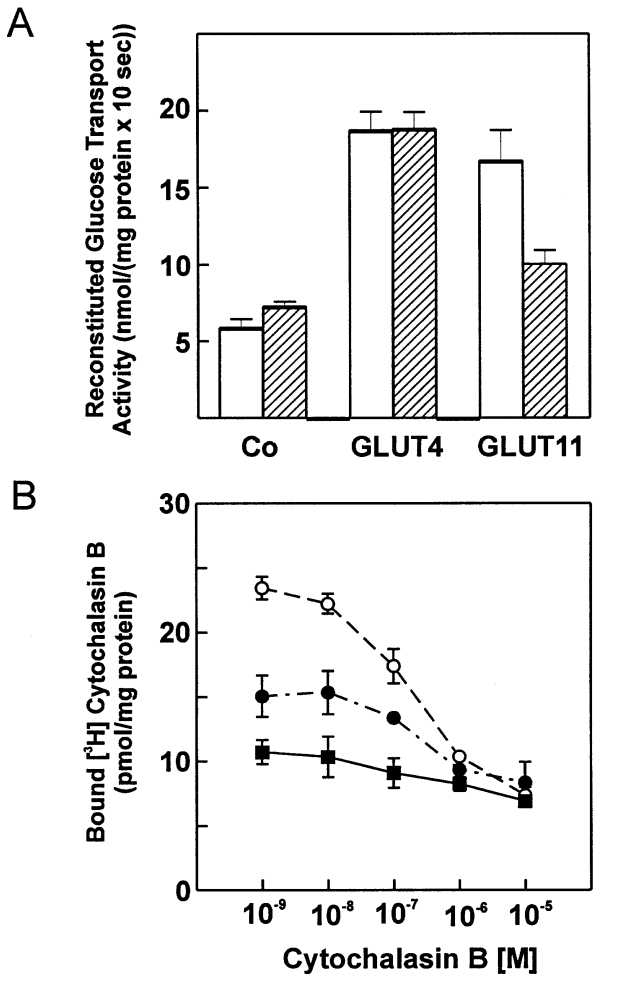

Figure 8 Glucose-transport activity and cytochalasin B binding in COS-7 cells transfected with GLUT11 cDNA

(**A**) Reconstitutable glucose-transport activity in membranes from COS-7 cells overexpressing GLUT11 was assayed as described in the text at a D-glucose concentration of 5 mM in the presence (hatched bars) or absence (open bars) of 5 mM fructose. Data represent means ± S.D. of triplicate samples from a representative experiment that was repeated four times with membranes from independent transfections. Co, control. (**B**) Specific binding of [^{3}H]cytochalasin B was assayed in plasma membranes from COS-7 cells transfected with GLUT11 (●), GLUT4 (○) or blank vector (■) as described. K_d values derived from the binding curves were 196.6 ± 29.7 nM for GLUT11 and 115.3 ± 4.9 nM for GLUT4 (means ± S.D. from three independent transfections).

Expression of GLUT11 in human and rat tissues

In human tissues, mRNA of GLUT11 was detected exclusively in heart and skeletal muscle (Figure 5A). In other tissues (brain, placenta, lung, liver, kidney and pancreas in Figure 5A; results are not shown for spleen, thymus, prostate, testis, ovary, intestine and leucocytes), no signal could be detected. The Northern blots showed the presence of a major transcript of 7.2 kb, and an additional band at approx. 3.6 kb. In rat tissues (Figure 5B), a 7 kb transcript was detected mainly in heart; lower amounts of mRNA were found in skeletal muscle. No transcript was detectable in rat adipose tissue (Figure 5B) or in 3T3-L1 adipocytes (results not shown).

In order to demonstrate the presence of the GLUT11 protein in human tissues, heart membranes were separated by SDS/PAGE and probed with the anti-GLUT11 antiserum. As illus-

trated in Figure 6(A), a specific immunoreactive band was detected at 45 kDa in plasma membranes and in the microsomal fraction from both atrium and ventricle. No signal was detected with the pre-immune serum (Figure 6B) or with serum that was blocked with the immunogenic peptide (results not shown). A control blot with anti-GLUT4 antiserum (Figure 6C), which was run to ascertain the quality of the membrane preparation, exhibited a somewhat different distribution of GLUT4.

Expression of GLUT11 cDNA in COS-7 cells

As is illustrated in Figure 7, transfection of COS-7 cells with the GLUT11 cDNA produced the expression of a 40 kDa membrane protein that was not present in membranes from cells transfected with blank vector. Antiserum against a C-terminal peptide of GLUT11 detected the transporter in the plasma-membrane fraction, and also in a fraction of high-density microsomes.

Reconstituted glucose-transport activity and cytochalasin B binding in membranes from COS-7 cells transfected with GLUT11 cDNA

Overexpression of the GLUT11 cDNA in COS-7 cells produced a 2–3-fold increase in the D-glucose transport reconstituted from isolated membranes (Figure 8A). A similar effect was produced by overexpression of GLUT4 in parallel samples. Furthermore, the presence of 5 mM fructose markedly inhibited the glucose-transport activity of GLUT11, but not that of GLUT4.

Membranes from transfected cells were incubated with [^{3}H]-cytochalasin B and increasing concentrations of unlabelled agent to determine specific binding of this ligand. As is illustrated in Figure 8(B), overexpression of GLUT11 produced a significant increase in specific binding of cytochalasin B. The K_d value derived from Scatchard plots of the binding curves (results not shown) was 196.6 ± 29.7 nM and was significantly higher than the K_d value of GLUT4 (115.3 ± 4.9 nM, means $\pm$ S.D. from three independent transfections).

DISCUSSION

The sequence of the GLUT11 presents all elements (sugar-transporter signatures) that are characteristic of the GLUT family and are required for their function as sugar transporters. Specifically, hydrophobicity plots predict a tertiary structure that includes 12 membrane-spanning helices [1]. In addition, the sequence presents conserved residues that have previously been shown to be essential for transporter function: several conserved arginine and glutamate residues on the cytoplasmic surface [13], the tryptophan residue in helix 11 corresponding with Trp-412 in GLUT1 [20,21], tyrosine residues in helix 4 and 7 corresponding to Tyr-146 and Tyr-292/293 in GLUT1 [22,23], and glutamines in helix 5 and 7 corresponding to Gln-161 and Gln-282 in GLUT1 [24,25]. As anticipated on the basis of these sequence characteristics, we detected reconstitutable glucose-transport activity when the GLUT11 cDNA was expressed in COS-7 cells. Thus the gene is a novel member of the family of sugar/polyol-transport facilitators and has been designated accordingly.

Our preliminary characterization indicates that GLUT11 is capable of transporting glucose. In contrast to that of GLUT4, however, the reconstituted glucose-transport activity of the GLUT11 is inhibitable by fructose. Thus GLUT11 appears to recognize fructose with comparable affinity to, possibly with even higher affinity than, glucose. This observation is consistent with the sequence similarity between GLUT11 and GLUT5. Like GLUT5, GLUT11 lacks the tyrosine residue in helix 10 that is conserved in GLUT1–4. However, it should be noted that more direct evidence is needed before a definitive conclusion as to the substrate specificity of GLUT11 can be drawn, and before GLUT11 can be considered a fructose transporter.

According to the multiple alignment of the human glucose transporters, the GLUT family can be divided into three subclasses. Class I comprises GLUT1–4, class II the fructose transporter GLUT5, GLUT11 and GLUT9 (GLUTX). Class III consists of the unusual transporters GLUT8, GLUT6 (previously GLUT9) and GLUT10. It is unclear whether the members of these subclasses are characterized by common functional features, or whether they merely reflect the evolutionary development of the GLUT family. The *Drosophila* genome (alignment of the GLUT families in [8]) comprises only a single class I, no class II and seven class III-like GLUT isotypes.

The recent cloning of five additional members of the GLUT family and the comparison of their sequences allows several general conclusions. First, all sequences are consistent with the 12-helix model originally developed on the basis of the sequence of GLUT1. In addition, all novel GLUT proteins appear to be glycosylated in loops facing the cell surface according to the model. Secondly, the sequences confirm the necessity of the sugar-transporter signatures that were defined in comparisons of GLUT1–5 and in mutational analyses [13]. Finally, comparison of the mammalian GLUT family with that of *Caenorhabditis elegans* and *Drosophila melanogaster* indicates that the family of sugar-transport facilitators underwent marked alterations during evolution. Most strikingly, the number of family members appears to decrease from yeast, *C. elegans* and *D. melanogaster* to *Homo sapiens*.

At present, we can only speculate as to the specific function of GLUT11. Since the protein is a close relative of GLUT5 [26], and because of the inhibition of its glucose-transport activity, it may be a fructose transporter. Furthermore, the present findings indicate that GLUT11 has striking muscle specificity. Thus this isoform is a candidate for the unkown compensatory transporter in GLUT4-null mice [27], and it remains to be shown whether it participates in the regulation of glucose homoeostasis *in vivo*.

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