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Sequence, tissue distribution, and differential expression of mRNA for a putative insulin-responsive glucose transporter in mouse 3T3-L1 adipocytes

(gene family/differentiation)

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ABSTRACT **The cDNAs for two putative glucose transporters from mouse 3T3-L1 adipocytes were isolated and sequenced. One of these cDNAs encodes the murine homolog of the human hepG2/erythrocyte glucose transporter, termed GT1. GT1 mRNA is most abundant in mouse brain and is expressed in both 3T3-L1 preadipocytes and adipocytes. The other cDNA encodes a glucose transporter-like protein, termed GT2, that has a unique amino acid sequence and tissue distribution. GT2 cDNA encodes a protein with 63% amino acid sequence identity and a similar structural organization to GT1. GT2 mRNA is found at high levels in mouse skeletal muscle, heart, and adipose tissue, all of which exhibit insulin-stimulated glucose uptake. GT2 mRNA is absent from 3T3-L1 preadipocytes but is induced dramatically during differentiation into adipocytes. This increase in mRNA content correlates closely with the acquisition of insulin-stimulated glucose uptake. We propose that GT2 is an insulin-regulated glucose transporter.**

The uptake of glucose for metabolism and growth is essential to most animal cells and is mediated by glucose-transporter proteins (1). Glucose is transported across the brush border membranes of kidney and intestine by Na^+-coupled active transport, whereas facilitative glucose transport is responsible for glucose uptake by other tissues—e.g., brain, liver, muscle, and adipose tissues. cDNAs encoding facilitative glucose transporters have been isolated from human hepatoma (2) and rat brain (3). This type of glucose transporter (the HepG2/erythrocyte transporter) is expressed at high levels in brain, erythrocytes, and certain transformed cell lines and at lower levels in adipose tissue and kidney. Related cDNAs have also been isolated from human and rat liver (4, 5) and human fetal skeletal muscle (6). However, to date a glucose transporter cDNA specific to the few cell types (adipocytes and skeletal and heart muscle) that exhibit insulin-stimulated glucose uptake has not been identified. Here we report the cloning and characterization of two glucose transporter-like cDNAs from mouse 3T3-L1 adipocytes. One of these transporter cDNAs encodes the murine homolog of the HepG2/erythrocyte glucose transporter, termed GT1. The other cDNA encodes a protein, termed GT2, that is expressed at high levels in tissues that exhibit insulin-stimulated glucose uptake.§ This and the fact that expression of the corresponding mRNA is induced during differentiation of adipocytes strongly suggests that GT2 is an insulin-responsive glucose transporter.

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EXPERIMENTAL PROCEDURES

Cells and Animals. Mouse 3T3-L1 preadipocytes were cultured and differentiated as described (7). Briefly, cells were grown to confluence, and 2 days later conversion into adipocytes was induced by feeding Dulbecco's modified Eagle's medium (DMEM) containing 10% (vol/vol) fetal bovine serum, isobutylmethylxanthine, dexamethasone, and insulin for 2 days. The cells were fed the same medium minus isobutylmethylxanthine and dexamethasone, but containing insulin, for an additional 2 days. For the next 7 days, cells were fed DMEM supplemented only with 10% fetal bovine serum (without added insulin) every other day. Three- to 5-week-old CD mice (Charles River Breeding Laboratories) were used for preparation of RNA from tissues.

Screening of 3T3-L1 Adipocyte cDNA Library and DNA Sequencing. A mouse 3T3-L1 adipocyte cDNA library (8) in λ ZAP (Stratagene) was screened initially using the human HepG2 glucose transporter cDNA (2). The HepG2 cDNA insert was radiolabeled with ^{32}P by random-priming (9) and then used as probe to screen 10^6 recombinants of the 3T3-L1 adipocyte library. Ten positive clones were purified and characterized and represent GT1, the murine homolog of the HepG2/erythrocyte glucose transporter (2, 3). The insert of one of these clones was used as probe in a low-stringency screen (4) of 700,000 recombinants of the same cDNA library. Four additional cDNA clones representing the same mRNA were isolated and sequenced in both directions by the dideoxynucleotide chain-termination method (10). This mRNA encodes a protein referred to as GT2. Hydropathy plots were generated using the algorithm of Kyte and Doolittle (11).

RNA Isolation and Analysis. Total cellular RNA was isolated by the guanidine thiocyanate method (12). For RNA blot analysis, RNA was denatured with glyoxal, electrophoresed on 1% agarose gels, transferred to Hybond N (Amersham), hybridized according to Thomas (13), washed to high stringency (65°C, 15 mM NaCl), and autoradiographed. The complete cDNA inserts of GT1 and GT2 were used as probes after labeling by random-priming (9). To control for RNA amounts, some blots were reprobed with an unrelated mouse cDNA clone referred to as pAL15 (14), which encodes a mRNA that is not altered during the differentiation of 3T3-L1 preadipocytes. The intensity of the bands was estimated by

Abbreviations: GT1, the murine homolog of the HepG2/erythrocyte glucose transporter; GT2, the murine adipocyte/muscle glucose transporter-like protein.

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§The sequences reported here have been deposited in the EMBL/GenBank data base (for GT2, accession no. M23383).

densitometry of the autoradiograms. The levels of expression of GT1 and GT2 mRNAs (see Fig. 6) estimated by densitometry were normalized to that of pAL15.

Glucose Uptake Assay. The rate of 2-deoxy-D-[U-^{14}C]-glucose (DuPont/NEN) uptake by 3T3-L1 cell monolayers was determined as described by Frost and Lane (15). Cell monolayers cultured in parallel were used for either 2-deoxyglucose uptake assays or RNA isolation.

RESULTS

Isolation and Sequence of Two Glucose Transporter-Like cDNA Clones. The cDNA insert of a human HepG2 cDNA clone (2) was used to screen a mouse 3T3-L1 adipocyte λ ZAP cDNA library. Ten clones were selected, plaque-purified, and characterized by sequencing. On the basis of nucleotide sequence and restriction mapping, it was concluded that all clones represent the same mRNA. The inserts of two of these clones were sequenced in their entirety. The size of these cDNA inserts, each ≈2570 base pairs, is in good agreement with the size of the mRNA estimated from Northern blots (see Figs. 4 and 5). The derived amino acid sequence of these cDNAs is >97% identical to that of the human HepG2 (2) and rat brain glucose transporters (3). The isolated cDNAs, therefore, represent the murine homolog of the HepG2/erythrocyte glucose transporter and are referred to as GT1. [See Fig. 2 for the translated amino acid sequence of GT1; the complete nucleotide sequence is deposited in the EMBL/

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                                                                                CCGTGGGTTGTGGCAGTGAGTCCCACCAGACCCGCCCT      42

TTGCACACGGCTTCCGAACGCCGGGGTCTCGTGCCGGCCAGGCCCGGACCCTATACCCTATTCATTTTTTTCTTATTGCAGCGCCTGAGTCTTTTCTTCTTTTAAAACAAG     153

ATG CCG TCG GGT TTC CAG CAG ATC GGC TCT GAC GTG AAG GAT GGG GAA CCC CCT CGG CAG CGA GTG ACT GGA ACA CTG GTC CTA      237
Met Pro Ser Gly Phe Gln Gln Ile Gly Ser Asp Val Lys Asp Gly Glu Pro Pro Arg Gln Arg Val Thr Gly Thr Leu Val Leu       28

GCT GTA TTC TCA GCT GTG CTT GGC TCC CTT CAG TTT GGC TAT AAC ATT GGG GTT ATC AAT GCC CCA CAG AAG GTG ATT GAA CAG      321
Ala Val Phe Ser Ala Val Leu Gly Ser Leu Gln Phe Gly Tyr Asn Ile Gly Val Ile Asn Ala Pro Gln Lys Val Ile Glu Gln       56

AGC TAC AAT GCA ACG TGG CTG GGT AGG CAA GGT CCT GGG GGA CCG GAT TCC ATC CCA CAA GGC ACC CTC ACT ACG CTC TGG GCT      405
Ser Tyr Asn Ala Thr Trp Leu Gly Arg Gln Gly Pro Gly Gly Pro Asp Ser Ile Pro Gln Gly Thr Leu Thr Thr Leu Trp Ala       84

CTC TCC GTG GCC ATC TTC TCT GTG GGT GGC ATG ATC TCT TCC TTT CTC ATT GGC ATC ATT TCT CAA TGG TTG GGA AGG AAA AGG      489
Leu Ser Val Ala Ile Phe Ser Val Gly Gly Met Ile Ser Ser Phe Leu Ile Gly Ile Ile Ser Gln Trp Leu Gly Arg Lys Arg      112

GCT ATG CTG GCC AAC AAT GTC TTG GCC GTG TTG GGG GGC GCC CTC ATG GGC CTA GCC AAT GCC GTG GCC TCC TAT GAG ATA CTC      573
Ala Met Leu Ala Asn Asn Val Leu Ala Val Leu Gly Gly Ala Leu Met Gly Leu Ala Asn Ala Val Ala Ser Tyr Glu Ile Leu      140

ATT CTT GGA CGG TTC CTC ATT GGC GCC TAC TCA GGG CTA ACA TCA GGG CTG GTG CCC ATG TAT GTG GGA GAA ATC GCC CCC ACT      657
Ile Leu Gly Arg Phe Leu Ile Gly Ala Tyr Ser Gly Leu Thr Ser Gly Leu Val Pro Met Tyr Val Gly Glu Ile Ala Pro Thr      168

CAT CTT CGG GGT GCC TTG GGA ACA CTC AAC CGA CTG GCC ATC GTC ATT GGC ATT CTG GTT GCC CAG GTG CTG GGC TTG GAG TCT      741
His Leu Arg Gly Ala Leu Gly Thr Leu Asn Arg Leu Ala Ile Val Ile Gly Ile Leu Val Ala Gln Val Leu Gly Leu Glu Ser      196

ATG CTG GGC ACA GCT ACC CTG TGG CCA CTG CTT CTG GCT CTC ACA GTA CTC CCT GCT CTC CTG CAG CTG ATT CTG CTG CCC TTC      825
Met Leu Gly Thr Ala Thr Leu Trp Pro Leu Leu Leu Ala Leu Thr Val Leu Pro Ala Leu Leu Gln Leu Ile Leu Leu Pro Phe      224

TGT CCT GAG AGC CCC AGA TAC CTC TAC ATC ATC CGG AAC CTG GAG GGG CCT GCC CGA AAG AGT CTA AAG CCC CTG ACC GGC TGG      909
Cys Pro Glu Ser Pro Arg Tyr Leu Tyr Ile Ile Arg Asn Leu Glu Gly Pro Ala Arg Lys Ser Leu Lys Pro Leu Thr Gly Trp      252

GCT GAT GTG TCT GAC GCA CTA GCT GAG CTG AAG GAT GAG AAA CGG AAG TTG GAG AGA GAG CGT CCA ATG TCC TTG CTC CAG CTC      993
Ala Asp Val Ser Asp Ala Leu Ala Glu Leu Lys Asp Glu Lys Arg Lys Leu Glu Arg Glu Arg Pro Met Ser Leu Leu Gln Leu      280

CTG GGC AGC CGC ACC CAC CGG CAG CCT CTT ATC ATC GCA GTG GTG CTG CAG CTG AGC CAA CAG CTC TCA GGC ATC AAT GCT GTT     1077
Leu Gly Ser Arg Thr His Arg Gln Pro Leu Ile Ile Ala Val Val Leu Gln Leu Ser Gln Gln Leu Ser Gly Ile Asn Ala Val      308

TTC TAC TAT TCA ACC AGC ATC TTC GAG TCG GCT GGG GTG GGA CAG CCA GCC TAC GCC ACC ATA GGA GCT GGT GTG GTC AAT ACG     1161
Phe Tyr Tyr Ser Thr Ser Ile Phe Glu Ser Ala Gly Val Gly Gln Pro Ala Tyr Ala Thr Ile Gly Ala Gly Val Val Asn Thr      336

GTC TTC ACG TTG GTC TCG GTG CTC TTA GTA GAA CGA GCT GGA CGA CGG ACA CTC CAT CTG TTG GGC CTG GCC GGC ATG TGT GGC     1245
Val Phe Thr Leu Val Ser Val Leu Leu Val Glu Arg Ala Gly Arg Arg Thr Leu His Leu Leu Gly Leu Ala Gly Met Cys Gly      364

TGT GCC ATC TTG ATG ACC GTG GCT CTG CTG CTG CTG GAA CGG GTT CCA GCC ATG AGC TAT GTC TCC ATC GTG GCC ATA TTT GGC     1329
Cys Ala Ile Leu Met Thr Val Ala Leu Leu Leu Leu Glu Arg Val Pro Ala Met Ser Tyr Val Ser Ile Val Ala Ile Phe Gly      392

TTT GTG GCC TTC TTT GAG ATT GGC CCT GGC CCC ATT CCC TGG TTC GTG GCA GAG CTC TTC AGC CAG GGC CCC CGC CCA GCC GCC     1413
Phe Val Ala Phe Phe Glu Ile Gly Pro Gly Pro Ile Pro Trp Phe Val Ala Glu Leu Phe Ser Gln Gly Pro Arg Pro Ala Ala      420

ATG GCT GTC GCT GGT TTC TCC AAC TGG ACC TGT AAC TTC ATT GTC GGC ATG GGT TTC CAG TAT GTT GCG GAT CGT ATG GGT CCT     1497
Met Ala Val Ala Gly Phe Ser Asn Trp Thr Cys Asn Phe Ile Val Gly Met Gly Phe Gln Tyr Val Ala Asp Arg Met Gly Pro      448

TAC GTC TTC CTT CTA TTT GCC GTC CTC CTG CTT GGC TTC TTC ATC TTC ACC TTC CTA AAA GTG CCT GAA ACC AGA GGC CGG ACG     1581
Tyr Val Phe Leu Leu Phe Ala Val Leu Leu Leu Gly Phe Phe Ile Phe Thr Phe Leu Lys Val Pro Glu Thr Arg Gly Arg Thr      476

TTT GAC CAG ATC TCA GCT GCC TTC CGA CGG ACA CCT TCC CTT TTA GAG CAG GAG GTG AAA CCC AGT ACA GAA CTT GAA TAC TTA     1665
Phe Asp Gln Ile Ser Ala Ala Phe Arg Arg Thr Pro Ser Leu Leu Glu Gln Glu Val Lys Pro Ser Thr Glu Leu Glu Tyr Leu      504

GGG CCA GAT GAG AAT GAC TGA GGGGCAAAACAGGGTGGGAGAGCCACCTCTCCACCCAGACTCCCTCCTTTCCTCTACAGCACTTTAGCCCTCTCTTCCCTGTT     1769
Gly Pro Asp Glu Asn Asp ---                                                                                        510

ACCTCCAGGTTGAAGGAACAGCAGCCTGGGGAACTGGGAAGCTGAAGGGAGGGGTGGTCCATGTACCCCTCATTCCCCCTGTGTGATTCTTTTGGATTATTTATGTGTGTG     1880

GCTAGGCTGTGGCCACCTAGATGGGCTTTCTCCGTCCTGCCTTCCTCCTGCCCCTACCCAGACTCAGCTCTAGACTACTTTCCTCCCCTTTCGAGAAGGGGTCTGCAGGAG     1991

GGTGGGGTGGCCCTGAATTCATCAGGATAAACAGCAGGGGTGGGTGTGTGAGCGAGTGCTTTCCTCCCACAAACTGGCACTTCCACTGAACTCTTGCCACACAGGCTCTGG     2102

GTGAAGGGGGTTGTCTTGACCCCTCCAGGGCAAAGGATACACCCTCCCAAAATCTAGCCCTGCCTCCCCACAGGCTCCACCCTCCCGGGCAAAGGAACACAATAGTACATA     2213

CCTGACAGGGCAAGGACGGTTAGAGCGCATCAGTCTCCATTTGGGGCCCTAGGTTGTTCCCAGGGCTGCAAAGCGTAGGTACCAACACTTTCTTGTTCCCCTCCAGGAAGG     2324

GTGCTAAACCCGAAAGCTTCTGACCAACTAAGGGCGGGAGGGGATTTGAAAGGCTGCCTATAAACACTGGTTGGGAGGGAGCCTTTGGTATTTTTGTATGTTTTGAAGAAC     2435

GGGATAGGGACAGAAACCCAAGGGCTGCTGTATTAAATGTGTATATAGAGATTCGTCCATAAAGTCACTGTTTGAAACCTGCAGC                             2520
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FIG. 1. Nucleotide sequence of the cDNA for a mouse 3T3-L1 adipocyte glucose transporter-like protein (GT2) and its predicted amino acid sequence. Nucleotide and amino acid numbers are indicated at the end of each line. The underlined nucleotide sequence (positions 184–195) is ambiguous due to compression.

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GT2 MPSGFQQIGSDVKDGEPPRQRVTGTLVLAVFSAVLGSLQFGYNIGVINAPQKVIEQSYNATWLGRQGPGGPDSIPQGTLTTLWALSVAIFSVGGMISSFL 100
    :              .:....::: :.::: .:::::::::::::::::::::. ::.::  : :   ..:: .:::::::.:::::::::::::.::
GT1 M--------------DPSSKKVTGRLMLAVGGAVLGSLQFGYNTGVINAPQKVIEEFYNQTWNHRIG----EPIPSTTLTTLWSLSVAIFSVGGMIGSFS  92

GT2 IGIISQWLGRKRAMLANNVLAVLGGALMGLANAVASYEILILGRFLIGAYSGLTSGLVPMYVGEIAPTHLRGALGTLNRLAIVIGILVAQVLGLQSMLGT 200
    .:.. ...:.:...::  :.:: ....:::...   :.:.:::::.::.:.:::.:.:::::::.:: :::::::::..:.::.:::.:::.:.:.:.
GT1 VGLFVNRFGRRNSMLMMNLLAFVAAVLMGFSKLGKSFEMLILGRFIIGVYCGLTTGFVPMYVGEVSPTALRGALGTLHQLGIVVGILIAQVFGLDSIMGN 182

GT2 ATLWPLLLALTVLPALLQLILLPFCPESPRYLYIIRNLEGPARKSLKPLTGWADVSDALAELKDEKRKLERERPMSLLQLLGSRTHRQPLIIAVVLQLSQ 300
    :.::::::::... .:::::: :::::::::::.: : :: :..:.. :: : : :::. .:.:.:.: :.. ::. ...:.:. :...:::::.:::::::::
GT1 ADLWPLLLSVVFVPALLQCILLPFCPESPRFLLINRNEENRAKSVLKKLRGTADVTRDLQEMKEEGRQMMREKKVTILELFRSPAYRQPILIAVVLQLSQ 282

GT2 QLSGINAVFYYSTSIFESAGVGQPAYATIGAGVVNTVFTLVSVLLVERAGRRTLHLLGLAGMCGCAILMTVALLLLERVPAMSYVSIVAIFGFVAFFEIG 400
    :::::::::::::::::.::: ::.:::::.:.:::.::.::...:::::::::::.::::: :::.:::.:: :::::.: :::.:::::::::::::.:
GT1 QLSGINAVFYYSTSIFEKAGVQQPVYATIGSGIVNTAFTVVSLFVVERAGRRTLHLIGLAGMAGCAVLMTIALALLERLPWMSYLSIVAIFGFVAFFEVG 382

GT2 PGPIPWFV-AELFSQGPRPAAMAVAGFSNWTCNFIVGMGFQYVADRMGPYVFLLFAVLLLGFFIFTFLKVPETRGRTFDQISAAFRRTPSLLEQEVKPST 499
    :::::::. :::::::::::: .:::::::::.:::::: :::::.. ::::::.:.:::. :::::..:::::.:::::.:. .::.. .  . .. .:..
GT1 PGPIPWFIVAELFSQGPRPARIAVAGFSNWTSNFIVGMCFQYVEQLCGPYVFIIFTVLLVLFFIFTYFKVPETKGRTFDEIAFGFRQGGAS-QSDKTPEE 481

GT2 ELEYLGPDEND                                                                                          510
     .. ::.:..
GT1 LFHPLGADSQV                                                                                          492
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FIG. 2. Comparison of the amino acid sequences of two mouse 3T3-L1 adipocyte glucose transporter proteins. Amino acids are indicated by single-letter code. Colons denote identical amino acids, and single dots indicate chemically similar amino acids. The sequence of GT2 is that shown in Fig. 1; the sequence of GT1 represents the predicted amino acid sequence of the murine 3T3-L1 adipocyte homolog of the HepG2/erythrocyte glucose transporter. The 12 putative transmembrane domains are underlined and overlined.

GenBank data base (accession no. M23384).] The full-length cDNA insert for GT1 was used in a subsequent low-stringency screen of the same library. Four additional clones that represented the same mRNA were distinct from GT1. The near full-length insert of 2520 base pairs of one clone (λ GT2) was sequenced in its entirety. This nucleotide sequence contains a single open reading frame encoding a protein of 510 amino acids that corresponds to a calculated molecular weight of 54,948 (Fig. 1). Despite the fact that none of the cDNA clones contained a poly(A) tail, the cDNA insert of λ GT2 is only slightly smaller than the mRNA estimated from Northern blots at 2.7 kilobases (see Fig. 4). The protein encoded by this cDNA is referred to as GT2.

Relationship of Mouse GT1 and GT2 to Other Members of the Glucose Transporter Gene Family. Reports (2–6) of the cloning of several glucose transporter-like cDNAs indicate the existence of a glucose transporter gene family. In Fig. 2, the sequences of mouse GT1 and GT2 are aligned with the identical and similar amino acids indicated. The overall amino acid sequence identity between GT1 and GT2 is 63%. GT2 also shares 49% identical amino acid sequence with the human liver glucose transporter (4) and 55% with the human

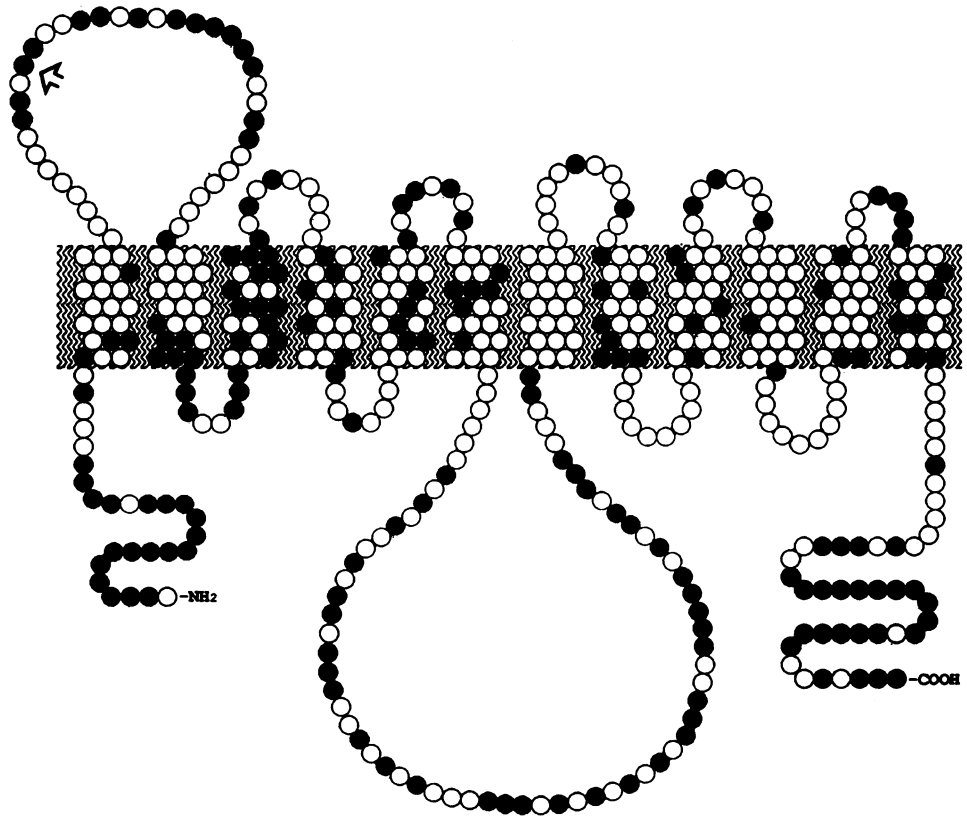

FIG. 3. Proposed model for the organization of GT2 in the membrane. Amino acids of GT2 are represented as circles. The model is based on the hydropathy plot for GT2 (data not shown) and its homology to the mouse glucose transporter GT1. Amino acids identical in GT1 and GT2 are represented as open circles (○); divergent amino acids are shown as solid circles (●). The arrow indicates a potential asparagine-linked glycosylation site.

fetal skeletal muscle glucose transporter (6). Hydropathy plots of GT2 and GT1 (data not shown) are virtually superimposable. The putative topology of GT2 based on this similarity is consistent with the occurrence of 12 membrane-spanning regions as appears to be the case for the other glucose transporters (Fig. 3). Most amino acid substitutions in the sequence of GT2 relative to that of the other glucose transporters are found in the cytoplasmic and extracellular domains and in transmembrane domain 3.

Tissue Distribution of GT1 and GT2 mRNA. Transfer blots of RNA prepared from various mouse tissues showed RNA transcripts of 2.7 kilobases for both GT1 and GT2 (Fig. 4). GT1 is expressed at high levels in brain and at much lower levels in the other tissues examined. This is a similar pattern of distribution to that found for the HepG2/erythrocyte transporter (3) and supports the view that GT1 is the mouse equivalent (Fig. 4*B*). The pattern of expression of GT2 (Fig. 4*A*) is quite different in that high glucose transporter RNA levels are found in skeletal muscle, heart, and adipose tissue. The transcript is far less abundant in lung and below the limit of detection in spleen, kidney, liver, and brain.

Expression of GT2 mRNA During Adipose Conversion Correlates with Acquisition of Insulin-Stimulated Glucose Uptake. The 3T3-L1 cell line is a well-characterized model system for preadipocyte differentiation (16–18). Within a few days after treatment with dexamethasone, isobutylmethylxanthine, and insulin, 3T3-L1 preadipocytes differentiate into cells exhibiting the morphological and biochemical characteristics of adipocytes. The mature adipocyte phenotype is attained 6–9 days after induction of differentiation (16–18). mRNA levels for both GT1 and GT2 were assayed during the time course of differentiation (Fig. 5). GT1 mRNA is present in preadipocytes as well as adipocytes (Fig. 5*B*) and its cellular level is not appreciably affected by differentiation. However, the level of GT2 mRNA, which is not detectable in preadipocytes, first appears 3 days after induction of differentiation and reaches a maximal level in the mature adipocyte (Fig. 5*A*).

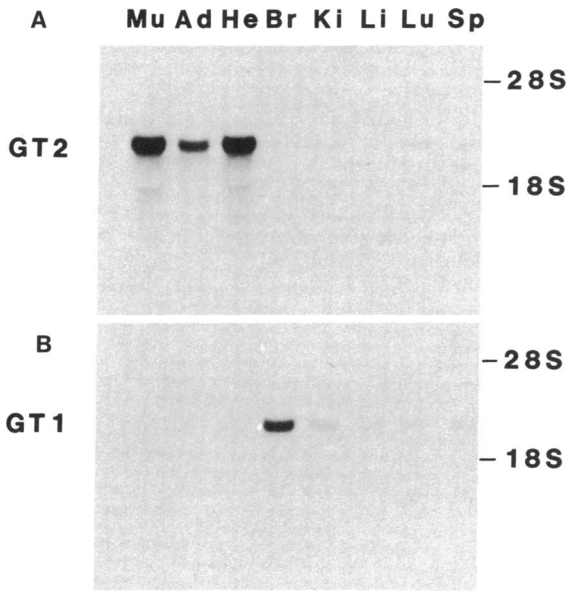

FIG. 4. Northern blot analysis of tissue distribution of mouse GT1 and GT2 mRNAs. Total RNA (15 μg) was electrophoresed after glyoxal denaturation on a 1% agarose gel, transferred to a nylon membrane, and hybridized to the GT2 (*A*) and GT1 (*B*) cDNA inserts labeled by random-priming. Both mRNAs detected are ≈2.7 kilobases in size. Tissues analyzed were as indicated by lane labels. Lanes: Mu, skeletal muscle; Ad, adipose; He, heart; Br, brain; Ki, kidney; Li, liver; Lu, lung; Sp, spleen.

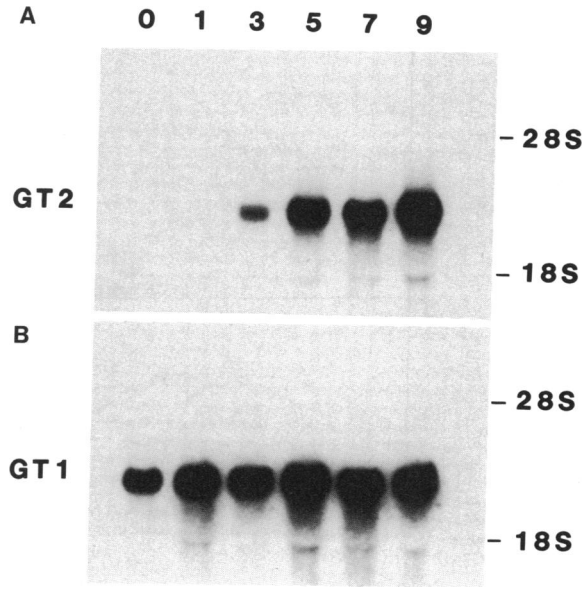

FIG. 5. Northern blot analysis of GT2 and GT1 mRNAs expressed during differentiation of 3T3-L1 preadipocytes. Replicate monolayers of 3T3-L1 preadipocytes were differentiated into adipocytes by induction with isobutylmethylxanthine, dexamethasone, and insulin. At various times after initiating differentiation, total RNA was isolated and subjected to Northern blot analysis. Northern blot analyses were performed as in Fig. 4, except that total RNA (15 μg) from 3T3-L1 cells was used. The lane labels indicate the day after induction of differentiation that RNA was isolated. Parallel blots were probed with GT2 (*A*) or GT1 (*B*) cDNA inserts.

The differentiation of 3T3-L1 preadipocytes also leads to a dramatic rise in the ability of the cells to carry out insulin-stimulated glucose uptake (19). Therefore, we compared the changes in insulin-stimulated hexose uptake with the changes in GT1 and GT2 mRNA levels in 3T3-L1 cells during the course of differentiation. As shown in Fig. 6, only the increase in GT2 mRNA closely paralleled the increase in insulin-stimulated glucose uptake, suggesting that GT2, and not GT1, might be responsive to stimulation by insulin.

DISCUSSION

The activation of glucose uptake by insulin occurs in only a few cell types, notably skeletal and heart muscle cells and adipocytes (1). Although the primary structures of several glucose transporter-like proteins have been determined, none has yet been reported for the glucose transporter(s) of the latter cell types. Northern blot analysis detects low levels of the HepG2/erythrocyte glucose transporter in adipose tissue (3). However, experiments using specific anti-peptide antibodies have revealed that the HepG2/erythrocyte glucose transporter accounts for only a small fraction of the glucose transporters present in adipocytes (20). The occurrence of the HepG2/erythrocyte-type glucose transporter in adipose tissue was confirmed by cloning its homolog from a mouse 3T3-L1 adipocyte cDNA library (Fig. 2). Consistent with the prediction by Oka *et al.* (20), we have shown conclusively by cloning and mRNA analysis that another glucose transporter-like protein (GT2) is present in adipocytes. High levels of GT2 mRNA were also found in the other tissues (i.e., skeletal muscle and heart) that exhibit insulin-stimulated glucose uptake (Fig. 4*A*). GT2 mRNA was not, however, found in the other tissues examined. We suggest that GT2 mRNA encodes an insulin-responsive glucose transporter. This view is supported by the finding that GT2 mRNA levels follow a similar pattern as the acquisition of insulin-stimulated hexose uptake during the differentiation of 3T3-L1 preadipocytes (Fig. 6).

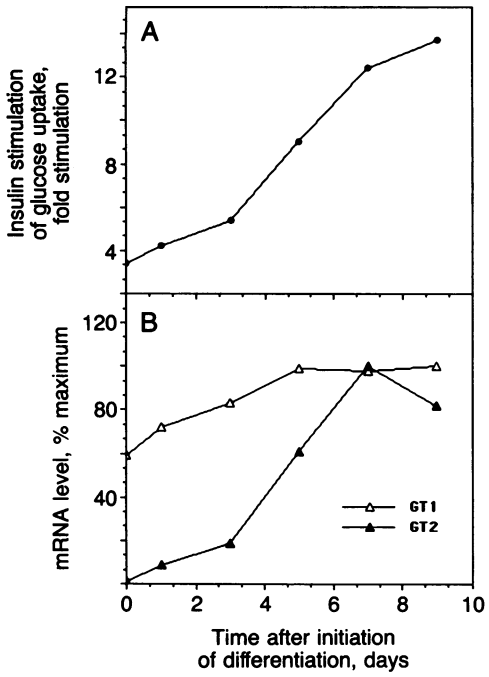

FIG. 6. Time course for acquisition of insulin-stimulated 2-deoxy-D-[U-^{14}C]glucose uptake capacity and for expression of glucose transporter mRNA levels during differentiation of 3T3-L1 cells. At various times after the induction of differentiation, the rate of 2-deoxy-D-[U-^{14}C]glucose uptake was measured before and after treatment of 3T3-L1 cells with 1 μM insulin for 10 min. (*A*) Fold stimulation by insulin is the ratio of uptake after preincubation with or without insulin. On day 9 the rates of 2-deoxyglucose uptake were 970 and 70 pmol per min per 10^6 cells treated with insulin or not, respectively. (*B*) For analysis of GT1 and GT2 mRNA levels, total RNA (15 μg) from parallel cell monolayers was subjected to Northern blot analysis as described in Fig. 5. mRNA levels were quantitated by densitometry, normalized, and expressed as percent of maximal levels.

The discovery of GT2 may be of pathophysiological importance, as it is a candidate for certain post-receptor defects in non-insulin-dependent diabetes mellitus.

The structure of GT2 is of interest (Fig. 3). Its high degree of similarity to other glucose transporters identifies it as a member of the glucose transporter gene family. The primary structure of GT2 is most similar to the other glucose transporters in its putative membrane-spanning domains (Figs. 2 and 3). This might reflect the functional importance of these domains in the facilitated transport of glucose across the membrane. The intra- and extracellular regions, however, are quite distinct and thus might be responsible for regulation by insulin and for the kinetic properties of the transporter. The availability of the two cDNA clones reported here should facilitate the identification of the functional domains of these glucose transporters.

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