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Golgi Localization and Functional Expression of Human Uridine Diphosphatase*

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A full-length E(ecto)-ATPase (Plesner, L. (1995) Int. Rev. Cytol. 158, 141–214) cDNA was cloned from a human brain cDNA library; it encodes a 610-amino acid protein that contains two putative transmembrane domains. Heterologous expression of this protein in COS-7 cells caused a significant increase in intracellular membrane-bound nucleoside phosphatase activity. The activity was highest with UDP as substrate and was stimulated by divalent cations in the following order: Ca$$^{2+}$$ > Mg$$^{2+}$$ > Mn$$^{2+}$$. The results of immunofluorescence staining indicate that this protein is located in the Golgi apparatus. UDP hydrolysis was increased in the presence of Triton X-100 or alamethicin, an ionophore that facilitates movement of UDP across the membrane, suggesting that the active site of this UDPase is on the luminal side of the Golgi apparatus. This is the first identification of a mammalian Golgi luminal UDPase gene. Computer-aided sequence analysis of the E-ATPase superfamily indicates that the human UDPase is highly similar to two hypothetical proteins of the nematode Caenorhabditis elegans and to an unidentified 71.9-kDa yeast protein and is less related to the previously identified yeast Golgi GDPase.

There is evidence that a UDPase is present on the luminal side of the Golgi apparatus of mammalian cells (1, 2). Proteins and lipids are glycosylated in the lumen of the Golgi apparatus. UDP-Gal, a sugar donor in glycosylation, is synthesized in the cytosol and translocated into the Golgi lumen via specific membrane carriers (3). After transfer of sugar residues to proteins and lipids by galactosyltransferases, the resulting UDP is hydrolyzed to UMP by UDPase (4). In this way, UDP, which is highly inhibitory to galactosyltransferases (5), does not accumulate in the luminal of the Golgi apparatus. UMP then exits the Golgi lumen by exchange with cytosolic UDP-galactose (for review, see Ref. 6).

A highly specific Saccharomyces cerevisiae Golgi UDPase has been described and purified to homogeneity (7). This enzyme appears to be involved in protein and lipid mannosylation, as the GDP is generated from the mannose donor, GDP-mannose, by mannosyltransferase. Null mutants of yeast GDPase accumulate GDP in the Golgi lumen and decrease mannosylation of proteins and lipids in this compartment (8). These mutant cells also have defects in the transfer of GDP-mannose into the Golgi lumen (9) because GMP exit is required for the entry of GDP-mannose. The gene encoding the yeast GDPase, GDA1 (8), is a member of the E-ATPase protein family (10) since it is similar in amino acid sequence to potato apyrase CD39 (12, 13). The genes encoding mammalian Golgi UDPase and GDPase have not been identified yet. Through a homology search of the GenBank Data Bank, we found a newly identified human partial cDNA (Kiaa0392) that encodes a novel E-ATPase (14). In this study, we show that this new E-ATPase is a Golgi luminal UDPase.

EXPERIMENTAL PROCEDURES

Materials—Nucleoside phosphates and Trichoderma viride amylase were purchased from Sigma. RPMI 1640 medium, fetal bovine serum, penicillin/streptomycin, Dulbecco's modified Eagle's medium, and l-glutamine were purchased from Life Technologies, Inc. Brefeldin A (BFA) was purchased from Molecular Probes, Inc. (Eugene, OR). Anti-Myc monoclonal antibody (9E10) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-pS6 monoclonal antibody that recognizes an epitope located on the 58-kDa Golgi peripheral membrane protein (15) was purchased from Sigma.

Cloning of a Full-length UDPase cDNA—The rapid amplification of 5'-cDNA ends (5'-RACE) method (16) was used to amplify the 5'-end upstream sequence of the human Kiaa0392 cDNA (GenBank accession number AB002390) (14) from a human brain Marathon-Ready cDNA library (CLONTECH, Palo Alto, CA). The sense PCR primer AP1 (5'-CCATCCTAATACGACTCACTATAGGGC) was provided by the manufacturer for amplifying the 5'-end adaptor sequence (5'-CTAATAGCAGTACTATAGGGC) was provided by the manufacturer for amplifying the 5'-end adaptor sequence (5'-CTAATAGCAGTACTATAGGGC) was provided by the manufacturer for amplifying the 5'-end adaptor sequence. The antisense primer AP2 (5'-TTTGCCGATCTGTCGACGAAGATCTCAA) was complementary to nucleotides 308–335 of the human brain Kiaa0392 cDNA (14). The PCR product was subcloned into pGEM3zf (Promega, Madison, WI) with a blunt end at the 5'-end and a XbaI site at the 3'-end (underlined above).

The remaining coding region of Kiaa0392 cDNA was also amplified by PCR. The nucleotide sequence of the sense primer S1 was complementary to that of primer A1. The antisense primer A2 (5'-GGTTCTGAGTGGAGCAGTGCTGGTCGGAGATCTCAAGCTTGGGAGATCTCAA) was complementary to nucleotides 1658–1684 of Kiaa0392 cDNA (14). Full-length cDNA was created by fusing this PCR product with the 5'-RACE PCR product using the XbaI and XhoI restriction sites and was subcloned into GW1-CMV mammalian expression vector (British Biotechnology, Oxford, United Kingdom) using NotI and XhoI sites. DNA was sequenced by the dyeodeoxy chain termination method with a Sequenase kit from U. S. Biochemical Corp.

Two primers were used to PCR-amplify cDNA encoding Myc epitope-tagged UDPase (Myc-UDPase). The sense primer contained a sequence identical to the T7 promoter of pGEM3zf vector. The antisense primer (5'-GGTCTAGAATTACAAGCTCCTCTATGAGTTTGTCCTGTCCTCAAGATCCCCGGGACTTGGTACGAGGATTCTTCTTCTATACTCGAGCTTTCGCTTCTAGGTCCTCCGTGGCATT) contained an XbaI site (underlined at the 5'-end, stop codon, an antisense sequence encoding the Myc epitope

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank Data Bank with accession number(s) AF016032.

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1 The abbreviations used are: BFA, brefeldin A; 5'-RACE, rapid amplification of 5'-cDNA ends; PCR, polymerase chain reaction; ACR, apryrase conserved region; RER, rough endoplasmic reticulum; E-ATPase, ecto-ATPase.
get tct tgg cat att tgg cta tct ccc gta tgg ggg gca cta acc aga gag gag cag aaa att cgg cag tga
tct tct ccc ggc ccc ggc tgg cag cag gac gag gag cag aaa att cgg cag tga
tct tct ccc ggc ccc ggc tgg cag cag gac gag gag cag aaa att cgg cag tga

**FIG. 1.** Nucleotide sequence of human UDPase cDNA together with the deduced amino acid sequence. Nucleotides and amino acid residues are numbered. Before the ATG initiation codon, two stop codons are identified (thick white letters). The four highly conserved apryrase regions (ACR 1–4) are in boldface. The putative membrane-spanning domains determined with the use of the algorithm of Kyte and Doolittle (23) are underlined. The two putative N-linked glycosylation sites are in boldface and underlined.

Human Golgi Luminal UDPase

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(TGQ AGC GGC GCC CGC GCC AGG AGA GAG GCC GGC GCC CTC CAG GAA GGC GCC TGG CCG 60
TGC AGA CCA CTG GTC TGT GCG GCC CCC TCC CGC CCC CCG GAC AAG GAG GCC TGC TCT 120
AAC TTC AGA ACA CCC CAG CAT TTG TTG ATT ATT TGG GAA GGA GCT AAT CCC GAC 180
GCA TTG CCT TTG TCC AGT ATG ggg agg att ggc atc tcc tgt ctt ctt ctt ctc 240
C G R I S L P V C C P R I L N T L 31
A G G CAA ATT GTG ATT AGT GTG CTC GCT GTT GCT GTT GCT GCT GTT TCA CTT TTA TAT TCT 366
F P L D I K R I N S L 51
GTC GTC AAT ATC CCA AAT TAT GGG CTA ACC AGA AGC AAG AAA TAT CAA GAG ATT TAC 426
V S N K Y R N K Y R L T R D K F Q R Y 71
cag gca cgg att gtt acc att gat gtt gct gtt gct gct gct gtt tca ctt tta tat tct tct tct 506
V V D C G S C S H V P V Y C W P R H 111
att ggc aat cca cat gtt tgg gat att acc aga cca acc aga gat tat ggg caa aca acc aag cca 560
N P N P P H D L K R I N S L 71
att ggc aat cca cat gtt tgg gat att acc aga cca acc aga gat tat ggg caa aca acc aag cca 560

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Northern Analysis—Polyadenylated RNA (2 μg/lane) from human cell lines was subjected to electrophoresis on a formaldehyde-containing 1.2% agarose gel and blotted by capillary flow onto a charge-modified nylon membrane (NEN Life Science Products). A human multiple-tissue Northern blot (2 μg of polyadenylated RNA/lane; CLONTECH) was used to determine tissue specificity of UDPase mRNA. The probes were generated from UDPase cDNA by PCR and then labeled with \[^{32}P\]dATP using a random priming kit (Life Technologies, Inc.). Prehybridization, hybridization, and washing of membranes were carried out following the rapid hybridization protocol from CLONTECH. Final washes were at 65 °C in 0.5% sodium saline citrate and 0.1% SDS. The blot was exposed to Biomax Mr film (Eastman Kodak Co.) with intensifying screens at 280 °C for 48 h. A human β-actin cDNA probe was used as a control.

Genomic Southern Analysis—Human genomic DNA (10 μg) was digested with either EcoRI or NdeI, electrophoresed on a 0.8% agarose gel, and transferred to a charge-modified nylon membrane. The membrane blot was then prehybridized and hybridized under high stringency conditions (42 °C, 50% formamide and 5× SSC). For the probe, a 404-base pair DNA fragment (nucleotide sequence 1024–1428) was generated by PCR. The primer sequences were 5'9-ATGGGCGGCGT-GTCGACT and 5'-TATAGGGAAAGTCGGTGGTCTC. This DNA fragment has neither EcoRI nor NdeI restriction sites. PCR amplification of human genomic DNA with these two primers resulted in a 400-base pair product (data not shown), indicating that there is no intron between these two primers. Final washes were at 65 °C in 0.5% sodium saline citrate and 0.1% SDS.

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Cell Culture and Transfection—COS-7 cells were grown in a humidified incubator with 5% CO₂ at 37 °C in Dulbecco’s modified Eagle’s medium supplemented with penicillin, streptomycin, glutamine, and 10% fetal bovine serum. COS-7 cells were transfected with cDNA encoding wild-type or Myc-tagged protein by LipofectAMINE reagent (Life Technologies, Inc.). The cells were harvested for immunoblotting or for enzymatic assay 48 h after transfection.

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(EQKLISEEDL), and a sequence complementary to nucleotides 2017–2134 in Fig. 1. The cDNA fragment was subcloned into GW1 mammalian expression vector using NotI and XbaI sites and then partially sequenced.
Preparation of COS-7 Crude Membranes—Crude membranes of the transfected COS-7 cells were prepared by the method of Coppi and Guidotti (17). Protein concentration was determined by the method of Peterson (18).

Electrophoresis and Immunoblotting—COS-7 cells transfected with control vector or Myc-UDPase cDNA were harvested, solubilized in reducing sample buffer (2% SDS, 10% glycerol, 1% β-mercaptoethanol, and 65 mM Tris-HCl, pH 6.8), and boiled for 5 min prior to loading. Samples were analyzed on 9% SDS-polyacrylamide gel, followed by immunoblotting with anti-Myc monoclonal antibody 9E10. Immunoreactive bands were visualized with horseradish peroxidase-conjugated goat anti-mouse antibody and the Renaissance chemiluminescence reagent (DuPont).

**Measurement of Nucleoside Phosphatase Activity**—To measure nucleoside phosphatase activity, COS-7 crude membranes (12 μg at 0.24 mg/ml) were preincubated for 5 min at 37 °C with 1 mM NaN₃, 0.5 mM Na₃VO₄, and 5 mM CaCl₂ in 45 μl of buffer A (20 mM HEPES/Tris, pH 7.4, 120 mM NaCl, 5 mM KCl, and 0.2 mM EDTA). The nucleoside phosphatase reaction was initiated by addition of 0.1 volume (5 μl) of the same buffer containing nucleoside phosphate substrates, pH 7.0, to give a final concentration of 0.1–1 mM. Nucleoside phosphatase activities were linear for at least 30 min (see Fig. 3C). The divalent cation-stimulated nucleoside phosphatase activity was determined by measuring the inorganic phosphate released as described by Lanzetta et al. (19) or by Ames (20) and by subtracting values obtained with 0.2 mM EDTA alone from those obtained with various concentrations of divalent cation (e.g., CaCl₂, MgCl₂, or MnCl₂) plus chelator. To examine the membrane orientation of the UDPase activity, alamethicin (final concentration of 0.2 mg/ml) or Triton X-100 (final concentration of 0.1% (v/v)) was added to facilitate transmembrane diffusion of UDP.

Intact or lysed COS-7 cells were used to determine whether the active site of this protein is located intracellularly or extracellularly. Transfected COS-7 cells were incubated with 10 mM EDTA to detach them from culture dishes and washed twice with buffer A. The intact cells were resuspended in buffer A with 1 mM NaN₃ and 0.5 mM Na₃VO₄ to a final density of 10⁸ cells/ml. Triton X-100 was added to a final concentration of 0.1% together with protease inhibitors including 1 mM phenylmethylsulfonyl fluoride and 2 μg/ml each aprotinin, chymostatin, pepstatin A, and leupeptin. The cells were allowed to lyse for 30 min on ice. In a separate experiment, cells were disrupted by homogenization with a Dounce homogenizer in the same buffer without Triton X-100. Both intact cells and cell lysates were then preincubated for 5 min at 37 °C with 180 μl of buffer A with 1 mM NaN₃ and 0.5 mM Na₃VO₄, with or without 5 mM CaCl₂. The nucleoside phosphatase reaction was initiated by addition of 0.1 volume (20 μl) of the same buffer containing UDP, pH 7.0, to give a final concentration of 1 mM. At the end of the incubation, cells that had not been homogenized or treated with Triton X-100 were still intact (>90%) as demonstrated by trypan blue exclusion. The calcium-activated UDPase activities were determined by measuring the inorganic phosphate released as described by Ames (20).
membranes were incubated for 5 min at 37 °C with 1 mM NaN₃, 0.5 mM MgCl₂, or MnCl₂, were determined by subtracting values obtained with 0.2 mM EDTA alone from those obtained with various concentrations of divalent cation plus chelator. Values are means ± S.D. of three independent experiments.

Immunofluorescence Staining—COS-7 cells grown on polylysine-coated culture chamber slides (Nunc Inc., Naperville, IL) were transfected at ~40–60% confluence with Myc-UDPase cDNA using the LipofectAMINE method. After exposure of cells to BFA (20 μg/ml) for 30 min at 37 °C, cells were washed twice with phosphate-buffered saline, pH 7.4, containing 1 mM MgCl₂ and 0.1 mM CaCl₂. The immunofluorescence staining was done by modifying the method described by Yoon and Guidotti (21). Cells were fixed with 2% paraformaldehyde, permeabilized with 0.1% Triton X-100, and stained with primary antibody. Subsequent staining was done by modifying the method described by Yoon and Guidotti (21).

RESULTS

DNA Cloning—We cloned the full-length coding sequence of a novel cDNA (Fig. 1) from a human adult brain cDNA library by the 5′-RACE method (16). The protein product of this cDNA is a new E-ATPase protein (14) since it contains apyrase conserved regions (ACR1–4) (Fig. 1) that are present in all known E-ATPase proteins (11). The initiation codon (ATG) at nucleotide sequence 199–201 is the most likely translational initiation site because there are two stop codons in the upstream sequence of the same reading frame (Fig. 1). The deduced polypeptide sequence from this initiation codon contains 610 amino acids. Hydrophobicity analysis using the Kyte and Doolittle algorithm (23) predicts two hydrophobic stretches in the polypeptide (amino acid residues 33–55 and 551–573), both sufficiently long to transverse the membrane (Fig. 1). There are two potential N-glycosylation sites between the two putative transmembrane domains (indicated in boldface and underlined in Fig. 1), suggesting that this protein may be a membrane glycoprotein.

Northern Blot Analysis—To determine whether this new E-ATPase is another ectoapyrase or ecto-ATPase, we examined its mRNA expression pattern in cells with or without ectoapyrase activity. The mRNA is expressed in cells with ectoapyrase activity (human B lymphoblast LG2) and without ectoapyrase activity (human T lymphoma Jurkat cells) (13) as determined by Northern blot analysis (Fig. 2A). We were able to detect at least three mRNA transcripts (3.0, 3.2, and 7.5 kilobases in length) reacting with the same probe (Fig. 2A). Reverse transcription-PCR analysis of human breast cancer MCF-7 cell lines (with ectoapyrase activity) and their multidrug-resistant derivatives, MCF-7ADR cells (ectoapyrase-deficient), indicated that both cells express this mRNA (data not shown). The presence of mRNA in these four cell lines suggests that this new E-ATPase is probably not a new ectoapyrase or ecto-ATPase.

To determine tissue-specific mRNA distribution, we analyzed RNA purified from human tissues by hybridization with a fragment of the full-length cDNA (1667 base pairs) (Fig. 1). mRNA was expressed in all the tissues examined, including heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas (Fig. 2A, lanes 3–10).

Genomic Southern Analysis—To determine the copy number of this new E-ATPase gene, human genomic DNA was digested with EcoRI (Fig. 2B, lane 1) or NdeI (lane 2) and then analyzed by Southern blotting with a cDNA probe (nucleotide sequence 1024–1428) under high stringency hybridization and wash conditions. The sequence of this cDNA probe has neither EcoRI nor NdeI sites and does not contain any intron within the fragment at the level of the genomic DNA (see “Experimental Procedures”). The presence of at least two bands in both digests (Fig. 2B) indicates that there might be two or more copies of this new E-ATPase gene in the human genome.

Functional Analysis—To study the function of this new E-ATPase, a control expression vector or the cDNA encoding a Myc-tagged protein (Myc-UDPase) was transfected into COS-7 cells. Transfected COS-7 cells expressed a ~69-kDa protein recognized with anti-Myc monoclonal antibody 9E10 (Fig. 3A). No protein was detected in the immunoblot of COS-7 cells transfected with control vector.

Expression of Myc-tagged protein significantly increased the
nucleoside phosphatase activity in COS-7 crude membranes. As shown in Fig. 3B, the UDPase activity (99.23 ± 2.46 nmol of Pi/min/mg) is 4.76-fold higher than the UDPase activity (20.85 ± 0.57 nmol of Pi/min/mg) of crude membranes from COS-7 cells transfected with control vector alone. The nucleoside phosphatase activity was measured in the presence of 1 mM azide (inhibitor of F-type ATPase) and 0.5 mM vanadate (inhibitor of P-type ATPase). It is known that activities of E-ATPases are not inhibited by these inhibitors (10).

Comparison of the substrate specificity of nucleoside phosphatase activities in COS-7 crude membranes is illustrated in Fig. 3B. It is important to note that these activities were determined under conditions where the activities were linear with respect to time and substrate concentration (Fig. 3, C and D). The activity was highest with UDP as the substrate; lower activity was obtained with GDP, CDP, and TDP. AMP, ADP, ATP, and UMP were not substrates. UTP, GTP, TTP, and CTP (data not shown) were hydrolyzed, but with rates lower than those of UDP, GDP, TDP, and CDP (Fig. 3B). The cleavage rates of these nucleoside triphosphates may not represent the real activity since hydrolysis of nucleoside triphosphates by other phosphatases would provide products (i.e. nucleoside diphosphates) that would be cleaved by the UDPase. Finally, UDP was a better substrate than GDP or other nucleoside diphosphates (data not shown) in the concentration range from 0.1 to 2.5 mM (Fig. 3C). We concluded that human E-ATPase is a UDPase.

The activity of this human UDPase was stimulated by Ca2+, whereas Mg2+ and Mn2+ had a lower or minimal effect (Fig. 4). The maximum activation was obtained at 5 mM Ca2+. We found that the enzymatic properties of human UDPase are similar to those of the previously reported rat liver cell Golgi luminal UDPase (1). First, the substrate specificities of human UDPase and rat liver Golgi UDPase are almost identical. Second, the activity of Golgi luminal UDPase in rat liver cells is also known to be activated by Ca2+ and not by Mg2+ or Mn2+ (1).

**Cellular Localization**—To study the cellular localization of this human UDPase, we compared the enzymatic activity of intact and disrupted cells to determine whether it is located intracellularly or extracellularly. The rational of the experiment is that if the enzyme is located inside the plasma membranes, the UDPase activity will increase after the cells are disrupted. On the other hand, the UDPase activity will not change if the enzyme is an ectoprotein. In fact, the UDPase activity of Myc-UDPase cDNA-transfected COS-7 cells increased significantly after the cells were treated with 0.1% Triton X-100 (~5-fold) (Fig. 5A) or were homogenized with a Dounce homogenizer (~4-fold) (data not shown). At the end of the incubation, cells not treated with detergent were still intact (>90%), as demonstrated by trypan blue exclusion. These results suggest that human UDPase is located inside the cell. The UDPase activity of COS-7 cells transfected with GW1 control vector increased after these cells were solubilized with 0.1% Triton X-100. Since UDPase is likely present in all mammalian cells, it is not surprising to find that COS-7 cells have endogenous UDPase activities. Interestingly, the UDPase activity of Triton-treated mock-transfected cells was even higher that of intact cells transfected with Myc-UDPase cDNA (Fig. 5A). These results further support the conclusion that UDPase is an intracellular enzyme.

The subcellular distribution of human UDPase was analyzed by immunofluorescence staining. COS-7 cells transfected with Myc-UDPase cDNA were stained with anti-Myc antibody in a

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**Fig. 6. Localization of UDPase protein.** COS-7 cells were transfected with Myc-UDPase cDNA. 48 h post-transfection, cells were treated with or without BFA (20 μg/ml) for 30 min at 37 °C. BFA was used here to induce enzymes of the Golgi stacks to redistribute into the endoplasmic reticulum and to cause the Golgi cisternae to disappear (22) (B and D). Fixed cells were stained either with anti-Myc monoclonal antibody 9E10 (1 μg/ml) (A and B) or with the monoclonal antibody against the 58-kDa Golgi peripheral membrane protein (1:50 dilution) (C and D), followed by fluorescein isothiocyanate-conjugated anti-mouse IgG, and then viewed by fluorescence microscopy (see “Experimental Procedures”).

**Fig. 7. Relatedness within the E-ATPase family.** The horizontal branch lengths are proportional to the mean number of differences per residues along each branch. The relationships were derived from Neighbor-Joining weighted progressive alignments using the CLUSTAL W program (36). The GenBank™ accession numbers for E-ATPases are as follows: rat ecto-apyrase, U81295; human ecto-ATPase, U91510; potato apyrase, U58597; pea NTPase, Z32743; yeast GDPase, U18799; hypothetical protein C33H5.14 of C. elegans, U41007; hypothetical protein R07E4.4 of C. elegans, U39652; hypothetical 71.9-kDa yeast protein, U18778; and NTPases 1 and 3 of T. gondii, U14322 and U14324, respectively. Sequence similarity and E values were obtained by comparing UDPase with other E-ATPases using the Gapped BLAST data base search program (37).
patch close to the nucleus corresponding to the Golgi complex (Fig. 6A). No stain was found in cells transfected with control vector (data not shown). Monoclonal antibodies against the 58-kDa Golgi peripheral membrane protein were used here as a Golgi marker (Fig. 6C) (15). After cells were exposed to BFA, the fluorescence signal of both Myc-UDPase (Fig. 6B) and the 58-kDa Golgi peripheral membrane protein (Fig. 6D) dramatically decreased. BFA has been shown in studies of numerous cell types to induce enzymes of the Golgi stacks to redistribute into the endoplasmic reticulum and to cause the Golgi cisternae to disappear (22). These results suggest that the UDPase protein is located in the Golgi apparatus.

This conclusion is supported by the experiment shown in Fig. 5B, in which the UDPase activity of COS-7 crude membranes increased 2-fold in the presence of Triton X-100 and 3-fold in the presence of alamethicin, an ionophore that facilitates transmembrane diffusion of nucleotides. We conclude that the active site of the UDPase is on the luminal side of Golgi complexes. Similar criteria have been used to localize other Golgi luminal enzymes, including galactosyltransferase and sialyltransferase (24) as well as a Golgi luminal protein kinase (25). We conclude that the human UDPase is a Golgi luminal enzyme.

Relatedness within the E-ATPase Protein Family—Sequence alignment of the E-ATPases are human Golgi UDPase, hypothetical protein C33H5.14 of C. elegans, hypothetical protein R07E4.4 of C. elegans, hypothetical 71.9-kDa yeast protein, yeast GDPase, rat ectoapyrase (CD39), and human ecto-ATPase (CD39L1). All these E-ATPases share the appyrase conserved regions (ACR1–4) and the two other regions (X1 and X2) that are different between UDPase/GDPase and ecto-ATPase/ectoapyrase are underlined.

![Fig. 8. Amino acid sequence alignments of UDPase and other E-ATPases.](http://www.jbc.org/Downloaded from /by guest on October 3, 2019)
other E-ATPases in the dendrogram, suggesting an ancient and independent line of evolution of the *T. gondii* NTPases.

**DISCUSSION**

We have cloned a new human E-ATPase gene, as the protein sequence contains the four apprarse conserved regions (ACR1–4) (11). Expression of the protein in COS-7 cells resulted in an increase in Ca\(^{2+}\)-dependent nucleoside phosphorylase activity. This activity was highest with UDP as substrate and was simulated by divalent cations in the following order: Ca\(^{2+}\) \(\gg\) Mg\(^{2+}\) \(\gg\) Mn\(^{2+}\). By immunofluorescence staining and enhancement of UDP hydrolysis in the presence of alamethicin, the protein was determined to be a Golgi luminal UDPase. Although yeast GDPase is not essential for UDPase. The second E-ATPase gene in yeast protein may have functions similar to that of human UDPase mRNA is present in all human tissues and all four cell lines examined here, suggesting that this UDPase is widely expressed in many human cells. Recently, the gene encoding the *S. cerevisiae* GDP-mannose transporter (Vrg4p) was identified; the lethal phenotype of a null vrg4 mutant suggests that glycosylation of protein and lipid in the Golgi apparatus is essential for viability (30). Therefore, Golgi UDPase might be essential for most, if not all, cells. It will be of interest to know whether there are genetic diseases affecting nucleoside phosphate transport or metabolism in the Golgi apparatus, in a manner analogous to diseases involving cysteine and sialic acid transport into lysosomes.

Genomic Southern analysis under high stringency hybridization conditions indicates that there might be at least two copies of the UDPase gene in the human genome. In fact, the presence of another UDPase activity in the rough endoplasmic reticulum (RER) has been described in rat liver cells. Like the Golgi UDPase, the RER UDPase also preferentially hydrolyzes UDP and GDP. However, these two enzymes are slightly different in at least two respects. First, the RER UDPase (which actually should be named RER GDPase) hydrolyzes nucleotides in the order GDP \(\gg\) UDP. Second, the activity of Golgi UDPase is preferentially activated by Ca\(^{2+}\), whereas the activity of RER UDPase is stimulated by both Ca\(^{2+}\) and Mg\(^{2+}\) (1). Further study is required to know whether the second gene is indeed a RER UDPase. Interestingly, *C. elegans* also has two copies of a UDPase-like gene (Figs. 7 and 8). In the genome of *S. cerevisiae*, there are only two copies of an E-ATPase gene. The hypothetical 71.9-kDa protein of chromosome V (Figs. 7 and 8) also shares high similarity in amino acid sequence with human UDPase. It is likely that the two *C. elegans* proteins and this yeast protein may have functions similar to that of human UDPase. The second E-ATPase gene in *S. cerevisiae* encodes the Golgi GDPase. Although yeast GDPase is not essential for cell viability and growth, null mutants of GDPase have a partial block in O- and N-glycosylation of secreted proteins as well as a dramatic decrease in mannosylinositol phosphorylceramide (8) and a decrease in transport of GDP-mannose into the Golgi lumen (9). Since glycosylation of proteins and lipids in the Golgi apparatus is essential for viability (30), we wonder whether the activity of the hypothetical 71.9-kDa protein may account for the viability of *gda1* null mutants. It will be interesting to know whether the yeast GDPase and the 71.9-kDa protein have different biological functions.

Members of the E-ATPase protein family all share high sequence similarity in four apprarse conserved regions (ACR1–4) (11). ACR1 and ACR4 sequences are similar to actin-hsp70-hexokinase-\(\beta\)-\(\gamma\) phosphate-binding motifs, indicating a possible role in nucleotide binding; however, the structures and functions of these ACRs are not yet known. To understand the catalytic mechanism of E-ATPase, it is important to point out the differences between various E-ATPase enzymes. Ecto-apyrase (CD39) is an ectoenzyme that hydrolyzes nucleoside tri- or diphosphates (13, 31, 32); ecto-ATPase (CD39L1) is also an ectoenzyme, but it preferentially hydrolyzes adenosine triphosphate (28, 33, 34). Ca\(^{2+}\) and Mg\(^{2+}\) have almost the same stimulatory effect on ecto-apyrase (13). Tubule ecto-ATPase is more active with Mg\(^{2+}\) than Ca\(^{2+}\) (35). The activity of human Golgi luminal UDPase is highest with UDP and GDP as substrates and is stimulated by divalent cations in the following order: Ca\(^{2+}\) \(\gg\) Mg\(^{2+}\) \(\gg\) Mn\(^{2+}\) (Fig. 4). Interestingly, amino acid sequence alignments between UDPase and other E-ATPases (Fig. 8) revealed that some regions of these E-ATPases may be important in determining substrate specificity. First, the sequence of the ACR1 region of UDPase/GDPase, DXGS/S/T/G/S/T/XXX, is different from that of ecto-ATPase/ectoapyrase, DXGSSHT/(NS)/XXX, in two amino acid residues (underlined). Since ACR1 is similar to the \(\beta\)-phosphate-binding motif of actin-hsp70-hexokinase, these two amino acid residues may be responsible for distinguishing nucleoside diphosphates from nucleoside triphosphates. Second, at least two other regions, X1 and X2, have significant differences between UDPase/GDPase and ecto-ATPase/ecto-apyrase; however, the functions of these regions are still unknown.

In summary, we have cloned a new human E-ATPase gene and shown that it encodes a Golgi luminal UDPase. This is the first identification of a mammalian Golgi UDPase gene. The wide distribution of UDPase mRNA in human tissues and cells makes it evident that metabolism of nucleoside phosphates in the Golgi lumen is essential for the functions of the Golgi apparatus, e.g. protein and lipid glycosylation. Finally, we have shown that human UDPase is highly related to one yeast and two worm hypothetical proteins.

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