Rat kidney MAP17 induces cotransport of Na-mannose and Na-glucose in *Xenopus laevis* oocytes

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Submitted 16 April 2003; accepted in final form 11 June 2003

Blasco, Tatiana, José J. Aramayona, Ana I. Alcalde, Julia Catalán, Manuel Sarasa, and Victor Sorribas. Rat kidney MAP17 induces cotransport of Na-mannose and Na-glucose in *Xenopus laevis* oocytes. Am J Physiol Renal Physiol 285: F799–F810, 2003.—Renal reabsorption is the main mechanism that controls mannose homeostasis. This takes place through a specific Na-coupled uphill transport system, the molecular identity of which is unknown. We prepared and screened a size-selected rat kidney cortex cDNA library through the expression of mannose transport in *Xenopus laevis* oocytes. We have identified a membrane protein that induces high-affinity and specific Na-dependent transport of D-mannose and D-glucose in *X. laevis* oocytes, most likely through stimulation of the capacity of an endogenous transport system of the oocyte. Sequencing has revealed that the cDNA encodes the counterpart of the human membrane-associated protein MAP17, previously known by its overexpression in renal, colon, lung, and breast carcinomas. We show that MAP17 is a 12.2-kDa nonglycosylated membrane protein that locates to the brush-border plasma membrane and the Golgi apparatus of transfected cells and that it is expressed in the proximal tubules of the kidney cortex and in the spermatids of the seminiferous tubules. It spans twice the cell membrane, with both termini inside the cell, and seems to form homodimers through intracellular Cys23, a residue also involved in transport expression. MAP17 is responsible for mannose transport expression in oocytes by rat kidney cortex mRNA. The induced transport has the functional characteristics of a Na-glucose cotransporter (SGLT), because D-glucose and α-methyl-D-glucopyranoside are also expressed substrates that are inhibited by phloridzin. The corresponding transporter from the proximal tubule remains to be identified, but it is different from the known mammalian SGLT-1, -2, and -3.

The special feature of being able to directly use mannose as an energy source (23). D-Mannose homeostasis seems to be controlled by the reabsorption activity of the kidney, because most of the ultrafiltered mannose is readily reabsorbed in the proximal tubule (25, 27, 29, 39). This important role has been studied for more than 30 years using in vivo and in vitro experiments and different animal species (5, 10, 22, 25, 27, 29, 30, 33, 39). These studies have concluded that the brush-border membrane of the renal tubular cells contains a high-affinity (Km ~0.1 mM) and very specific Na-dependent D-mannose transport system. Stoichiometric determinations have evidenced Na-D-mannose relationships of 1:1 (10, 22) and 2:1 (5), a difference that can be explained by the use of different animal species, as well as experimental and data analysis approaches. With respect to specificity, despite being inhibited by D-glucose, α-methyl-D-glucoside, and phloridzin, the Na-mannose cotransport system seems to be different from the known Na-dependent D-glucose transport system (5, 10, 22, 25, 27, 29, 30, 39). Several groups have also reported a strong inhibition of renal mannose transport by D-fructose, which could be explained by direct competition for the same transport system (5, 22, 25, 27, 29).

The molecular characterization of this renal transport was initiated by our group a few years ago (5). We reported that the size of the rat kidney RNA responsible for D-mannose transport expression in *Xenopus laevis* oocytes was exceptionally small (~1 kb) compared with all other known transporters (2–5 kb). The transport induced by the 1-kb-fraction-enriched mRNA in oocytes was also very small (~100% greater than in water-injected oocytes), but it exhibited kinetic characteristics similar to the transport measured by the renal brush-border membrane vesicles.

To determine the physiological relationship of this small RNA to mannose transport in the kidney, we have identified by expression cloning the cDNA responsible for the Na-coupled D-mannose transport induction in *X. laevis* oocytes. The kinetic behavior is similar to that in the data published for mannose transport in rat kidney and other animal models. The cDNA se-

DIETARY SUPPLY IS THE MAIN SOURCE OF D-mannose in humans. Once absorbed, the fate of mannose depends on the cell type, but it is mainly focused on membrane protein glycosylation. In fact, mutations in enzymes involved in mannose handling for glycosylation and metabolism cause serious syndromes, such as several congenital disorders of glycosylation (1) and α-mannosidosis (3). In addition to glycosylation, sperm has the special feature of being able to directly use mannose as an energy source (23). D-Mannose homeostasis seems to be controlled by the reabsorption activity of the kidney, because most of the ultrafiltered mannose is readily reabsorbed in the proximal tubule (25, 27, 29, 39). This important role has been studied for more than 30 years using in vivo and in vitro experiments and different animal species (5, 10, 22, 25, 27, 29, 30, 33, 39). These studies have concluded that the brush-border membrane of the renal tubular cells contains a high-affinity (Km ~0.1 mM) and very specific Na-dependent D-mannose transport system. Stoichiometric determinations have evidenced Na-D-mannose relationships of 1:1 (10, 22) and 2:1 (5), a difference that can be explained by the use of different animal species, as well as experimental and data analysis approaches. With respect to specificity, despite being inhibited by D-glucose, α-methyl-D-glucoside, and phloridzin, the Na-mannose cotransport system seems to be different from the known Na-dependent D-glucose transport system (5, 10, 22, 25, 27, 29, 30, 39). Several groups have also reported a strong inhibition of renal mannose transport by D-fructose, which could be explained by direct competition for the same transport system (5, 22, 25, 27, 29).

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sequence reveals that it is the rat counterpart to the human 17-kDa membrane-associated protein MAP17/ DD96 (16, 17), whose molecular characteristics are very different from those of all known transporters. MAP17 was first cloned as an mRNA that was over-expressed in most carcinomas, which then led to the identification of type 1 PSD95-Dlg-zona occludens-1 (PDZK1), a PDZ domain-containing globular protein that interacts with the COOH terminus of MAP17 (18).

In this work, we also show evidence of the need for interaction with an additional protein(s) in the oocyte, which is functionally similar to, but different from, the known Na-glucose cotransporters SGLT-1, -2, and -3 (38).

**MATERIALS AND METHODS**

**Preparation, injection, and uptake assays with oocytes.** The general methods for handling *X. laevis* and their oocytes and for transport assays have been described previously (37). Oocytes were injected with different amounts of capped cRNA, using an automatic nanoliter injector (World Precision Instruments, Hertfordshire, UK). In vitro transcriptions were performed with an mMESSAGE mMACHINE kit (Ambion, Austin, TX). Transport assays were performed at room temperature for 30 or 60 min using 5 Ci/ml [35S]methionine (Amersham, Buckinghamshire, UK) at 40–80 µCi/ml as a tracer, according to the cold substrate concentration. For Na-dependent uptake, groups of 10 oocytes were incubated in an uptake medium [in mM] 100 NaCl, 2 KCl, 1 CaCl2, 1 MgCl2, and 10 HEPES-Tris, pH 7.5] containing the cold and radiolabeled substrates. For Na-independent uptake, NaCl was equimolecularly substituted by choline chloride. Before the uptake assay, the oocytes were washed for 2 min in uptake solution without Na at room temperature, and after the specific incubation time the radioactive medium was recovered, and the oocytes were washed four times with 4 ml each of ice-cold washing solution. Except where indicated to the contrary, all data show transport in the presence of NaCl.

**Cloning of MAP17.** Directional cDNA library construction and expression cloning were performed as published (14, 31) using the SuperScript Plasmid System (Invitrogen, Paisley, UK). The first cDNA strand was methylated using 5-methyl-dCTP, transformed into Epicurian Coli XL-Gold ultracompetent cells (Stratagene, La Jolla, CA), and screened at 500 colonies/plate. Sequencing was automatically performed using ABI Prism 310 (PerkinElmer Life Sciences, Boston, MA) and Vector NTI Suite software.

**Culture and transfections of opossum kidney cells.** Cells culture and uptake assays were performed as reported (32). For transient transfections, the cDNAs in pCMV Script (Stratagene) were transfected using Lipofectamine Plus reagent, and 8 h later, 0.5 µg total RNA were electrophoresed in formaldehyde-denaturing agarose gels as explained elsewhere (2) and vacuum-blotted onto nylon membranes (Biodyne, Pall Gelman, Ann Arbor, MI). After hybridization in an ULTRAhyb solution (Ambion) with a 660-bp MAP17-derived 32P-labeled ribo-probe, signals were obtained by exposure to Kodak Biomax MS film at −80°C for 4–8 h.

In situ hybridization histochemistry was done as published elsewhere (34). Five-micrometer cryosections, thaw-mounted onto gelatin-coated slides, were air dried, fixed in paraformaldehyde, and dehydrated in ethanol. Forty-mer antisense oligonucleotides (5′-AGATGGGCTGTGATCCAAAGGAGGTGAAGGCTGCTTGTT) were 3′-labeled with 32P-labeled New England Nuclear (both from Amersham Biosciences). In vitro translation of rat MAP17 was done using rabbit reticulocyte lysate in the absence or presence of canine pancreatic microsomal membranes (both from Promega, Madison, WI). Posttranslation modifications were analyzed by a combination of microsome addition and endoglycosidase H digestion exactly as published (15).

**Northern blot analysis and in situ hybridization.** Total RNA was purified using a QuickPrep Total RNA Extraction kit (Amersham Biosciences). For Northern blot analysis, 20 µg total RNA were electrophoresed in formaldehyde-denaturing agarose gels as explained elsewhere (2) and vacuum-blotted onto nylon membranes (Biodyne, Pall Gelman, Ann Arbor, MI). After hybridization in an ULTRAhyb solution (Ambion) with a 660-bp MAP17-derived 32P-labeled ribo-probe, signals were obtained by exposure to Kodak Biomax MS film at −80°C for 4–8 h.

Mutant constructions. The hemagglutinin antigen (HA; YPYDVPDYA) was introduced in positions 5, 23, and 66 of the MAP17 protein by site-directed mutagenesis using a QuikChange kit (Stratagene) and the following primers (HA-encoding sequences are underlined): HA5, 5′-TTGGCCCTCTACCCATAGGCGTCAAGGTTCAGATCAGTCCGAGTTGCTGCTC; HA23, 5′-GGCTGAACCAATATCCCATAGCGACCTGCCCCAGACTACCGGCGGTGATAGGAAACC; HA66, 5′-GACAGCGATGTACCCATAGGCGTGCTCACTACCGGAGTTAAGCTCAGTG.

For cystein mutants, they were mutated to serines using the following primers (point mutations for Cys conversion are underlined): Cys20 (C20S), CGGAGATGGCAGCCTGGCAGTCGCA; and Cys25 (C55S), GCTCTTCTGATCTCTAAGAGATGGCTTGAAGATGGTGTCAG.
mounting media (Merck, Darmstadt, Germany). Cells were visualized by epifluorescence with either an Olympus BX60 or a confocal Carl Zeiss LSM 310 microscope. Golgi staining and colocalization were performed using the ceramide analog BODIPY-TR (Molecular Probes, Eugene, OR) following the manufacturer’s guidelines. In short, BODIPY-TR (1 mM) was dissolved in ethanol and incubated with the fixed cells as BSA-complexes, for 10 min at 5 μM, after a washing of the secondary antibody. When necessary, the cells were incubated for 30 min at 37°C with 10 μg/ml brefeldin A (Molecular Probes) before fixation, from a stock of 5 mg/ml in ethanol.

Hybrid depletion in oocytes. Hybrid depletion experiments were performed as described previously (31) using six oligodeoxynucleotides derived from the rat MAP17 sequence as follows: sense oligos: 105–124 ATGTTGGCCCT-CAGTCTGCT (S105), 251–270 CGTCAACCATTCTGTCGGC (S251), and antisense oligonucleotides: 105–124 AGCA-GACTGAGGGCCACAT (AS124), 335–352 TATCTGCACTTATGGCC (AS335), and 430–449 TCATATGTTGTGTCGAGGA (AS430). Rat kidney cortex (poly)A/H11001 TCCATGCCC (AS335), and 430–taining (in g/l) 8 NaCl, 1.15 Na2HPO4, 0.2 KCl, 0.2 KH2PO4, 0.132 CaCl2 were washed once in PBS2, the oocytes were lysed by vortexing for 20 s in 20 μl/oocyte of a nondenaturing homogenization buffer (120 mM NaCl, 50 mM Tris-HCl, pH 8, and 0.5% Nonidet P-40) supplemented with protease inhibitors (CompleteMini; Roche Diagnostics, Mannheim, Germany) and incubated briefly on ice. The lysates were then centrifuged at 16,000 g for 10 min and 4°C, and the supernatants were stored at −80°C. Incorporation radioactivity was determined by scintillation counting of trichloroacetic acid precipitates from aliquots.

Immunoprecipitation was performed with polyclonal anti-HA antibody (Clontech) bound to protein G plus/protein A-agarose (Calbiochem); 10 μl of 0.1 μg/μl anti-HA were coupled to 30 μl protein G/A-agarose slurry/group of oocytes by slow rotation for 2 h at 4°C. Equal amounts (2 × 10^6 cpm/μl) of precleared lysates were mixed with the coupled beads and shaken slowly overnight at 4°C. The beads were washed four times in buffer containing 100 mM NaCl, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 0.5% Nonidet P-40, and 500 mM LiCl and four times in the same buffer without LiCl. The beads were resuspended in an SDS-PAGE loading buffer and heated at 65°C for 15 min. When necessary, β-mercaptoethanol (5% final) was added to the samples. After electrophoresis in 15% SDS-polyacrylamide, the gels were fixed in 3% glycerol, 10% acetic acid, and 20% methanol for 30 min, then treated with Amplify (Amersham) for 15 min, dried, and exposed to Eastman Kodak Biomax films for 4 days at −80°C.

RT-PCR. The cloning of an 1,808-bp fragment of rat PDZK1, thereby encoding the full open reading frame, was done by two-step RT-PCR using the Superscript Preamplification System and Platinum Taq DNA Polymerase High Fidelity (both from Invitrogen). The following primers were designed from the GenBank sequence: sense 5'-TTCTCAGAATGAGGCGATT-3' and antisense 5'-CAGCTAAGCTTTCATTCAAAT-3'. The product was cloned into PCR-Script (Stratagene), and the capped cDNA was tailing kit (PolyA) (Ambion).

Biochemical procedures. Treatment with p-chloromercuribenzoate (pCMB) was done as published elsewhere (11). Oocytes were incubated for 5 min in uptake medium (without substrates) containing 1 mM pCMB, rinsed, and then incubated 5 min in the same medium with or without 5 mM β-mercaptoethanol. Before substrate uptake, the oocytes were again rinsed three times. The methanethiosulfonate (MTS) (Toronto Research Chemicals, Downsview, Ontario, Canada) [2-(trimethylammonium)ethyl] methanethiosulfonate (MTSET) and 2-aminoethyl methanethiosulfonate (MTSET) were dissolved in DMSO and used at 1 or 5 mM for 5 or 10 min of preincubation with the oocytes.

Coimmunoprecipitation. Groups of 30 oocytes were injected with 10 ng HA23-MAP17 cRNA, alone or in combination with 20 ng of rat kidney cortex poly(A)+ RNA, and metabolically labeled, as explained above. For cross-linking assays, groups of oocytes were washed twice in PBS2+ containing (in g/l) 8 NaCl, 1.15 Na2HPO4, 0.2 KCl, 0.2 KH2PO4, 0.132 CaCl2 × 2H2O, and 0.1 MgCl2 × 6H2O and incubated in 3 ml glycerol buffer (10% glycerol, 0.1% saponin, 1 mM orthovanadate, in PBS2+) for 20 min at 4°C. Oocytes were then washed once in ice-cold glycerol buffer and incubated with 2 ml cross-linker buffer (0.25 M sucrose, 1 mM orthovanadate in PBS2+) containing the cleavable cross-linker 3,3'-dithio-bis(sulfo succinimidyl propionate) (DTSSP; Pierce, Rockford, IL). This was added from a freshly made 100× stock solution in DMSO to a final concentration at 1 mM. Cross-linking was allowed for 2 h at 4°C. Then, the oocytes were washed once in PBS2+, and a buffer containing 100 mM glycine in PBS2+ was added to stop the reaction. After a final wash in PBS2+, the oocytes were lysed by vortexing for 20 s in 20 μl/oocyte of a non-denaturing homogenization buffer (120 mM NaCl, 50 mM Tris·HCl, pH 8, and 0.5% Nonidet P-40) supplemented with protease inhibitors (Complete-Mini; Roche Diagnostics, Mannheim, Germany) and incubated briefly on ice. The lysates were then centrifuged at 16,000 g for 10 min and 4°C, and the supernatants were stored at −80°C. Incorporated radioactivity was determined by scintillation counting of trichloroacetic acid precipitates from aliquots.

RESULTS

Expression cloning of MAP17. A cDNA library was prepared from size-selected rat kidney cortex poly(A)+ RNA (1 kb as the mean) according to our previous results (5), and it was screened by expression cloning in X. laevis oocytes. A single cDNA clone of 0.8 kb was obtained out of 40,000 colonies, which induced about 150% Na-coupled D-mannose transport above the endogenous, water-injected level of the cell when assayed at 0.1 mM D-mannose for 1 h (Fig. 1A). Dose-response and expression-time course experiments revealed that the maximal transport rate was already obtained using 0.5 ng cRNA/oocyte after 1 day of expression time and that the uptake was linear for at least 4 h (see Fig. 1A; other data not shown). Despite the low level of expres-
tion that prevented an accurate kinetic characterization, we found that MAP17 induces high-affinity Na-coupled D-mannose transport (Fig. 1B), with a Hill coefficient $>2$ for Na activation (Fig. 1C), which is in agreement with our previous results (5). The fit analysis is summarized in Table 1, whereby we conclude that the effect of MAP17 most likely consists of an increase in the capacity of the endogenous uphill transport system of the oocyte.

Table 1. Kinetic analysis of MAP17-induced transport in Xenopus laevis oocytes

<table>
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<tr>
<th></th>
<th>$V_{\text{max}}$ pmol-oocyte $^{-1}$ h$^{-1}$</th>
<th>$K_m$ pmol</th>
<th>$K_d$ pmol</th>
<th>DF, r</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>D-Mannose</td>
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<td></td>
<td>Saturation kinetics</td>
<td>$V_{\text{max}}$</td>
<td>$K_m$</td>
<td>$K_d$</td>
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<tr>
<td>H$_2$O</td>
<td>1.00±0.47</td>
<td>0.12±0.06</td>
<td>7.25±0.46</td>
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<td>MAP17</td>
<td>1.62±0.53</td>
<td>0.06±0.03</td>
<td>9.64±0.69</td>
<td>55, 0.6728</td>
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<td>Net</td>
<td>1.56±1.00</td>
<td>0.23±0.10</td>
<td>—</td>
<td>5, 0.9742</td>
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<tr>
<td>H$_2$O</td>
<td>Na activation</td>
<td>$V_{\text{max}}$</td>
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<tr>
<td>H$_2$O</td>
<td>1.25±0.08</td>
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<td>MAP17</td>
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<td>18.52±2.00</td>
<td>1.98±0.41</td>
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<td>Net</td>
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<td>16.35±3.95</td>
<td>2.32±0.84</td>
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<tr>
<td>H$_2$O</td>
<td>D-Glucose</td>
<td>$V_{\text{max}}$</td>
<td>$K_m$</td>
<td>$n$</td>
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<tr>
<td>H$_2$O</td>
<td>3.59±0.58</td>
<td>0.88±0.15</td>
<td>0.71±0.06</td>
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<td>MAP17</td>
<td>7.34±1.45</td>
<td>1.01±0.22</td>
<td>0.92±0.13</td>
<td>63, 0.9543</td>
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<tr>
<td>Net</td>
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<td>1.34±0.33</td>
<td>—</td>
<td>5, 0.9761</td>
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<tr>
<td>H$_2$O</td>
<td>Na activation</td>
<td>$V_{\text{max}}$</td>
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<tr>
<td>H$_2$O</td>
<td>0.24±0.10</td>
<td>43.72±35.12</td>
<td>1.31±0.73</td>
<td>60, 0.5071</td>
</tr>
<tr>
<td>MAP17</td>
<td>0.46±0.10</td>
<td>25.45±11.51</td>
<td>1.33±0.59</td>
<td>63, 0.6067</td>
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<tr>
<td>Net</td>
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<td>17.53±9.30</td>
<td>1.56±1.37</td>
<td>5, 0.8297</td>
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The specificity of the induced transport was assayed in two ways. First, the uptake of 0.05 mM D-mannose was completely inhibited only by D-mannose, D-glucose, and phloridzin (Fig. 2A). However, no significant effects were observed with sugars such as L-mannose, D-fructose, phloretin, the α-mannosidase inhibitor 1-deoxymannojirimycin (DMM), and the amino acids and ions indicated in Fig. 2A. Second, according to the inhibition results, a direct measure of the uptake of several radioactive sugars showed that 0.5 mM D-$[^{14}$C]$\text{glucose}$ and α-methyl-D-$[^{14}$C]$\text{glucopyranoside}$ were transported similarly to D-mannose by MAP17-expressing oocytes (Fig. 2B). However, 0.5 mM D-$[^{14}$C]$\text{galactose}$, 3-O-methyl-D-$[^{14}$C]$\text{glucopyranoside}$, and D-$[^{14}$C]$\text{fructose}$ were excluded. Therefore, a kinetic characterization of expressed glucose transport was also performed (Table 1), thereby obtaining a reduced affinity for glucose compared with mannose (Fig. 2C) and a similar stoichiometry (Fig. 2D). As with mannose, the effect of MAP17 was mainly on the $V_{\text{max}}$ of glucose transport. Nevertheless, these results should be considered as an approximation due to the low level of transport expression and therefore the general error of the fits (Table 1).

Sequencing and secondary structure. Two-direction sequencing revealed an 816-bp cDNA that encodes an open reading frame of 114 amino acid proteins and 12,243 Da (Fig. 3). This was confirmed by SDS-PAGE of in vivo (Fig. 4A) and in vitro (Fig. 4B) translated cRNA. In addition, a thiol-dependent dimerization seems to occur according to the 24-kDa signal observed under nonreducing conditions (Fig. 4B). A comparison of the nucleotide and amino acid sequences via BLAST found 82% identity with the human MAP17/DD96 (16, 17; GenBank accession NM_005764), an orphan protein selectively up-regulated in human carcinomas. A wide variety of weak identities was also found with several ATP-synthases, ATPases, Na-solute symporters, and transferases. The rat MAP17 cDNA was communicated to GenBank with accession no. AF402772.

Hydropathy analysis (20) confirmed that rat MAP17 is an integral membrane protein with several possible models. These include one membrane-spanning do-
main (SAPS and PredictProtein-PHD servers) that, in addition, could contain an NH$_2$-terminal signal peptide (SOSUIsignal, TMHMM, and SignalP), or two membrane-spanning domains without signal sequence (DAS, SosuiTM, and TMpred), a prediction problem that is common in membrane proteins with hydrophobic NH$_2$ ends. In vitro translation using rabbit reticulocyte lysates in the presence of canine microsomes (Fig. 4B, lanes 2 and 5) showed no evidence of a signal sequence, as no change in mobility was observed compared with the absence of microsomes (lanes 1 and 4). The number of transmembrane domains was also directly determined by tagging MAP17 with HA in positions 5, 23, and 66 (see positions in Fig. 3). Given that all three mutants were fully functional in oocytes, they were transiently expressed in OK cells, and the proteins were immunodetected using an anti-HA primary and FITC-conjugated secondary antibodies, with or without saponin (Fig. 4C). Only HA23-expressing cells were immunodecorated without permeabilization of the membrane, therefore suggesting that this part of the protein is located extracellularly and that MAP17 contains two transmembrane domains, with both NH$_2$ and COOH termini inside the cell. In addition, MAP17 is nonglycosylated (NetOGlyc 2.0 and the lack of an effect by endoglycosidase H, Fig. 4B, lanes 3 and 6) and contains several potential phosphorylation sites in serines, threonine, and tyrosine (Fig. 3). Additional searches found an anion-exchangers family 1 alignment at residues 56–103 (ProfiScan) and a phosphomannomutase/phosphoglucomutase block at 73–82 (Fig. 3). The PDZ-binding site at the COOH terminus (amino acids 111–114) for interaction with the globular protein PDZK1/diphor1 (9, 18) is also shown underlined in Fig. 3.

**Tissue and cell distribution of MAP17.** Northern blot analysis showed that, as expected, MAP17 mRNA is very abundant in the rat kidney cortex, but also in the testis, and less so in the urinary bladder (Fig. 5A). We did not find expression in the duodenum, jejunum, ileum, colon, liver, spleen, lung, heart, brain cortex, brain stem, cerebellum, skeletal muscle, or adipose tissue. We also assayed several renal cell line RNAs, obtaining hybridization signals in Madin-Darby canine kidney (dog), LLC-PK1 (pig), and MCT (mouse) cells; OK cells do not seem to express MAP17. Further localization studies were performed by in situ hybridization. In the kidney, the expression was...
similar to the human MAP17, that is, restricted to the proximal tubules, but from both superficial and deep nephrons (Fig. 5B). In the testis, MAP17 cRNA was expressed in seminiferous tubules (Fig. 5C), and Nomarski microscopy revealed a precise location in the spermatids (Fig. 5D).

A more detailed (subcellular) analysis of the epithelial expression of MAP17 was made by confocal laser immunofluorescence in OK cells permanently transfected with HA23-tagged MAP17. Figure 6 shows that, in the absence of saponin (A in figure; no permeabilization), MAP17 is located at the apical-most end of the cell membrane. Permeabilization with saponin, however, evidenced an additional immunodecoration in the cytoplasm, reminiscent of Golgi network staining, and a subtle staining of the nuclear membrane (Fig. 6B).

Double staining of the cells with the Golgi marker BODIPY-TR showed that MAP17 colocalizes with a specific subset of the Golgi apparatus (Fig. 7, A–F). Finally, this specific staining was also dispersed after treatment with the Golgi toxin brefeldin A (Fig. 7G).

**Structure-function relationship.** Pretreatment of MAP17-expressing oocytes for 10 min with 1 mM of thiophilic pCMB inhibited the net Na-D-mannose cotransport by 60% (Fig. 8A). Treatment with thiol-oxidating methylthiosulfonates MTSEA or MTSET showed no effect at either 1 or 5 mM and for 5, 10, or 30 min, most likely because the reagents were not accessible to the cysteins (Fig. 8A). Next, we mutated the two cysteins, Cys20 (extracellular) and Cys 55 (intracellular), of HA23-tagged MAP17 (Fig. 8B) to check whether they were involved in the pCMB effect. We found that only Cys55 and the double mutant Cys-less exhibited reduced mannose transport, similar to the pCMB effect.

Moreover, Western blot analysis showed that these mutants also could not form homodimers (Fig. 8C). This therefore suggests a relationship between the quaternary conformation of MAP17 and transport induction, given that the abundance (measured by densitometry) of the homodimer conformation signal in these experiments was only ~20% that of the monomer band, but it was responsible for 60% of the induced transport.

**Hybrid depletion of MAP17.** To determine the role of MAP17 in mannose transport expressed in *X. laevis* oocytes by total kidney cortex mRNA, we performed hybrid depletion with three sense and three antisense 20-mer oligonucleotides. The small mRNA-induced Na-D-mannose cotransport in oocytes was abolished by the three antisense oligos (all located inside the open reading frame, at either the 5'- and 3'-ends or the center),
with no effect provoked by the sense oligos (Fig. 9).

Therefore, MAP17 is responsible for mannose transport expressed by rat kidney cortex mRNA in oocytes.

Evidence for the involvement of additional proteins. The small size of MAP17 and the low expression level of sugar transport induced in oocytes make it unlikely that this protein by itself represents a hexose transport system. Indirect evidence for the participation of additional proteins in the oocyte expression system arose from dose-response experiments of HA23-tagged MAP17 expression. Figure 10A shows that transport saturation appeared after 0.01 ng of MAP17 cRNA/oocyte. This could be explained, for example, by a saturation of the translation and/or by processing of the protein. However, a quantification of the synthesized protein by Western blotting and densitometric analysis showed that the protein plateau started after 0.1 ng/oocyte, in either the 12- or 24-kDa band (Fig. 10, B and C). As MAP17 does not have extracellular free amino groups, biotinylation of MAP17 to exclusively determine the protein inserted into the plasma membrane could not be performed. One possible interpretation of these results is that the expression of mannose transport in oocytes by MAP17 is restricted by the need for one or more endogenous proteins to be present in a limited amount, and therefore the increasing amounts of MAP17 would saturate the activity.

We then assayed a direct approach to find possible collaboration between MAP17 and the characterized accumulative glucose transporters. The fact that MAP17, in addition to mannose, induces Na-D-glucose and Na-α-methyl-D-glucopyranoside cotransport (Fig. 2, B and C) and that it is inhibited by phloridzin (Fig.

Fig. 4. Molecular analysis of MAP17. A: 35S metabolic labeling of Xenopus laevis oocytes injected with water or MAP17 cRNA and electrophoresis in reducing conditions, showing the predicted 12.2-kDa molecular size (arrow). B: in vitro translation of MAP17 with reticulocyte lysates (lanes 1 and 4), in the presence of microsomes (lanes 2 and 5), and digested with Endo H (lanes 3 and 6). Lanes 4–6 contain 100 mM DTT. C: opossum kidney (OK) cells transiently transfected with the MAP17 cDNA tagged in positions 5, 23, and 66 were immunolabeled with anti-HA antibody + saponin. Without detergent, the antibody only finds the corresponding antigen in the HA23 mutant, thereby confirming the 2-membrane hydropathy model.

Fig. 5. Analysis of MAP17 RNA expression in rat tissues and cell lines. A: Northern blot analysis showing the hybridization signal in superficial renal cortex (SC), juxtamedullar cortex (JM), urine bladder (BL), and testis (TS). No expression was found in the duodenum (DU), jejunum (JE), ileum (IL), colon (CO), spleen (SP), brain cortex (BC), brain stem (BS), cerebellum (CE), lung (LU), heart (HE), liver (LI), skeletal muscle (SM), or fat (FT). The hybridization signal was also found in Madin-Darby canine kidney, LLC-PK1, and MCT, but not in the OK cell line. Top and bottom: gel photomontage of the corresponding 18S ribosomal bands to the scale of the corresponding blot, respectively. B: darkfield view of MAP17 expression in the superficial and juxtamedullar cortex of the kidney, labeling the proximal tubules of the outer and deep nephrons. C: darkfield view of the seminiferous tubule walls of the testis labeled with a MAP17-derived probe. D: further analysis with Nomarski imaging, which revealed that the expression is exclusive of the spermatids.

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2A) indicates that a member of the SGLT family could be interacting or being modified by MAP17. As the SGLT-1 substrates D-galactose and 3-O-methyl-D-[14C]glucopyranose were not transported (Fig. 2B), SGLT-1 should be excluded (38). Despite this, we directly tested all three SGLT members characterized to date, namely, rat SGLT-1 and SGLT-2 and pig SGLT-3, expecting that if one of them were interacting with MAP17 in the kidney to transport D-mannose, then coexpression of both in the oocyte would further increase the uptake shown by MAP17 alone. The corresponding cRNAs were injected separately or coinjected with either MAP17 by itself or in combination with MAP17 plus PDZK1 cRNAs (Fig. 11A). The globular, PDZ-containing protein PDZK1 was assayed, given that it was the first protein reported to interact with MAP17. As a result, all SGLT cRNAs induced Na-D-glucose cotransport at the expected intensities (38). However, none of them was able to induce significant net Na-D-mannose cotransport, either alone or in different combinations with MAP17 and/or PDZK1. Subsequently, we similarly coinjected MAP17 and total rat kidney cortex mRNA into X. laevis oocytes. As Fig. 11B shows, again poly(A)+ RNA and MAP17 induced a similar level of Na-mannose cotransport, and the coinjection of both elicited a slight but significant stimulation above the mRNA injection level.

Finally, several coimmunoprecipitations were performed in oocytes expressing either HA23-MAP17 alone or in combination with rat kidney cortex mRNA. In the absence of the cross-linker DTSSP (see MATERIALS AND METHODS), 2A) indicates that a member of the SGLT family could be interacting or being modified by MAP17. As the SGLT-1 substrates D-galactose and 3-O-methyl-D-[14C]glucopyranose were not transported (Fig. 2B), SGLT-1 should be excluded (38). Despite this, we directly tested all three SGLT members characterized to date, namely, rat SGLT-1 and SGLT-2 and pig SGLT-3, expecting that if one of them were interacting with MAP17 in the kidney to transport D-mannose, then coexpression of both in the oocyte would further increase the uptake shown by MAP17 alone. The corresponding cRNAs were injected separately or coinjected with either MAP17 by itself or in combination with MAP17 plus PDZK1 cRNAs (Fig. 11A). The globular, PDZ-containing protein PDZK1 was assayed, given that it was the first protein reported to interact with MAP17. As a result, all SGLT cRNAs induced Na-D-glucose cotransport at the expected intensities (38). However, none of them was able to induce significant net Na-D-mannose cotransport, either alone or in different combinations with MAP17 and/or PDZK1. Subsequently, we similarly coinjected MAP17 and total rat kidney cortex mRNA into X. laevis oocytes. As Fig. 11B shows, again poly(A)+ RNA and MAP17 induced a similar level of Na-mannose cotransport, and the coinjection of both elicited a slight but significant stimulation above the mRNA injection level.

Finally, several coimmunoprecipitations were performed in oocytes expressing either HA23-MAP17 alone or in combination with rat kidney cortex mRNA. In the absence of the cross-linker DTSSP (see MATERIALS AND METHODS),
AND METHODS), only the 12- and 24-kDa bands were precipitated (Fig. 12, arrows). However, with DTSSP additional proteins of 15, 19, and 28 kDa (arrowheads) were pulled. Of these, the 15- and 19-kDa bands represent oocyte proteins, because they were already precipitated by MAP17 injection alone. The 28-kDa band seems to represent a renal protein because it appeared exclusively in the MAP17/mRNA oocyte extracts. All other proteins were also precipitated in the water-injected oocytes.

DISCUSSION

By expression cloning, we have identified MAP17 as a cDNA that induces Na-activated transport of D-glucose and D-mannose when expressed in X. laevis oocytes (Fig. 1A). Furthermore, we have shown that MAP17 is necessary for rat kidney cortex (poly)A+ RNA to induce mannose transport in oocytes (Fig. 9). Nevertheless, the question of whether MAP17 is directly involved in the renal handling of D-mannose is not yet clear. Both the structural simplicity of MAP17 (see below) and kinetic analysis of the induced transport suggest that MAP17 is not solely responsible for the expressed transport, but rather it is acting as an activator of the capacity of an oocyte's endogenous transporter (Table 1). The expressed transport fits the general kinetic characteristics observed in both in vitro and in vivo renal mannose uptake assays: Na-dependent mannose transport of high affinity and low capacity (5, 10, 22, 27, 29, 30; Fig. 1, B and C) and strong inhibition by the classic substrates of the SGLT family of transporters (38), D-glucose, α-methyl-D-glucopyranoside, and phloridzin (5, 10, 22, 39; Fig. 2A). The main difference in the mammalian system arises from

![Fig. 8. Role of cysteins on function and structure. A: induced mannose transport (filled bars) is inhibited by the mercurial p-chloromercuribenzoate (pCMB), and its effect is reversed by β-mercaptoethanol (βM). 2-(N,N-Trimethylammonium)ethy1 methanethiosulfonate (MTSET) and 2-aminoethyl methanethiosulfonate (MTSEA) have no effect in either water (open bars)- or cRNA-injected oocytes. B: only the mutation of Cys55 inhibited the induced transport. *Significantly different from wild MAP17-induced transport (P < 0.05). C: Cys55 mutants are not able to show the 24-kDa band under nonreducing conditions.](http://ajprenal.physiology.org/)

![Fig. 9. Hybrid depletion experiments on mRNA-induced Na-D-mannose cotransport in oocytes. Oocytes were injected with water or rat kidney cortex mRNA (30 ng/oocyte), alone or annealed with rat MAP17-derived 20-mer oligonucleotides. Six oligos (within the open reading frame) were used: sense oligos S105, S251, and S407; and antisense oligos AS105, AS335, and AS430 (see MATERIALS AND METHODS). D-Mannose transport was measured 5 days after injection. *Significant compared with MAP17-induced transport (P < 0.05).](http://ajprenal.physiology.org/)

![Fig. 10. Dose-response relationships. Increasing amounts of HA23-MAP17 cRNA were injected into oocytes to compare mannose transport induction (A) vs. membrane protein expression, shown as a Western blot ± DTT (B), and densitometries of the signals in reducing conditions (C). Whereas transport saturation started with 0.01 ng/oocyte, the protein plateau started after 0.1 ng/oocyte, in either the 12- or 24-kDa band.](http://ajprenal.physiology.org/)
the specificity of the transport, given that most of the previous studies indicate that, even if D-glucose inhibits D-mannose transport, both sugars do not share the same route. This conclusion was deduced not only from the type of inhibition (22, 30) but also from the fact that D-mannose was not able to successfully inhibit D-glucose transport (22, 39). However, we have shown that MAP17 also induces Na-coupled D-glucose transport in *X. laevis* oocytes (Fig. 2, B–D), a result that could still be in agreement with our data if, as we postulate, MAP17 is interacting/activating an SGLT-related transporter other than SGLT-1, -2, or -3. In this event, D-mannose would not completely inhibit the transport of D-glucose, because additional SGLT members that do not collaborate with MAP17 are present along the nephron (38). In any case, it would not be surprising if the putative SGLT-like protein of the *X. laevis* oocyte had some functional differences with respect to its mammalian kidney counterpart in accordance with their evolutionary distance.

MAP17 was previously cloned in a search for mRNAs upregulated in renal carcinomas, but it also turned out

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**Fig. 11.** Coexpression in *X. laevis* oocytes. A: MAP17, the SGLT-1, -2, and -3 carrier cRNAs, and PDZK1 cRNA were coexpressed as indicated to induce D-glucose (filled bars) and mannose (open bars) transport. Although all SGLTs expressed Na-glucose cotransport at the expected intensities, only MAP17 was able to induce D-mannose transport above the level in water-injected oocytes 2 days postinjection. *Significantly different mannose uptake vs. water (P < 0.05).* B: Oocytes were injected with 20 ng of rat kidney cortex messenger RNA, 1 ng of MAP17 cRNA, or a combination of both. Uptake was measured 5 days after the injection. *Significantly different from water-injected oocytes (P < 0.05).* #Significantly different from mRNA-expressing cells.

**Fig. 12.** Coimmunoprecipitation of MAP17. Oocytes were metabolically labeled and injected with either water, HA23-MAP17 cRNA, or a combination of HA23-MAP17 and rat kidney cortex poly(A) RNA. Before the lysis, some oocytes were treated with the cross-linker 3,3'-dithio-bis(sulfosuccinimidyl propionate) (DTSSP), and then MAP17 was immunoprecipitated with anti-HA in nondenaturing conditions. In the absence of DTSSP (A), only the 12- and 24-kDa bands were seen (arrows). However, with DTSSP (B), HA antibody additionally pulled bands of 15, 19, and 28 kDa (arrowheads), most of which disappeared with β-mercaptoethanol. The 28-kDa band is only seen in the mRNA-injected oocytes.
to be overexpressed in most colon, lung, and breast carcinomas (16, 17). Now, we have also shown a physiological expression in the testis, more precisely in the spermatids (Fig. 5). Initial hydrophatic analysis suggested the existence of a signal peptide and a transmembrane domain, because the software tools for molecular analysis cannot differentiate between signaling peptides and transmembrane domains when the amino acid sequence starts with a hydrophobic NH$_2$ terminus. However, we have now shown by mutation analysis that the predicted signal peptide is actually a first transmembrane domain, and therefore that MAP17 spans twice the brush border of the tubular cells (Fig. 4C). In addition, we have also shown that MAP17 seems to form homodimers of 24 kDa (e.g., Fig. 4B), most likely through a disulfide bond between intracellular Cys$^{55}$ residues (Fig. 8C). The presence of Cys$^{55}$ also seems to be necessary for the complete expression of mannose transport by MAP17 (Fig. 4B), which indicates that either the homodimer conformation is implicated in the transport or, alternatively, Cys$^{55}$ participates directly in the activation.

To understand the function of MAP17, human PDZK1 was initially identified as a globular protein containing four PDZ domains, the protein of which encoded the heavy chain of a heteromultimeric immunoglobulin (10). The presence of four PDZ domains, followed by a single cytosolic region consisting of the last 48 amino acids. Second, we have inserted the 9 amino acid-HA tags in 3 different positions (MAP17 is only 114 amino acids long), and, surprisingly, all the resultant constructs are still able to induce the same mannose and glucose transport as the native protein. It is therefore evident that the structural requirements of MAP17 to induce its effects are not as sophisticated as they seem to be for the real transporters, even in a homodimer conformation. On the other hand, the binding of membrane proteins to different PDZ proteins seems to be a new mechanism of regulation, given that it is the CFTR chloride channel that binds either to PDZK1, which remains in the plasma membrane, or to CFTR-associated ligand, which continues to accumulate in the Golgi (7). Because MAP17 is expressed in both the plasma membrane and the Golgi, its expression could also be regulated in a similar way.

In summary, to our knowledge we are the first group to report a functional role of MAP17 in the cell, i.e., the induction of uphill transport of mannose and glucose in oocytes, most likely through the interaction or modulation of at least one other protein. We have also clarified its molecular structure and its location in the cell and tissues. Identification of all proteins interacting with MAP17 will help to understand the complete function of MAP17 in the kidney.

We are indebted to M. A. Hediger (Harvard Institutes of Medicine, Boston, MA) for the kind gifts of rat SGLT-1 and -2 cDNA and E. M. Wright (UCLA School of Medicine, Los Angeles, CA) for providing pig SGLT-3 and useful comments on the manuscript. We also thank J. Biber for critiquing the manuscript and making helpful suggestions and H. Murer for supplying Madin-Darby canine kidney and LLC-PK1 cells (Institut of Physiology, Univ. of Zurich). Finally, we are grateful to M. Levi (Univ. of Colorado Health Sciences Center, Denver, CO) for providing MCT cells.

DISCLOSURES

This work was supported in part by two grants to V. Sorribas, one from the Government of Aragon (CONSID-P078/99) and the other from the Spanish Ministry of Science and Technology (MCYT-BIO2000/1608). T. Blasco was supported by a fellowship from the Government of Aragon (CONSID B197/98).
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