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

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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 ultrafiltrated 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-

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quence 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 D-[3H(2,6)]mannose (Am-bion, Austin, TX). Additional methods for handling the hybrid were performed as published (31). After SDS-PAGE, the gel was purified 1:50, and incubated for 1 h. Mowiol 4-88 (Calbiochem, San Diego, CA) was used as an antifading agent in the immunofluorescence and confocal imaging. Crude membrane preparations were obtained from the oocytes expressing the HA-tagged MAP17 as published (31). Western blot analysis was performed using a rabbit polyclonal anti-HA antibody (Clontech, Palo Alto, CA), a goat anti-rabbit IgG secondary antibody conjugated to horseradish peroxidase (Chemicon, Temecula, CA), and enhanced chemiluminescence detection (ECL Plus, Amersham Biosciences). Opossum kidney (OK) cells expressing HA-tagged MAP17 were used as a control, and were fixed in 4% paraformaldehyde, and dehydrated in ethanol. Forty-mer antisense oligonucleotides (5′-AGATGGCTGTGATTCAA-GAGAGGTGAGAGGTCAGCTTGTT) were 3′-labeled with [32P]dATP (New England Nuclear). RNA membranes from vegetal 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.

In situ hybridization histochemistry was done as published elsewhere (34). Five-micrometer cryosections, thawed onto gelatin-coated slides, were air dried, fixed in paraformaldehyde, and dehydrated in ethanol. Forty-mer antisense oligonucleotides (5′-AGATGGCTGTGATTCAA-GAGAGGTGAGAGGTCAGCTTGTT) were 3′-labeled with [32P]dATP (New England Nuclear). RNA membranes from vegetal membranes were hybridized with the tissue sections overnight under Nescofil coverslips in a humid chamber at 42°C in a hybridization buffer. The slides were washed at high stringency, dehydrated with ethanol, dipped into LM-1 emulsion (Amersham Biosciences), and examined after development, using darkfield and Nomarski microscopy (Olympus BX60). As controls for in situ hybridization histochemistry, Northern blotting, the melting temperature of the hybrids formed, competition in cohybridization experiments, and hybridization with probes for other mRNAs were all used as explained elsewhere (34). The melting temperature of the hybrids was determined during the washing procedure and found to be close to the values predicted by Primer Analysis software (Oligo 6.0, MedProbe).

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′-TTGGCCCTCTTACCCATACGAGCCGACGTCGTGCTC
- HA23, 5′-GCTGTCACAAATCCCACTACGAGCCGACGTCGTGCTC
- HA66, 5′-GAGACGATGTACACCCATACGAGCCGACGTCGTGCTC
- HA110, 5′-GAGTGGCCCTCTTTACCCATACGAGCCGACGTCGTGCTC

For cystein mutants, they were mutated to serines using the following primers (point mutations for Cys conversion are underlined): Cys20 (C20S), CCGAAGTGGCACCTGCCAG-ACTCCGGCGGTCTAGGGAACC

and Cys55 (C55S), GCTCTTCCTGGCTCCAG-ACCTACGCGGGTCTAGGGAACC

Acclimation of type 1 PSD95-Dlg-zona occludens (PDZK1), a PDZ domain-containing globular protein induced to interact with an additional protein(s) in the oocyte is essential for sugar transport.
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 breflidin 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 oligodeoxyribonucleotides derived from the rat MAP17 sequence as follows: sense oligos: 105–124 ATGTGGGCCCC- CAGTTGTGCT (S105), 251–270 CTGCTACCTTTGCTG-GCC (S251), and 407–426 TGGTCTGGAGGAGGGCGA (S407); and antisense oligonucleotides: 105–124 AGCA-GACTGAGGGCAGAAT (AS124), 355–352 TTATCTGCCATTCA-TGGGCCC (AS355), and 430–449 TCACATGGTGTTGCTG-CTGCAG (AS430). Rat kidney cortex (poly)A/H11001 containing (in g/l) 8 NaCl, 1.15 Na2HPO4, 0.2 KCl, 0.2 KH2PO4, 0.132 CaCl2, and 2H2O, and 0.1 MgCl2, pH 8, was used. Rat kidney cortex RNA was prepared from size-selected rat kidney cortex poly(A)/H11001 RNA (1 kb as the mean) according to our previous method. A single cDNA clone of 0.8 kb was selected from a library of rat kidney cortex RNA (1 kb as the mean) according to our previous method. The cloning of an 1,808-bp fragment of rat PKD1, 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’-GGTTCCAA- GACTAGTTGATGCTA-3’ and antisense 5’-CAGCTAAGCT- TTAATG-3’. The product was cloned into PCR-Script (Stratagene), and the capped cRNA was tailing using a Poly(A) Tailing kit (Ambion). Cross-linking was allowed for 2 hr at 4°C. This was added from a freshly made 100 mM solution 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 MgCl2×2H2O and incubated in 3 ml glycerol buffer containing 1 mM pCMB, rinsed, and then incubated with 10 ng HA23-MAP17 cRNA, alone or in combination with 20 ng of rat kidney cortex poly(A)+ RNA. A single cDNA clone of 0.8 kb was selected from a library of rat kidney cortex RNA (1 kb as the mean) according to our previous method. The cloning of an 1,808-bp fragment of rat PKD1, 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’-GGTTCCAA- GACTAGTTGATGCTA-3’ and antisense 5’-CAGCTAAGCT- TTAATG-3’. The product was cloned into PCR-Script (Stratagene), and the capped cRNA was tailing using a Poly(A) Tailing kit (Ambion).

Biological procedures. Treatment with p-chloromercuribenzenzoate (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 for 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) reagents (Toronto Research Chemicals, Downsview, Ontario, Canada) [2-(trimethylammonium)ethyl] methanethiosulfonate (MTSET) and 2-aminomethyl methanethiosulfonate (MTSEA) were dissolved in DMSO and used at 1 or 5 mM for 5 or 10 min of preincubation with the oocytes.

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 from 40,000 colonies, which induced ~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. 10A; 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

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<th>D-Mannose</th>
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<td></td>
<td>$V_{\text{max}}$, pmol-oocyte$^{-1}$·hour$^{-1}$</td>
<td>$K_m$, mM</td>
<td>$K_d$, mM</td>
<td>DF, r</td>
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<td>H2O</td>
<td>1.00 ± 0.47</td>
<td>0.12 ± 0.06</td>
<td>7.25 ± 0.46</td>
<td>58, 0.8205</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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<tr>
<td>Net</td>
<td>1.56 ± 1.00</td>
<td>0.23 ± 0.10</td>
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<td>5, 0.9742</td>
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<td>$n$, 10^5</td>
<td>DF, r</td>
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<td>H2O</td>
<td>1.25 ± 0.08</td>
<td>21.60 ± 3.00</td>
<td>1.80 ± 0.40</td>
<td>72, 0.8160</td>
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<td>MAP17</td>
<td>2.89 ± 0.15</td>
<td>18.52 ± 2.00</td>
<td>1.98 ± 0.41</td>
<td>76, 0.8192</td>
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<tr>
<td>Net</td>
<td>1.64 ± 0.17</td>
<td>16.35 ± 3.95</td>
<td>2.32 ± 0.84</td>
<td>5, 0.9652</td>
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<td>$V_{\text{max}}$, pmol-oocyte$^{-1}$·hour$^{-1}$</td>
<td>$K_m$, mM</td>
<td>$K_d$, mM</td>
<td>DF, r</td>
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<tr>
<td>H2O</td>
<td>3.59 ± 0.58</td>
<td>0.88 ± 0.15</td>
<td>0.71 ± 0.06</td>
<td>67, 0.8215</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>
<td>6.30 ± 0.43</td>
<td>1.34 ± 0.33</td>
<td>—</td>
<td>5, 0.9761</td>
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<td></td>
<td>$V_{\text{max}}$, pmol-oocyte$^{-1}$·hour$^{-1}$</td>
<td>$K_m$, mM</td>
<td>$n$, 10^5</td>
<td>DF, r</td>
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<tr>
<td>H2O</td>
<td>0.24 ± 0.10</td>
<td>43.72 ± 35.12</td>
<td>1.31 ± 0.73</td>
<td>60, 0.5071</td>
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<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>
<td>0.23 ± 0.06</td>
<td>17.53 ± 9.30</td>
<td>1.56 ± 1.37</td>
<td>5, 0.8297</td>
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Values are means ± asymptotic SE. DF, degrees of freedom; r, regression coefficient.

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-[14C]glucose and α-methyl-D-[14C]glucopyranoside were transported similarly to D-mannose by MAP17-expressing oocytes (Fig. 2B). However, 0.5 mM D-[14C]galactose, 3-O-methyl-D-[14C]glucopyranoside, and D-[14C]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, lanes 1–3, and see Fig. 10B). 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-syntases, 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 NH2-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 NH2 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 NH2 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 serine, threonine, and tyrosine (Fig. 3). Additional searches found an anion-exchangers family 1 alignment at residues 56–103 (ProfiScan) and a phospho-mannomutase/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 co-transport by 60% (Fig. 8A). Treatment with thiol-oxidating methylthiolosulfonates 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), Fig. 3. Sequence analysis of human membrane-associated protein (MAP17). Nucleotide and single-letter code amino acid sequences are shown. The 2 transmembrane domains are indicated as TM1 and TM2, the PDZ-binding site at the COOH terminus is underlined, and the potential phosphorylation sites are labeled with asterisks and letters in bold. The amino acids encoding a phosphomannomutase block (PMM/PGM) are framed, and the 3 alternative hemagglutinin antigen (HA) insertion sites are indicated with arrowheads. A polyadenylation signal is in bold and underlined.
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. 4A)

![Fig. 4. Molecular analysis of MAP17.](image)

**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.](image)

**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.
A) 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), MAP17 associated with both MAP46 and MAP45 but not with MAP15 or MAP24. However, when DTSSP was used, MAP17 coimmunoprecipitated with MAP46/45 as well as MAP15 and MAP24 (Fig. 10). The presence of MAP15 and MAP24 in the MAP17 preparation further confirmed that these two proteins interact with MAP17.

Fig. 6. Subcellular expression of MAP17 in OK cells. Confocal sections were obtained every 1.5 μm from the apical to the basal membrane of OK cells permanently transfected with HA23-tagged MAP17 and immunodecorated with anti-HA antibody in the absence (A) or presence (B) of saponin. Without permeabilization, MAP17 is located at the apical membrane; however, the use of saponin revealed an uneven staining of the cytoplasm, with a pattern reminiscent of the Golgi network.

Fig. 7. MAP17 colocalizes with specific regions of the Golgi apparatus. Permeabilized OK cells permanently expressing HA23-tagged MAP17 were double labeled with anti-HA antibody (green, A) and with the Golgi marker BODIPY-TR (red, C). E: colocalization is shown by the emergence of the yellow staining of MAP17/Golgi. B, D, and F: Z-sections along the axis shown in A, C, and E, respectively. The anti-HA staining pattern was lost by pretreatment with brefeldin A (G).
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/H11001 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

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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-(Trimethylammonium)ethyl] 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.

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).

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.
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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...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 residues (Fig. 8C). The presence of Cys also seems to be necessary for the complete expression of mannose transport by MAP17 (Fig. 8B), which indicates that either the homodimer conformation is implicated in the transport or, alternatively, Cys 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 interacted with the COOH terminus of MAP17 (19). PDZK1 also interacts with the organic anion transporter cMOAT (18) and the CFTR chloride channel (7), and recently, a direct interaction between MAP17 and NaPi-2a has also been reported (13). Counterparts do so with the Na-phosphate cotransporter cMOAT (18) and the CFTR chloride channel (7), and PDZK1 also interacts with the organic anion transporter cMOAT (18) and the CFTR chloride channel (7), and recently, a direct interaction between MAP17 and NaPi-IIa has also been reported (13). Therefore, the challenge now is to identify the renal protein that interacts with MAP17 and transports $\alpha$-mannose. In addition to the structural simplicity of MAP17, experimental evidence for the collaboration of MAP17 with another protein to produce the transport expression arises from dose-response experiments, giving that the induction of mannose transport with increasing amounts of MAP17 cRNA does not parallel the expression of MAP17 protein in the oocyte (Fig. 10). A similar lack of correlation was reported, for example, for the amino acid transporter 4F2hc (4, 36), which encodes the heavy chain of a heteromultimeric complex. In this case, the low expression in oocytes was due to the use and depletion of the endogenous light chain of the oocyte (8). The function of PDZK1 could consist of either simply maintaining MAP17 in the plasma membrane or acting as an intermediary that joins MAP17 and a third protein with the characteristics of SGLT. Interestingly, the SGLT members known to date do not contain PDZ-binding domains, a characteristic that agrees with the lack of increase in the induction of mannose transport by MAP17 when MAP17/PDZK1 is coexpressed with SGLT-1, -2, and -3 (Fig. 11A). Independently of the sugar transporter implicated, the interactions with MAP17 seem to be very weak, as deduced from the lack of high bands communoprecipitated with MAP17 (Fig. 12). The same result has been observed with the Na-phosphate cotransporter NaPi-IIa (26), a protein that interacts with MAP17 through its NH$_2$ terminus, as shown in yeast two-hybrid assays: the interaction is so weak that pull-down experiments did not corroborate the interaction.

The interaction of MAP17 with the NaPi-IIa cotransporters is, at first, puzzling. The physiological meaning of this interaction is not yet known, but it adds additional appeal to the cell biology of this little protein, because its functions must not be restricted to sugar handling. For example, MAP17 could modulate the activity or organization of membrane transporters by direct interaction as the RS1 modifier (35), or through competition for PDZ-binding places to alter the stoichiometry of the transporters-PDZ proteins. In this way, MAP17 could force other PDZK1-associated proteins to reorganize in a PDZK1-independent way. The structural simplicity of MAP17 supports this simple regulatory role: first, MAP17 is integrated into the membrane with 2 spanning 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 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.

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