Functional characterization of ACDP2 (ancient conserved domain protein), a divalent metal transporter

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Goytain, Angela, and Gary A. Quamme. Functional characterization of ACDP2 (ancient conserved domain protein), a divalent metal transporter. Physiol Genomics 22: 382–389, 2005. First published May 17, 2005; 10.1152/physiolgenomics.00058.2005.—We have begun to identify and characterize genes that are differentially expressed with low magnesium. One of these sequences conforms to the ancient conserved domain protein, ACDP2. Real-time RT-PCR of mRNA isolated from distal epithelial cells cultured in low-magnesium media relative to normal media and in kidney cortex of mice maintained on low-magnesium diets compared with those animals consuming normal diets confirmed that the ACDP2 transcript is responsive to magnesium. Mouse ACDP2 was cloned from mouse distal convoluted tubule cells, expressed in Xenopus laevis oocytes, and studied with two-electrode voltage-clamp studies. When expressed in oocytes, ACDP2 mediates saturable Mg^{2+} uptake with a Michaelis constant of 0.56 ± 0.05 mM. Transport of Mg^{2+} by ACDP2 is rhesogenic, is voltage-dependent, and is not coupled to Na^{+} or Cl^{-} ions. Expressed ACDP2 transports a range of divalent cations: Mg^{2+}, Ca^{2+}, Mn^{2+}, Sr^{2+}, Ba^{2+}, Cu^{2+}, and Fe^{2+}; accordingly, it is a divalent cation transporter with wide substrate selectivity. The cations Ca^{2+}, Cd^{2+}, Zn^{2+}, and Ni^{2+} did not induce currents, and only Zn^{2+} effectively inhibited transport. The ACDP2 transcript is abundantly present in kidney, brain, and heart with lower amounts in liver, small intestine, and colon. Moreover, ACDP2 mRNA is upregulated with magnesium deficiency, particularly in the distal convoluted tubule cells, kidney, heart, and brain. These studies suggest that ACDP2 may provide a regulated transporter for Mg^{2+} and other divalent cations in epithelial cells.

Magnesium

Magnesium is an essential element that is required for the catalytic activity of numerous metalloenzymes and serves a structural role by stabilizing the conformation of certain metal-dependent protein domains, such as Ca^{2+}-binding proteins and receptors. Magnesium deficiency by either intestinal malabsorption or renal wasting can have devastating effects leading to muscle paresis and seizures. Moreover, magnesium depletion has been implicated in a variety of vascular diseases ranging from hypertension and cardiac arrhythmias to asthma and migraines (1). Intracellular free Mg^{2+} concentration is in the order of 0.5 mM, which is 1–2% of the total cellular magnesium (19). Intracellular Mg^{2+} level is maintained well below the concentration predicted from the transmembrane electrochemical potential, as extracellular magnesium is ~0.7 mM and the membrane voltage is near ~70 mV. This is possible because of membrane transporters located either within the plasma membranes or in intracellular organelles.

Despite the extensive evidence for unique mammalian Mg^{2+} transporters, few proteins have been biochemically identified to date that fulfill this role.

A number of proteins belonging to four different gene families have been identified recently that effect Mg^{2+} transport in mammalian cells. Two members of the transient receptor potential melastatin family (TRPM) of cation channels have been demonstrated to be primarily Mg^{2+} channels, although they also transport other divalent cations (15, 23). TRPM7 is ubiquitously expressed among tissues and has been reported to be essential in cellular viability (15). A related transporter, TRPM6, has been implicated in intestinal and renal Mg^{2+} absorption based on the observation that mutations in the TRPM6 gene cause a syndrome termed autosomal recessive hypomagnesemia with secondary hypocalcemia (HSH) that is characterized by dietary Mg^{2+} malabsorption and renal Mg^{2+} wasting (22, 27). TRPM6 is confined to the nephron, testis, lung, intestine, and perhaps several other tissues (22). TRPM7 and TRPM6 are characterized by six transmembrane segments, a highly conserved pore-forming region, and a Pro-Pro-Pro motif following the last transmembrane domain. Unlike the other members of the TRPM family, TRPM6 and TRPM7 possess a unique structure in that they have an α-kinesin domain in the COOH-terminal region (15, 20, 22). Recently, Dorovkov and Ryazanov (6) have shown that this α-kinesin phosphorylates and activates annexin, which interacts with many proteins and membranes and plays a pleiotropic role in cell proliferation, inflammation, and apoptosis (6). Chubanov et al. (3) have shown that TRPM6 and TRPM7 associate in heteromultimeric complexes that form functional Mg^{2+}/Ca^{2+} channels at the cell surface (3). Voets et al. (25) have reported that TRPM6 is located along the apical membrane of the small intestine and distal convoluted tubule, transports both Mg^{2+} and Ca^{2+}, and is associated with paralbumin and calbindin-D_{28K}, two divalent cation-binding proteins. Several researchers have also shown that other divalent ions such as Zn^{2+} and Co^{2+} were also transported by TRPM6 and TRPM7, supporting the earlier observations demonstrating that these proteins are permissive metal ion transporters (3, 12, 25). Nevertheless, Mg^{2+} appears to be the preferred substrate so that TRPM6 and TRPM7 are considered as primary Mg^{2+} entry channels. A second Mg^{2+} transport protein family, termed Mrs2, has been isolated from yeast, mouse, and human mitochondria (2, 33). Mrs2 appears to be a distant relative of the prokaryote CorA Mg^{2+} transport protein family (33). Mrs2 has been shown to be a major Mg^{2+} transporter of the mitochondria (13). We have recently demonstrated that the eukaryotic solute carrier, SLC41A1, transports Mg^{2+} when expressed in Xenopus oocytes (9), supporting the notion of Wabakken et al. (26) who proposed that members of the SLC41 family of proteins were Mg^{2+} transporters. There are three members of the SLC41 family.
SLC41A1 is not selective, as it transports a variety of divalent cations. Computer analysis of the SLC41A1 protein structure reveals that the protein consists of 10 putative transmembrane domains and includes 2 distinct regions similar to the integral membrane of the bacterial MgtE protein family (26). Finally, we have identified and characterized a novel Mg$^{2+}$-transporter family that we have called MagT (8). The MagT proteins form a novel family with no known similarity to any other reported transport family of proteins. The two members, MagT1 and MagT2 (N33), are located in many mammalian tissues; MagT1 is selective to Mg$^{2+}$, but MagT2 transports other divalent cations such as Fe$^{2+}$, Cu$^{2+}$, and Mn$^{2+}$ (Ref. 8 and Goytain A and Quamme GA, unpublished observations). In summary, a number of novel transport proteins have recently been recognized that will increase our understanding of cellular Mg$^{2+}$ homeostasis.

The kidney is the predominant organ that controls magnesium balance. A large number of hormones and factors influence renal magnesium reabsorption, but these are not selective to magnesium (4). We have shown that the selective regulation of magnesium transport is due to transcriptional control of proteins involved with cellular magnesium homeostasis (19). Epithelial cells cultured in low-magnesium growth media demonstrate a greater transport rate compared with normal cells (5). With this knowledge, we have begun to identify genes that determine epithelial Mg$^{2+}$ transport (8). One approach is to use differential expression to search for novel genes responsive to magnesium. We have used this approach to identify MagT and SLC41 protein families as eukaryotic Mg$^{2+}$ transporters (8, 9). Continuing with this method, we show here that the ancient conserved domain gene, Acdp2, is responsive to magnesium and encodes a protein that, when expressed in Xenopus oocytes, mediates Mg$^{2+}$ transport and other divalent cations. Interestingly, this extends the earlier observations of Wang and colleagues (30, 31). They cloned a novel gene family that they termed the “ancient conserved domain protein” family because all members share a domain that is evolutionarily conserved in a large number of species from bacteria to man. The Acdp genes are conserved with homology to the microbial CorC protein, which is involved with bacterial Co$^{2+}$ resistance (7, 14). Acdp genes are widely distributed, and the associated gene products are predominately localized to the plasma membrane (31). On the basis of the similarity to the bacterial transporter, Wang et al. (31) postulated that ACDP proteins are involved with ion transport in mammalian cells. The present expression studies support the notion that ACDP2 mediates divalent metal transport, is regulated by cellular magnesium levels, and is likely to play a role in magnesium balance.

**EXPERIMENTAL PROCEDURES**

**Animal preparation and cell culture.** Male mice were maintained for 5 days on a low-magnesium diet (ICN diet no. 902205; Nutritional Biochemicals, Cleveland, OH) or on this diet supplemented with 0.05% MgSO$_4$, which is comparable to normal mouse chow. The plasma concentrations were 0.13 ± 0.01 and 0.75 ± 0.09 mM, respectively, confirming that the mice consuming a magnesium-restricted diet were relatively magnesium deficient (19).

Mouse distal convoluted tube (MDCT) cells were originally derived and immortalized by Pizzonia et al. (17). The MDCT cell line has been extensively used by us to study renal magnesium transport (4). Cells were grown in basal DMEM-Ham’s F-12 (1:1) media (GIBCO) supplemented with 10% fetal calf serum (Flow Laboratories, McLean, VA), 1 mM glucose, 5 mM t-glutamine, 50 U/ml penicillin, and 50 µg/ml streptomycin in a humidified environment of 5% CO$_2$-95% air at 37°C. Where indicated, subconfluent MDCT cells were cultured in Mg$^{2+}$-free media (Stem Cell Technologies, Vancouver, BC, Canada) for 16 h. Other constituents of the Mg$^{2+}$-free culture media were similar to those in the complete media.

**Quantitative analysis of ACDP2 transcripts by real-time RT-PCR.** Total RNA of cells was extracted by TRIzol (Invitrogen). Genomic DNA contamination was removed by a DNA-free kit (Ambion) before first-strand cDNA was made. Standard curves were constructed by serial dilution of a linear pGEM-T vector (Promega) containing the ACDP2 gene. The primer set of mouse ACDP2 was as follows: forward 5’-TGAGCCCATGAGCGCAACCT-3’ and reverse 5’-CTCATCTGGCAAGCCTGCTAT-3’. The PCR product was sequenced to ensure specificity of the amplification. PCR products were quantified continuously with AB7000 (Applied Biosystems), using SYBR Green fluorescence, according to the manufacturer’s instructions. The relative amounts of ACDP2 RNA were normalized to mouse β-actin transcripts.

**Plasmid construction and generation of expression constructs.** An RT-PCR strategy was used to isolate mouse ACDP2 cDNA from the MDCT cell line. The cDNA comprising the open reading frame (ORF) of ACDP2 was amplified from the MDCT cDNA using the cloning primers (forward 5’-CCCGTGCAGAGAGATGGAGAAG-3’ and reverse 5’-GGGTCGTAGAGCGCTGCTG-3’) and subcloned into the pUC119 vector (Stratagene). The isolated clones were sequenced to confirm sequence integrity. Standard protocols were used for Escherichia coli cloning procedures and DNA sequencing (21). To synthesize complementary RNA (cRNA), the cDNA constructs were linearized and then transcribed with T7 polymerase in the presence of $^{32}$P-gppG cap, using the mMESSAGE MACHINE T7 KIT (Ambion) transcription system.

**Protein motifs were identified using standard techniques with BLASTP and the SWISSPROT data base (9).** Membrane topology was predicted by the SOSUI program based on Kyte-Doolittle hydrophobicity analysis using a window of 20 amino acids for the transmembrane domain. Expression of ACDP2 in Xenopus oocytes and current measurements. Xenopus oocytes were prepared and injected with cRNA, and electrophysiological recordings were performed according to techniques previously described (8). Briefly, defolliculated stage V–VI oocytes were typically injected with 25 ng of cRNA in 50 nl of H$_2$O. Oocytes were incubated at 18°C for 3–5 days in multiwell tissue culture plates containing Barth’s solution [88 mM NaCl, 1.0 mM KCl, 2.4 mM NaHCO$_3$, 1.0 mM MgSO$_4$, 1.0 mM CaCl$_2$, 0.3 mM Ca(NO$_3$)$_2$, 10 mM HEPES-NaOH, pH 7.6, 2.5 mM Na-pyruvate, 0.1% BSA, 10,000 U/ml penicillin, 100 µg/ml streptomycin]. To record expressed membrane currents, the oocytes were placed in a recording chamber (0.3 ml) and perfused with modified Barth’s (96 mM NaCl, 10 mM HEPES-NaOH) containing various concentrations of MgCl$_2$, as indicated, in substitution for osmotically equivalent amounts of NaCl. All experiments were performed at room temperature (21°C).

Steady-state membrane currents were recorded with the two-microelectrode voltage-clamp technique using a Geneclamp 500 amplifier (Axon Instruments.). Electrophysiology consisted of a voltage-clamp step profile consisting of a holding potential of −15 mV, followed by eight episode series of +25-mV steps of 2-s duration, from −150 to +25 mV within an episode duration of 6.14 s. Each episode recorded 1,536 data points collected at 4-ms intervals. The data were filtered at the appropriate frequency before digitization. To assess the permeability of different divalent cations, we used the shift in the reversal potentials of the respective cation from the reversal potentials of Mg$^{2+}$ currents, $\Delta$E$_{rev}$, and calculated by the permeability ratio.
where $R$, $T$, and $F$ have their standard meanings (15). Voltage-clamp episodes in the presence of extracellular test cations were corrected against episodes in the absence of external test cations.

All experimental conditions were performed on oocytes harvested from a minimum of three different animals.

**RESULTS**

**Identification of ACDP2 as a magnesium-responsive gene.** With the knowledge that differential gene expression is involved with selective control of epithelial cell magnesium conservation, our strategy was to identify candidates that were upregulated with low magnesium. Using real-time RT-PCR, we first showed that ACDP2 mRNA is increased 2.2 ± 0.2-fold in kidney tissue of animals maintained on low-magnesium. Using real-time RT-PCR, the expression level of the mouse ACDP1 transcript was normalized to that of the mouse MDCT, mouse distal convoluted tubule. Relative median values are noted by the horizontal bars, and significance ($P < 0.05$) is indicated by an asterisk (*).

**ACDP2 elicits Mg$^{2+}$ currents in expressing Xenopus oocytes.** To determine whether ACDP2 encodes a functional Mg$^{2+}$ transporter, we cloned the mouse ACDP2 cDNA from MDCT cells, prepared cRNA and injected it into Xenopus oocytes, and characterized Mg$^{2+}$-evoked currents using two-microelectrode voltage-clamp analysis. The electrophysiological data gave evidence for a rheogenic process with large inward currents in ACDP2 cRNA-injected oocytes, whereas there were no appreciable currents in control H2O- or total poly(A)$^+$ RNA-injected cells from the same batch of oocytes. Figure 2A illustrates a representative experiment demonstrating the clamping profile and typical currents measured in control or ACDP2-expressing oocytes with either nominally magnesium-free solution or 2.0 mM magnesium in the external solution. The mean data for all external magnesium concentrations are shown as a current-voltage ($I$-$V_{m}$) plot (Fig. 2B). The Mg$^{2+}$-evoked currents, measured at a fixed time point, were saturable (Fig. 2C), demonstrated a Michaelis constant of 0.56 ± 0.05 mM ($n = 29$; Fig. 2C, inset), and were similar among the larger negative clamping voltages (data not shown).

**Characterization of Mg$^{2+}$ currents expressed in Xenopus oocytes.** The Mg$^{2+}$-evoked currents were not altered with deletion of external NaCl by substitution with choline-Cl or the replacement of NaCl and MgCl$_2$ with appropriate amounts of Na-gluconate and Mg$_2$SO$_4$ (data not shown), arguing against Mg$^{2+}$-Na$^+$- and Mg$^{2+}$-Cl$^-$-coupled transports, which have been postulated in the literature (19).

A variety of divalent cations were used to determine the selectivity of the expressed ACDP2 channel. A number of divalent cations produced currents based on the permeability ratio using the reversal potential; these include Mg$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Cu$^{2+}$, and Fe$^{2+}$ (Fig. 3A). The permeability sequence measured under the given conditions is illustrated in Fig. 3B. Large concentrations (2 mM) of Ca$^{2+}$, Ni$^{2+}$, Zn$^{2+}$, and Cd$^{2+}$ did not produce appreciable currents in the absence of Mg$^{2+}$ in ACDP2-expressing oocytes. In the experiments shown, currents were corrected for changes in membrane resistance caused by the respective divalent cation using values from H$_2$O-injected oocytes (Fig. 3A).

Next, we determined whether the cations that were not transported by ACDP2 would inhibit Mg$^{2+}$-evoked currents. Relatively large concentrations (0.2 mM) of the respective inhibitors, Zn$^{2+}$, Ni$^{2+}$, and Cd$^{2+}$, and 2.0 mM Ca$^{2+}$ were tested in the presence of 2.0 mM MgCl$_2$ (Fig. 3C). Ca$^{2+}$ (2.0 mM) and Ni$^{2+}$ (0.2 mM) modestly inhibited Mg$^{2+}$ currents, whereas Zn$^{2+}$ (0.2 mM) markedly inhibited transport as reflected by the change in $\Delta E_{rev}$ for Mg$^{2+}$ (Fig. 3D). Cd$^{2+}$ did not affect the expressed Mg$^{2+}$ currents. On balance, these data indicate that ACDP2 cRNA-induced transport in oocytes supports a number of divalent cation substrates, but only Zn$^{2+}$ inhibited Mg$^{2+}$ transport.

**Sequence analysis of mouse ACDP2.** The isolated mouse ACDP2 cDNA contains an ORF of 2,079 bp, which predicts a protein of 693 amino acids with a calculated molecular mass of 77 kDa. (Fig. 4). The mouse ACDP2 cDNA is similar (98%) to the human sequence (GenBank ascension no. NP_060119).

Hydropathicity analysis using the Susui program predicts an integral membrane protein containing four hydrophobic transmembrane-spanning (TM) regions (Fig. 4). Analysis of the amino acid sequence by the NetNGlyc 1.0 and NetPhos 2.0 server programs reveals several consensus sites for posttrans-
ACDP2 encodes a divalent metal transporter.

Fig. 2. ACDP2 mediates Mg\(^{2+}\) currents in Xenopus oocytes. A: representative voltage-clamping profile and current measurements in control and ACDP2 cRNA-injected oocytes. Shown are current tracings determined in the absence and presence of 2.0 mM MgCl\(_2\) and the difference with and without Mg\(^{2+}\). B: current-voltage (I-V) relationships obtained from linear voltage steps from −150 to +25 mV in the presence of Mg\(^{2+}\)-free solutions or those containing the indicated concentrations of MgCl\(_2\). Oocytes were clamped at a holding potential of −15 mV and stepped from −150 to +25 mV in 25-mV increments for 2 s at each of the concentrations indicated. Shown are average I-V curves obtained from control H\(_2\)O-injected (n = 3) or MagT1-expressing (n ≥ 3) oocytes. Note the positive shift in reversal potential with increments in magnesium concentration. Values are means ± SE of observations measured at the end of each voltage sweep for the respective Mg\(^{2+}\) concentration. C: summary of concentration-dependent Mg\(^{2+}\)-evoked currents in ACDP2-expressing oocytes using a holding potential of −125 mV. Mean ± SE values are those given in Fig. 2A. Inset: an Eadie-Hofstee plot of concentration-dependent Mg\(^{2+}\)-evoked currents demonstrating a Michaelis constant (K\(_m\)) of 0.56 ± 0.05 mM. K\(_m\) was independent of the respective holding potential.
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Fig. 3. Characterization of ACDP2-mediated currents in *Xenopus* oocytes. A: substrate specificity of ACDP2 following application of test cations (2.0 mM) in the absence of external Mg^{2+}. For clarity, only Mg^{2+}, Mn^{2+}, Cu^{2+}, and Zn^{2+} are represented. Oocytes were clamped at a holding potential of −15 mV and stepped from −150 to +25 mV in 25-mV increments for 2 s for each of the cations. Note the reversal potentials used to calculate the permeability ratios given in Fig. 2B. Values are means ± SE of currents measured at the end of each voltage sweep for the respective divalent cation. B: summary of permeability sequence of the tested divalent cations. Shows average permeability ratios illustrated in Fig. 3A. C: inhibition of Mg^{2+}-evoked currents with 0.2 mM Ni^{2+}, Cd^{2+}, or Zn^{2+} or 2.0 mM Ca^{2+} in the presence of 2.0 mM external Mg^{2+}. For clarity, only Mg^{2+}, Ni^{2+}, Zn^{2+}, and Ca^{2+} are illustrated. Values are means ± SE of currents measured at the end of each voltage sweep for the respective cation. D: summary of potency sequence of inhibition by multivalent cations based on the change in *E*_{rev} (see EXPERIMENTAL PROCEDURES) given in Fig. 3C. The inhibitor was added with MgCl₂, and voltage clamp was performed ∼5 min later.

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There are four possible glycosylation sites at amino acid residues N-345, N-409, N-575, and N-684 on the last putative extracellular loop. ACDP2 contains no putative cAMP-dependent protein kinase phosphorylation sites but four possible protein kinase C phosphorylation sites at residues T-113, T-411, S-463, S-583, and S-613. There are also 13 predicted casein kinase II motifs at residues T-263, T-287, T-347, T-386, T-430, T-452, S-552, S-561, S-581, S-637, S-646, T-651, and T-656 and 6 myristoylation sites, G-22, G-28, G-34, G-120, G-573, and G-594. The presence of protein kinase C phosphorylation consensus sequences might indicate regulation of phosphorylation-dependent localization and function.

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Query of GenBank revealed genomic sequences that were identical to ACDP2 (GenBank accession no. NM_033569), and alignment with ACDP2 cDNA predicts the gene structure. The coding sequence possesses eight exons, six comprising ~150 bp and the first and last exon of ~1,000 bp in length. The mouse ACDP2 gene is localized to chromosome 19C3, and the human genomic sequence of ACDP2 (GenBank accession no NM_017649) was assigned to chromosome band 10q24.33.

Tissue distribution of ACDP2 mRNA expression and the response to magnesium. We used quantitative RT-PCR analysis to show that ACDP2 transcript is present in many tissues of normal mice (Fig. 5). The kidney and brain possesses the most transcript, but mRNA is abundant in heart and liver with lesser amounts in the small intestine and colon. The variability, particularly in the heart and brain, shown here may be due to the different cells comprising

![Fig. 4. Molecular characterization of ACDP2. Amino acid sequence of mouse ACDP2. Transmembrane segments are underlined, and predicted N-linked glycosylation sites (●), protein kinase C phosphorylation (●), N-myristoylation (X), and casein kinase (●) sites are indicated.](image-url)

![Fig. 5. Tissue distribution and responsiveness to magnesium of mouse ACDP2 expression. The design of the experiments is described in legend to Fig. 1. Relative tissue distribution of ACDP2 mRNA normalized to the lowest data point, found in the colon, the tissue with the lowest levels of transcript. Also shown is the response of ACDP2 mRNA to low-magnesium balance in these tissues. Data (means ± SE) were obtained from 5 experiments, and the significance is indicated by an asterisk (*).](image-url)
these tissues, as we did not attempt to isolate specific cell types.

Next, we determined the change in expression levels of ACDP2 transcripts in renal tissues with a decrease in magnesium balance. Mice were placed on low magnesium- or normal magnesium-containing diets for 5 days before death. The mean plasma concentration was 0.75 ± 0.09 mM for normal animals and 0.13 ± 0.01 mM for mice maintained on low-magnesium diets. The respective urinary magnesium concentrations were 13.2 ± 1.2 and 1.1 ± 0.3 mM. ACDP2 mRNA was increased by ~30-fold in heart and 3-fold in total kidney cortex tissue and in isolated distal convoluted tubule cells (Fig. 5). The distal convoluted tubule is the major nephron segment that actively reabsorbs filtered magnesium (4). ACDP2 transcript was increased by about twofold in brain and by ~30% in liver and colon, whereas there was no detectable change in the small intestine (Fig. 5). Taken together, the data show that ACDP2 is widely distributed among tissues and is responsive in some organs to magnesium balance.

**DISCUSSION**

The ACDP family of proteins is conserved in some form from bacteria to humans (31). Wang et al. (30) identified four distinct genes, *ACDP1–ACDP4*, of the human *ACDP* family (30). Human *ACDP1* has been linked to an inheritable disease termed urofacial syndrome (UFS) or Ochoa syndrome (16, 28, 29). The UFS syndrome is a rare genetic disorder affecting facial expression and urinary tract function, but the role of ACDP1 in this disorder is not known (28, 29). The early studies of Wang and coworkers (30) reported that ACDPs were localized in the nucleus, which led others to suggest that it is a cyclophilin-like protein (30). However, Wang et al. (31) have subsequently showed that mouse ACDP1 protein was localized to the plasma membrane. On the basis of this knowledge and looking at the secondary structure, they suggested that ACDPs might be some kind of metal transporter or receptor (31).

Recently, Yang et al. (32) provided evidence that mutants of *MAM3* (‘mitochondria aberrant morphology’), a member of the ACDP family of *Saccharomyces cerevisiae* proteins, confer resistance to manganese toxicity. They showed that MAM3 is an integral protein of yeast vacuolar membranes and speculated that it might function as an Mn$^{2+}$ transporter, although they could not demonstrate accumulation of manganese in the vacuole. More recently, Guo et al. (11) reported that the overexpression of ACDP4 in HEK-293 cells results in enhanced toxicity to metal ions including copper, manganese, and cobalt. Although they proposed that ACDP4 is a family of transport proteins, there was no direct evidence for this conclusion in their study. A bacterial ACDP-like polypeptide known as CorC of *Salmonella typhimurium* has been implicated in magnesium and cobalt homeostasis in bacteria (14). Mutations in *corC* are associated with cobalt resistance; however, there is no evidence that CorC is a metal transporter (7). Although another bacterial protein, CorA, is clearly an Mg$^{2+}$ transporter, the function of CorC proteins and their role in Mg$^{2+}$ homeostasis are not apparent (14, 24). CorC is only 18% similar to CorA and does not possess apparent transmembrane domains. Finally, a gapped alignment of the human ACDP2 and bacterial CorC sequences showed only 35% identical amino acid residues and those extended throughout most of both proteins. Accordingly, it is difficult to judge the relationship of ACDP2 functions with those of the bacterial CorC.

In this study, we show that ACDP2 is regulated by cellular magnesium balance and mediates divalent cation transport when expressed in *Xenopus* oocytes. Our evidence is that renal and intestinal epithelial magnesium transport is determined, in part, by differential gene expression of transport proteins that mediate magnesium transport (19). Because the ACDP2 transcript is regulated by magnesium, it was satisfying to find that ACDP2 transports Mg$^{2+}$. Although ACDP2 transports a number of divalent cations, Mg$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Cu$^{2+}$, and Fe$^{2+}$, we conclude that it is primarily an Mg$^{2+}$ transporter. The Michaelis constant, 0.54 mM, is appropriate for an extracellular and intracellular Mg$^{2+}$ concentration of 0.75 and 0.50 mM, respectively. The other cations, Co$^{2+}$, Mn$^{2+}$, Sr$^{2+}$, Ba$^{2+}$, Cu$^{2+}$, and Fe$^{2+}$, are not found at these levels, whether intracellular or extracellular.

We have recently identified two novel families of transporters, MagT and SLC41 (Refs. 8 and 9 and Goytain A and Quamme GA, unpublished observations). The first member of the MagT family, MagT1, is characterized by a very selective substrate specificity in that it transports only the divalent cation, Mg$^{2+}$ (8). However, it is inhibited by a number of multivalent cations. The potency sequence of cationic inhibition of the MagT1 intracellular transporter at 0.2 mM (the presence of 0.2 mM inhibitor) is Ni$^{2+}$ > Zn$^{2+}$ > Mn$^{2+}$ > Cd$^{2+}$ > Cu$^{2+}$ > Co$^{2+}$. The second member of this family, N33, is more permissive, with a cation selectivity (in the presence of 2.0 mM substrate) of Fe$^{2+}$ > Mn$^{2+}$ > Mg$^{2+}$ > Cu$^{2+}$ (Goytain A and Quamme GA, unpublished observations). The potency sequence of cationic inhibition of N33 was Cd$^{3+}$ > Zn$^{2+}$ > Mn$^{2+}$ > Ni$^{2+}$ > Co$^{2+}$. Compared with these transporters, SLC41A1 is a relatively nonspecific divalent cation channel (9). Relative selectivity is Mg$^{2+}$ = Sr$^{2+}$ >> Fe$^{2+}$ = Zn$^{2+}$ = Cu$^{2+}$ = Co$^{2+}$ = Ba$^{2+}$ > Cd$^{2+}$, determined under the same conditions as given for MagT1 and N33. Only the trivalent cation, Gd$^{3+}$, inhibited SLC41A1-mediated Mg$^{2+}$ currents. ACDP2 is much more like a divalent cation transporter in that it accepts many cations, Mg$^{2+}$ = Co$^{2+}$ > Mn$^{2+}$ > Sr$^{2+}$ = Ba$^{2+}$ > Cu$^{2+}$, and Fe$^{2+}$, and is inhibited by Zn$^{2+}$, Ca$^{2+}$, and Ni$^{2+}$, in that order, as measured with the same protocol as MagT and SLC41A1. Accordingly, the Mg$^{2+}$ transporters identified to date have very different properties. They may be distinguished by these differences: MagT1 transports only Mg$^{2+}$, and Ni$^{2+}$ inhibits MagT1- and N33-mediated Mg$^{2+}$ transport but not SLC41A1-induced Mg$^{2+}$ transport. SLC41A1 and ACDP2 transport Sr$^{2+}$, whereas MagT transporters do not. Interestingly, none of these different gene products transports Ca$^{2+}$ nor are they inhibited by Ca$^{2+}$. This differentiates these transporters from the TRPM family of divalent cation channels, which translocates Mg$^{2+}$ and Ca$^{2+}$ as well as other divalent cations (22, 25).

Our data suggest that ACDP2 is a nonspecific divalent cation transporter. However, its role in the cellular metabolism of these metals is not clear. Because ACDP2 is upregulated by low magnesium, it is interesting to speculate that the transport of these multivalent cations may be increased in magnesium deficiency. We have reported that cadmium uptake into the renal epithelial distal tubule cell line Madin-Darby canine kidney was greater after cellular magnesium depletion than in control cells (18). These studies used fluorescence changes of mag-fura-2 to sensitively measure cellular free Cd$^{2+}$ activity to
determine Cd$^{2+}$ transport. This observation led to the notion that heavy metal intoxication may be more severe in individuals with low magnesium (17). ACDP2 does not transport cadmium but it does transport other metals such as cobalt, manganese, strontium, barium, copper, and iron so that the cellular accumulation and intracellular distribution of these metals may be altered in magnesium deficiency. On the other side, it would be interesting to see if deficiency of these metals, for instance of copper or iron, affects magnesium metabolism.

In summary, we show that ACDP2 mediates the transport of a variety of divalent cations. Because the Michaelis constant of ACDP2 for Mg$^{2+}$ is near the physiological range of extracellular and intracellular Mg$^{2+}$ concentration, it supports the notion that ACDP2 is a physiologically relevant Mg$^{2+}$ transporter. Furthermore, we demonstrate that ACDP2 mRNA is regulated by magnesium, which suggests that it might play a role in regulation of cellular magnesium content.

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