Solute carrier family SLC41: what do we really know about it?

Andrea Fleig,† Monika Schweigel-Rüntgen‡ and Martin Kolisek§

The 41st family of solute carriers (SLC41) comprises three members A1, A2, and A3, which are distantly homologous to bacterial Mg\(^{2+}\) channel MgtE. SLC41A1 was recently characterized as being an Na\(^+\)/Mg\(^{2+}\) exchanger (NME; a predominant cellular Mg\(^{2+}\)-efflux system). Little is known about the exact function of SLC41A2 and SLC41A3, although, these proteins have also been linked to Mg\(^{2+}\) transport in human (animal) cells. The molecular biology (including membrane topology, cellular localization, transcriptomics, and proteomics) of SLC41A2 and SLC41A3 compared with SLC41A1 has only been poorly explored. Significantly more data with regard to function, functional regulation, involvement in cellular signaling, complex-forming ability, spectrum of binding partners, and involvement in the pathophysiology of human diseases are available for SLC41A1. Three recent observations namely the identification of the null mutation, c.698G>T, in SLC41A1 underlying the nephronophthisis-like phenotype, the recognition of a putative link between SLC41A1 and Parkinson’s disease, and the observation that nearly 55% of preeclamptic placental samples overexpress SLC41A1, marks the protein as a possible therapeutic target of these diseases. A potential role of the SLC41 family of Mg\(^{2+}\) transporters in the pathophysiology of human diseases is further substantiated by the finding that SLC41A3 knockout mice develop abnormal locomotor coordination. © 2013 WILEY-VCH Verlag GmbH & Co. KGaA, Weinheim.

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INTRODUCTION

The importance of magnesium (Mg) for normal cellular, tissue, organ, and body physiology has been described in many original papers and reviews. However, the molecular entities responsible for Mg\(^{2+}\) transport in higher eukaryotes and their intracellular distribution have started to be identified only recently. During the last decade, several genes have been shown to encode for putative or confirmed Mg\(^{2+}\)-transport systems (homeostatic factors) in humans, mammals, and other higher eukaryotes (Table 1). In view of the large spectrum of processes that involve Mg, this count is likely not the final.

An exciting journey aimed at identifying genes encoding for Mg\(^{2+}\)-transporter molecules was begun in 1976 with the pioneering work of Park et al. who identified that corA, corB, and mgt mutants affect Mg\(^{2+}\) transport in Escherichia coli.\(^{20}\) In 1986, the group around M. E. Maguire cloned a predominant bacterial Mg\(^{2+}\)-transport system corA from Salmonella typhimurium.\(^{21}\) Shortly afterwards this was followed by the cloning of Mg\(^{2+}\)-regulated MgtA and MgtB P-type ATPases (both from S. typhimurium)\(^{22}\) and of MgtE, an Mg\(^{2+}\) channel with limited phylogenetic distribution (cloned from Prowidencia stuartii).\(^{23}\) In 2003, Wabbaken et al. cloned a human homolog of MgtE named solute carrier family 41 member A1 (SLC41A1; given as A1 henceforth).\(^{1}\) Goytain and Quamme demonstrated...
**TABLE 1** | Putative or Confirmed Mg\(^{2+}\)-Transport Systems (Homeostatic Factors) in Humans, Mammals, and Other Higher Eukaryotes

<table>
<thead>
<tr>
<th>Mg(^{2+})-Transporter</th>
<th>Gene</th>
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<th>Localization</th>
<th>References</th>
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<td>GA, PGV</td>
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<td>GA, PGV</td>
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<td>CM, IMC, N</td>
<td>17–19</td>
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NU, nuclear; CM, cytoplasmic membrane; IMC, intracellular membrane compartments; IMM, inner mitochondrial membrane; GA, Golgi apparatus; PGV, post-Golgi vesicles; ?, possible, putative.

that A1 and also SLC41A2 and SLC41A3 (both also distantly homologous with MgtE; both cloned from *Mus musculus*; given as A2 and A3 henceforth) from the same protein family were able to conduct the electrogenic transport of Mg\(^{2+}\) when heterologously expressed in *Xenopus laevis* oocytes.\(^5,8,24\) The A1 or A2-dependent electrogenic transport of Mg\(^{2+}\) seen in *Xenopus* oocytes was not observed in mammalian or avian cellular systems transfected with human A1 or A2.\(^2,6\) The molecular biology and physiology of A3 remains unexplored. In regards to A2, only limited knowledge to its exact function(s) and mode of operation is currently available. However, A1, despite initial thoughts that it might represent an ion channel mechanism, has now been shown to function as an Na\(^{+}\)/Mg\(^{2+}\) exchanger (NME), at least in human and mammalian cells.\(^3\) The discovery of A1 being an NME has physically bridged a large pool of knowledge concerning the physiology and pathophysiology of Na\(^{+}\)/Mg\(^{2+}\) exchange (a mechanism known for three decades as being extant) with an opportunity to perform a molecular examination of NME suspected to be directly or indirectly involved in a plethora of ailments of mankind.

**TRANSCRIPTOMICS, CELLULAR LOCALIZATION, TOPOLOGY, AND COMPLEX-FORMING ABILITIES OF SLC41A1, A2, AND A3**

Transcripts of the human genes SLC41A1 (1q31-32), SLC41A2 (12q23.3), and SLC41A3 (3q21.2) have been identified in various organs and tissues. The A1 transcript is abundantly expressed in heart, testis, and also in the adrenal and thyroid glands, prostate and ovaries whereas lower levels have been detected in all other tested tissues. To date it has also been detected in all tested cell lines such as HEK-293, Tom-1, BV173, Reh, Jurkat, and JVM-131–3 (Kolisek et al. unpublished). Therefore, we can assume that member A1 is ubiquitously expressed in human cells. Romanuik et al.\(^{25}\) have identified A1-expression as being responsive to androgens. No information is available about the expression of human A2 and A3 across human tissues and organs in peer-reviewed bibliography. However, some information can be extracted from www.proteinatlas.org.

All three members of the SLC41 family (human A1, 513 aa, 56 kDa; human A2, 573 aa, 62.3 kDa; human A3, 513 aa, 56 kDa)
and human A3, 500 aa, 54.6 kDa) were predicted and also experimentally confirmed by functional studies as being proteins integral to the cytoplasmic membrane (A1 and A2 in both human and mouse models, A3 only in the mouse model). \(^2,3,5,6,8,24\) However, Sahni and Scharenberg in their recent review \(^7\) advocate the targeting of A2 into membranes of intracellular compartments.

The membrane topology of A1 is controversial. Initially, the 10 transmembrane helices (TM) ‘inside-in’ model of A1 topology was predicted (Figure 1(a), Table 2). \(^1\) Results of the computer-predictions of A1 topology are summarized in Table 2. The results of independent studies have led to a consensus concerning the intracellular orientation of the N-terminus of member A1, \(^2,3,4,33\) However, data regarding the C-terminus orientation are contradictory. Based on their epitope-tagging studies, the group of Mandt \(^43\) has proposed a model possessing 11 TM and a C-terminus oriented extracellularly (Figure 1(a)). In contrast, Sponder et al. \(^44\) by utilizing the split-ubiquitin functional assay in yeast, demonstrate that ‘A1 C-terminally tagged with a Cub-LexA-VP16 reporter cassette is targeted to cytoplasmic membrane and that the C-terminus is oriented such that it allows the reconstitution of functional ubiquitin and is,

**FIGURE 1** | Models of membrane topology of SLC41A1 (a) and SLC41A2 (b) constructed according to available peer-reviewed bibliography. Plot c depicts a computer predicted-model (TMPred) of SLC41A3 topology.
therefore, intracellular’. It conforms with the study of Nestler et al.45, claiming that the bait vector pBTT3-STE, carrying the Cub-LexA-VP16 moiety fused to the C-terminus of A1, is suitable for split-ubiquitin yeast two hybrid assay (SU-YTH) with SLC41A1 serving as the bait (intracellular/intracytosolic orientation of the reporter moiety is the precondition for performing SU-YTH assay). Thus, the data given by Sponder et al.44 support the initially proposed 10 TM ‘inside-in’ model (Figure 1(a)). Only further research, e.g., showing that A1 C-terminally tagged with Cub-LexA-VP16 is functional, will shed more light on the exact organization of A1 topology.

The strongly preferred computer-predicted model of SLC41A2 topology (TMPred at www.ch.embnet.org) possesses 10 TM with both termini being oriented extracellularly. However, Sahni et al. have demonstrated experimentally that the N-terminus of A2 is oriented extracellularly and the C-terminus intracellularly when overexpressed in DT40 chicken cells and, therefore, they have proposed the 11 TM ‘outside-in’ model of membrane topology of A2 (Figure 1(b)).6 Considering the structure–function relationship determined for MgtE, Sahni and Scharenberg have recently concluded that, in their pilot study,6 A2 has most probably been mistargeted to the cytoplasmic membrane as a result of its overexpression. Their assumption that A2 is primarily functional in the membranes of the intracellular compartments must be further tested (Figure 1(b)).7 The online-generated prediction of A3 topology is shown at Figure 1(c). The computer analyses do not reveal any specific targeting-(signal)-sequences, therefore, a more specific intracellular localization of A2 or A3 may not be assumed (http://www.cbs.dtu.dk/services/SignalP/).

Kolisek et al. have shown that A1 forms protein complexes with high molecular mass in vivo.2 Moreover, they propose that these complexes have a hetero-oligomeric character.2 Recently, Nestler et al. have utilized SU-YTH assay and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MLDI-TOF-MS) and identified multiple binding partners of A1.45 The most prominent binding partners of A1 identified are 3β-hydroxysteroid-Δ(8),Δ(7)-isomerase (emopamil-binding protein) and B-cell receptor associated-protein 31. Other binding partner candidates include IER3IP1, PPIB, UPF0480 protein C15orf24, SPINT2, C14orf1/PEBP28, NIFIE14, YIPF6, KCP2, SLC31A2, SLC35B1, SLC39A13, CRACM1, MITCH2, ACCA1, UBB, ATX2L, HSP7C, and TBB. These are mostly proteins integral to membranes constituting the endoplasmic reticulum (ER) and Golgi apparatus (GA; Figure 2(a)) and playing a role in proteoneogenesis, proper folding, maturation, secretion, anterograde transport, and the regulation of apoptosis (Figure 2(b)). Interestingly, among the binding partners of A1 other members of the SLC superfamily involved in lysosomal Cu2+, GA Zn2+ and ER sugar-transport have been identified. Nestler et al. therefore speculate that these proteins undergo similar modifications with regard to their maturation (posttranslational modification in ER and GA) as A1.45

So far, no information is available about the complex-forming abilities of A2 and A3.

Goytain and Quamme24 have proposed N-glycosylation as a possible posttranslational modification of A1. The NetNGlyc 1.0 prediction machine (DTU) indeed recognizes four putative glycosylation sequons within the sequence of A1. Three of them are located within the N-terminal cytoplasmic domain, and only the fourth is located in the putative extracellular loop (aa463–aa483; TMPred at ch.EMBNet.org). Therefore, the identification of a component of oligosaccharyltransferase (KCP2) among putative A1-binding partners further substantiates N-glycosylation as a possible posttranslational modification of A1.45

Several functional studies have demonstrated that phosphorylation mediated via cAMP-activated protein kinase A (PKA)61 and PKC64 plays a key role in the regulation of Na+/Mg2+ exchange. Indeed, Kolisek et al.3 have shown that phosphorylation mediated by cAMP-activated PKA is an important event for the activation of A1-mediated Mg2+ efflux (Na+/Mg2+ exchange). The N-terminal flanking sequence of A1, which seems to be an important regulatory region,3,6,44 possesses, in addition to the predicted PKC-phosphorylation sites, multiple putative phosphorylation hotspots for p38MAPK, cdc2, GSK3, cdk5, DNAPK, and CKII (NetPhos 1.044). Involvement of these protein kinases in the regulation of A1 function remains to be explored.

Moreover, ubiquitination seems to play a role in the regulation of A1 turnover. Computer prediction has revealed ubiquitination hotspots at positions 4, 8, 58, 92, 146, and 339 (http://bdmpub.biocuckoo.org). The role of ubiquitination in the turnover/degradation of A1 has been substantiated by MALDI-TOF-MS analysis combined with in-gel trypsin digestion, performed on Coomassie-stained protein bands resulting from the electro-separation of affinity-purified strep-A1 (performed under native conditions) and its potential binding partners.45 Nestler et al. have demonstrated that a protein band with a molecular mass well exceeding 170 kDa comprises A1, ACCA1, and ubiquitin, therefore, indicating that A1, or ACCA1, or both are prone to ubiquitination.45
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SLC41 FAMILY OF Mg$^{2+}$ CARRIERS

Experimental data in favor of two mechanisms have been presented in the literature (ion channel$^{24}$; or ion exchanger$^{2,3}$). Structural data have revealed that ion channels form aqueous pores across the plasma membrane, whereas exchangers can have either large aqueous vestibules reaching deep into the bilayer of the membrane or form hourglass-like funnels with a narrow, and possibly water-excluding selectivity filter.$^{46}$ Channels and exchangers can be defined and distinguished by biophysical characteristics, such as ion flux rates (slower in exchangers), temperature-sensitivity (more so in exchangers), and dependence on both membrane voltage and concentration gradients of the transported ion species.

Several reports have presented experimental data suggesting that A1, A2, and A3 represent Mg$^{2+}$-transport proteins.$^{2,3,5,6,8,24,43}$ A1 and A2 were originally considered to be putative Mg$^{2+}$ transporters based on distant sequence homologies with the prokaryotic Mg$^{2+}$ transporter MgtE.$^1$ Their initial functional characterizations used complementary RNA (cRNA) expression in *Xenopus* oocytes and membrane current recordings with two-electrode voltage-clamp (TEVC).$^5,8,24$ As the original A3 data were presented within a review article,$^8$ only limited information is available about buffers composition and experimental procedures. Therefore, these will not be further discussed. Currents were compared between oocytes injected with H$_2$O and A1 or A2 cRNA. The membrane potential was held at $-15$ mV and currents were elicited with voltage steps of 2-second duration and ranging from $-150$ mV to $+25$ mV. Increasing extracellular Mg$^{2+}$ concentrations from 0.2 mM to 10 mM gave rise to a saturable inward current at hyperpolarized potentials with an assessed reversal potential of about $-20$ mV. Outward currents also seemed to increase in parallel, although this was less clear because of the absence of recordings above $+25$ mV. Importantly, when using extracellular divalents other than Mg$^{2+}$, the oocyte experiments delineated a decrease in inward currents, with Ca$^{2+}$ being particularly ineffective in eliciting any measurable currents. A1 and A2 were concluded to represent an Mg$^{2+}$-transport mechanism with channel-like properties,$^{5,24}$ as the presented data could accommodate both, a constitutively active ion channel or an electrogenic ion exchange mechanism. Unfortunately, kinetic current data that could have shed light on this issue were not provided in these publications$^{5,24}$ and hence no definitive conclusion can be reached.

One way to differentiate between an ion channel and ion exchanger mechanism is to exploit the mandatory presence of both substrates for ion exchange to
Occur. Ion channels do not have this prerequisite as long as the permeating ion is available in sufficient quantities to move down its concentration gradient. The study of A1 by using Xenopus oocyte TEVC tested for the possibility that A1 could be NME by removing Na\(^{+}\) from the extracellular solution.\(^{24}\) This experimental manipulation did not alter the measured inward currents and thus favored the concept of a constitutively active ion channel mechanism, if one assumed that the inward currents were indeed carried by A1. However, one of the major limitations of the TEVC Xenopus oocyte system is the inability to control experimentally the internal milieu of the oocyte. Thus, any exogenously introduced protein could potentially activate unrelated endogenous currents such as swelling- and/or Ca\(^{2+}\)-activated chloride (Cl\(^{-}\)) currents. As is well known, oocytes injected with Ca\(^{2+}\), and to some extent with other divalent ions, induce Cl\(^{-}\) currents\(^{47-49}\) and the expression of A1 or A2 might lead to such activation. In this context, it should be mentioned that the overexpression of A1 in HEK293 cells leads to the secondary activation of endogenous ATP-sensitive Cl\(^{-}\) currents.\(^{2}\) The removal of external Cl\(^{-}\) from the solution, as mentioned in the experimental approach for A1\(^{24}\) does not help clarify whether the measured inward currents are carried by divalent ions or Cl\(^{-}\) ions, as Cl\(^{-}\) is a negatively charged ion and inward currents would reflect Cl\(^{-}\) efflux. For the same reason, the experiments in which Na\(^{+}\) was removed also remain open to this alternative explanation.

The first, and so far only, attempts at measuring A1 or A2 related whole-cell currents by using the patch-clamp technique were performed in tetracycline-inducible cell systems.\(^{2,6,43}\) Whereas no obvious currents could be seen in A2-expressing DT40 chicken B-cells,\(^{6}\) overexpression of A1 in HEK293 cells caused a secondary activation of an endogenous ATP-sensitive Cl\(^{-}\) current that was completely inhibited by the Cl\(^{-}\) channel blocker DIDS.\(^{2}\) The latter experimental manipulation did not uncover any additional currents. Regardless, A1 or A2 expression clearly enables Mg\(^{2+}\) transport, as supercharging extracellular Mg\(^{2+}\) levels to 10 mM leads to an obvious increase in intracellular Mg\(^{2+}\) concentrations\(^{2,6,43}\) and Mg\(^{2+}\)-transporter-deficient bacteria (Salmonella sp.) survive a Mg\(^{2+}\)-deprived environment when overexpressing A1.\(^{2}\) The greatest challenge of the ion channel hypothesis relates to the gating of the protein. If one takes the data acquired by TEVC in the oocyte system at face value and if the measured currents are carried by A1/A2, one would have to consider SLC41 proteins as being constitutively active ion channels, since removal of external Na\(^{+}\) had no apparent effect on the currents and would therefore seem to exclude an electrogenic exchange mechanism.\(^{5,24}\) However, experiments performed using whole-cell patch-clamp conditions do not give rise to any measurable currents,\(^{2,6}\) indicating that the SLC41 proteins are not constitutively active ion channels. Instead they might represent an ion channel mechanism whose
gating mechanism awaits discovery or they are carriers that produce electrophelial ion exchange and remain undetectable electrophysiologically.

The most straightforward evidence implicating A1 as a Mg\(^{2+}\) transporter has emerged from functional complementation experiments in Mg\(^{2+}\)-transport-deficient Salmonella sp. strain MM281 transformed with pUC18-hSLC41A1.\(^2\) Here, an increase in extracellular Mg\(^{2+}\) concentrations to 10 mM significantly enhances intracellular Mg\(^{2+}\) concentrations. Given an experimental Na\(^{+}\) concentration of 0.5 mM [Na\(^{+}\)], with an estimated 3 mM [Na\(^{+}\)], the driving ion in this situation is most likely to be Mg\(^{2+}\) with a concentration ratio of approximately 20 compared with a ratio of approximately 6 for Na\(^{+}\). This allows Mg\(^{2+}\) flux into the bacteria, as these typically have highly negative membrane potentials when metabolically active.\(^51\) The very negative membrane potential also helps to explain the partial rescue of Salmonella growth in only 100 μM extracellular Mg\(^{2+}\). Under these experimental conditions both Mg\(^{2+}\) and Na\(^{+}\) have similar concentration ratios (8 and 6, respectively) and with a theoretical reversal potential of NME around −26 mV under these conditions, the exchanger could potentially work in reverse mode, allowing Mg\(^{2+}\) influx and efflux of Na\(^{+}\).

The situation in mammalian cells, particularly in electrically nonexcitable cells, is more complicated. Here, Na\(^{+}\) is the relevant driving ion for Na\(^{+}\)/Mg\(^{2+}\) exchange, as the ratio of its concentration across the membrane is at least sixfold larger than that for Mg\(^{2+}\) (0.5 mM [Mg\(^{2+}\)]/[1 mM [Mg\(^{2+}\)] \text{vs} 12 mM [Na\(^{+}\)]/[145 mM [Na\(^{+}\)]). Since the direction of ion transport is defined by the equilibrium potential of the transported ion, A1 would normally extrude Mg\(^{2+}\) and import Na\(^{+}\) and reverse only when concentration gradients are experimentally manipulated to define artificially the driving ion species. This makes experiments with intact cells difficult to interpret, as the intracellular milieu is not easily manipulated, and alterations of extracellular ion conditions need to consider resulting changes in the transporter’s reversal potential, the direction of transport, the relationship between driven and driving ion species and even the ability to transport ions. Considering that resting HEK293 cells may have a membrane potential of around −15 mV,\(^52\) a resting Mg\(^{2+}\) concentration of 0.2 mM,\(^53\) and that the external Na\(^{+}\) is usually high (145 mM), at the physiological level of 1 mM external Mg\(^{2+}\), Na\(^{+}\) will be the driving ion. The relatively positive reversal potential (approximately +40 mV) for NME that can be assumed under these conditions would favor constant Mg\(^{2+}\) extrusion. Indeed, Mg\(^{2+}\) extrusion plays a critical role in transcellular Mg\(^{2+}\) transport as seen during Mg\(^{2+}\) reabsorption in the distal convoluted tubule of the kidney.\(^53\) This basolateral efflux of Mg\(^{2+}\) is thought to be Na\(^{+}\)-dependent.\(^54\)

Experimentally increasing external Mg\(^{2+}\) to high concentrations such as 10 mM would result in a shift of the driving ion from Na\(^{+}\) to Mg\(^{2+}\), which now would have an estimated concentration ratio of 50 (10 mM/0.2 mM), thus driving Mg\(^{2+}\) influx and transporting Na\(^{+}\) against its concentration gradient. This indeed has been observed in HEK293 cells with or without overexpression of SLC41A1, whereby the presence of SLC41A1 has an amplifying effect on Mg\(^{2+}\) influx.\(^5\) Furthermore, high external Mg\(^{2+}\) concentrations (15 mM) rescue cell growth and proliferation of TRPM7-deficient DT40 chicken B cells overexpressing A1\(^4\) or A2.\(^6\) The removal of external Mg\(^{2+}\) while maintaining Na\(^{+}\) levels should also make Mg\(^{2+}\) the driving ion species for the exchanger, resulting in Mg\(^{2+}\) efflux at more positive potentials. Furthermore, this process should be inhibited by simply removing the external Na\(^{+}\), and this is indeed observed in HEK293 cells overexpressing A1.\(^3\)

**SLC41A1 AS A Na\(^{+}/Mg\(^{2+}\) EXCHANGER**

The basal [Mg\(^{2+}\)], of most cells is maintained between 0.5 to 1.2 mM and measurable Mg\(^{2+}\) transport has long been known to take place only when it is decreased or increased, e.g., by extracellular Mg\(^{2+}\) deficiency or after hormonal stimulation. Thus, to overcome this problem for Mg\(^{2+}\)-transport studies, various methods such as Mg\(^{2+}\) depletion by incubation in Mg\(^{2+}\)-free media and artificial Mg\(^{2+}\) loading or the application of metabolic inhibitors\(^55\),\(^56\) must be used to generate conditions favoring the uptake or efflux of the ion.

As the first functional data concerning A1-related transport suggested a higher influx capacity in A1-overexpressing cells, Kolisek et al.\(^2\) designed their experiments to promote Mg\(^{2+}\) uptake. Cells were Mg\(^{2+}\)-depleted by incubation in totally Mg\(^{2+}\)-free media and most experiments were performed under inwardly directed Mg\(^{2+}\) gradients ([Mg\(^{2+}\)] \text{e} < [Mg\(^{2+}\)] \text{i}: 2, 5, or 10 mM). This lead to an [Mg\(^{2+}\)] \text{i} increase composed of an A1-independent linear component (most probably channel-mediated) and an A1-related temperature-sensitive component. The latter was insensitive to cobalt(III)hexaammine, a known inhibitor of bacterial/mitochondrial\(^14\),\(^57\) and mammalian\(^2\) Mg\(^{2+}\)-channels. Most interestingly, A1-overexpressing HEK293 cells, although Mg\(^{2+}\)-deficient showed a significant loss of Mg\(^{2+}\) when incubated under Mg\(^{2+}\)-free conditions ([Mg\(^{2+}\)] \text{i} > [Mg\(^{2+}\)] \text{e}: 0 mM). Measurements of the
total \([\text{Mg}]\) via atomic absorption spectroscopy (AAS) confirmed net \(\text{Mg}^{2+}\) transport showing a 12.7\% increase and 25.6\% loss of total \([\text{Mg}]\) after a 20-min incubation in a medium containing 5 mM or zero \(\text{Mg}^{2+}\), respectively. Together, these results characterize A1 as a functional \(\text{Mg}^{2+}\) carrier and give the first evidence for the ability of the protein to mediate the efflux of the ion.

Kolisek et al.\(^3\) have recently performed a second study with A1-overexpressing HEK293 cells to test whether the protein represents the molecular correlate of one of the postulated \(\text{Mg}^{2+}\)-efflux systems. As any efflux activity is dependent on the \([\text{Mg}^{2+}]\),\(^5\) experiments were carried out with HEK293 cells that had been \(\text{Mg}^{2+}\)-loaded by a 20-min incubation in a 10 mM \(\text{Mg}^{2+}\)-containing medium. The A1-related \(\text{Mg}^{2+}\) extrusion was not directly linked to anion (\(\text{Cl}^-, \text{HCO}_3^-\)) transport. However, it showed typical features of NME such as (1) activation by an increased \([\text{Mg}^{2+}]\),\(^5\) (2) strict dependence on extracellular \(\text{Na}^+\),\(^5\)\(^9\)\(\)\(^6\)\(^0\)\(^2\)\(^3\)\(^4\)\(^5\)\(^6\)\(^7\)\(^8\)\(^9\)\(^\circ\)\(^\text{inhibition by imipramine and quinidine}\(^6\)\(^1\)\(^\circ\) and, (4) regulation via cAMP-dependent PKA.\(^6\)\(^1\) Thus, unquestionably, A1 represents NME, the predominant \(\text{Mg}^{2+}\)-efflux system in mammalian cells.\(^6\)\(^0\)\(^2\)

As known for other \(\text{Na}^+\)-dependent exchangers (e.g., \(\text{Na}^+/\text{Ca}^{2+}\) antiport, \(\text{Na}^+/	ext{H}^+\) exchanger), the driving force for A1-mediated \(\text{Na}^+/\text{Mg}^{2+}\) exchange is, in the first instance, the inwardly directed concentration gradient for \(\text{Na}^+\).\(^3\) This makes the transporter directly dependent on extracellular \(\text{Na}^+\) and indirectly dependent on the \(\text{Na}^+/\text{K}^+\)-pump and thereby on ATP.\(^3\) In comparison with controls, A1-overexpressing HEK293 cells have a high \([\text{Na}^+]\) (about 40 mM) but reduced \([\text{Mg}^{2+}]\) (about 0.1 mM). Therefore, increasing the extracellular \(\text{Mg}^{2+}\) concentration will reduce the driving force for an electroneutral \(\text{Mg}^{2+}\) efflux and makes it possible that the exchanger switches to the reverse mode, thereby performing \(\text{Mg}^{2+}\) uptake. This mode of operation has been shown in erythrocytes, heart muscle cells, and rumen epithelium cells\(^6\)\(^3\)\(\)–\(^6\)\(^6\) and might explain difficulties in identifying the natural function of A1 in some studies.\(^4\)

In general, for a correct interpretation of the results of functional studies, consideration of the functions of cells and/or tissues is essential. For example, in view of the function of NME for \(\text{Mg}^{2+}\) transport across the basolateral membrane of intestinal, renal, and placental epithelia,\(^6\)\(^7\)\(^6\)\(^8\) its transcription upregulation in specific cell types, e.g., renal distal tubule cells exposed to low \(\text{Mg}^+\),\(^8\) is not surprising. \(\text{Mg}^{2+}\) influx systems such as TRPM6/TRPM7 or MagT1 are rapidly inactivated at normal \(\text{Mg}^{2+}\) concentrations. Thus an increased expression/activity of A1 could be interpreted as a mechanism to maintain a driving force for \(\text{Mg}^{2+}\) uptake under Mg-deficient conditions.\(^6\)\(^9\) For this, only local changes of \([\text{Mg}^{2+}]\), are sufficient; these can also be induced by the hormonal activation of A1\(^7\)\(^0\).

**SLC41 FAMILY AND ITS INVOLVEMENT IN HUMAN DISEASES**

Systemic and intracellular magnesium insufficiency has long been suspected to contribute to the development and progress of cardiovascular, metabolic, neurological (neurodegenerative), and psychiatric diseases and also conditions associated with pregnancy such as preeclampsia and eclampsia.\(^7\)\(^1\)\(^7\)\(^2\) Several reports have recently been published linking A1 (as a key component of cellular and perhaps also of systemic \(\text{Mg}^{2+}\) homeostasis) with serious human illnesses.

Among the most exciting findings has been the discovery that A1 is part of a novel Parkinson’s disease (PD) susceptibility locus PARK16. In this regard, Tucci et al.\(^7\)\(^3\) identified a rare coding variant of A1 p.A350V putatively linked to PD. This study was shortly followed by a report of Yan et al.\(^7\)\(^4\) identifying additional genetic variants of A1 present in a Chinese PD cohort, namely p.L146G, p.P480P, and c.552+50G>A. Interestingly, a conditional knockout mouse line, designated as Slc41a3tm1a(KOMP)Wtsi (http://www.knockoutmouse.org/martsearch/search?query=Slc41a3), which was generated as part of the International Knockout Mouse Consortium program, displays abnormal locomotor coordination (http://en.wikipedia.org/wiki/SLC41A3).

Kolisek et al.\(^7\)\(^2\) have performed an analysis of the transcriptional activities of magnesium-responsive genes in human placenta samples from the Mexican Mestizo cohorts of normoevolutive women and preeclamptic women. They found A1 to be the only magnesium-responsive gene with deregulated expression in the placentas of preeclamptic woman, with A1 being significantly overexpressed in approximately 55\% of tested preeclamptic samples and in only 9.5\% of normoevolutive samples.\(^7\)\(^2\) These findings strongly suggest an important role of A1 in the pathophysiology of preeclampsia.

A recently identified variant of A1, c.698G>T, has been associated with the nephronophthisis-like phenotype (NPHP-LP).\(^4\) This mutation results in the skipping of exon 6 of A1, leading to an in-frame deletion of a transmembrane helix.\(^4\) Hurd et al.\(^4\) suggest that defects in the maintenance of renal \(\text{Mg}^{2+}\) homeostasis caused by mutated A1 lead to tubular defects that result in NPHP-LP.
CONCLUSION

At the molecular and physiological level A1 is the best-characterized protein out of the three members of the SLC41 family. From the experimental evidences it is obvious that A1 is an NME, a primary Mg\(^{2+}\)-efflux system (able to operate also in a reverse mode under specific physiological conditions), and not a cation (Mg\(^{2+}\)) channel as was assumed in the pilot report of Goytain and Quamme.\(^{24}\) Little is known about the function of A2 and A3 in the context of intracellular Mg\(^{2+}\) homeostasis or about the exact mode of their operation. However, based on the homology between A1 and the two other members of SLC41 family it could be assumed, that both A2 and A3 also function as Mg\(^{2+}\) (X\(^{+}\)) carriers.

Cellular localization and membrane topology of A2 and A3 also awaits further examination. Consensus is lacking on the orientation of the C-terminus of A1.

Nothing is known about complex-forming abilities of A2 and A3. A1 has been demonstrated to form transient heterooligomeric complexes with proteins probably being involved in its posttranslational maturation (modification), proper folding, anterograde transport, and vesicular sorting. The interactions of A1 and its binding partners identified with the split-ubiquitin yeast two-hybrid assay await further examination.

Phosphorylation plays an important role in the regulation of A1 Na\(^{+}/\)Mg\(^{2+}\)-exchange activity. The stimulating effect of cAMP-dependent PKA on A1 was already demonstrated. The effects of other protein kinases, which have been computer-predicted to phosphorylate A1, remain to be examined experimentally. Moreover, the predicted N-glycosylation and ubiquitination of A1 need further exploration.

No link has been established between A2 or A3 and any known disease. However, A1 has been identified to be a part of the PD susceptibility locus PARK16 and its genetic variants exclusively associated with PD have been identified, predominantly in Asian (Chinese) cohorts. Moreover, recently, a link between preeclampsia and elevated expression of A1 in placental tissues has been demonstrated. The A1 null mutation c.698G>T has been associated with the nephronophthisis-like phenotype. Further research will be needed to elucidate the exact role of A1 (or its variants) in the pathophysiology of these diseases. Disturbed Na\(^{+}/\)Mg\(^{2+}\) exchange has been associated with cardiovascular and metabolic diseases. Therefore, any linkage of A1 with such ailments in the future will be unsurprising. The wide spectrum of diseases involving A1 (or Na\(^{+}/\)Mg\(^{2+}\) exchange) might also indicate an important role of A1 in regulation of systemic Mg homeostasis.

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