Chapter 4
Magnesium Homeostasis in Mammalian Cells

Andrea M.P. Romani

Contents

A.M.P. Romani (✉)
Department of Physiology and Biophysics, School of Medicine,
Case Western Reserve University, 10900 Euclid Avenue,
Cleveland, OH 44106-4970, USA
e-mail: amr5@po.cwruy.edu

L. Banci (ed.), *Metallomics and the Cell*, Metal Ions in Life Sciences 12,
DOI 10.1007/978-94-007-5561-1_4,

Abstract Magnesium, the second most abundant cation within the cell, plays an important role in numerous biological functions. Experimental evidence indicates that mammalian cells tightly regulate cellular magnesium ion content through specific mechanisms controlling Mg^{2+} entry and efflux across the cell membrane and the membrane of various cellular organelles as well as intracellular Mg^{2+} buffering under resting conditions and following hormonal and metabolic stimuli. This chapter will provide an assessment of the various mechanisms controlling cellular Mg^{2+} homeostasis and transport, and the implications changes in cellular Mg^{2+} content play under physiological and pathological conditions.

Keywords cell membrane • cytoplasm • endoplasmic reticulum • Mg^{2+} homeostasis • Mg^{2+} transport • mitochondria

Please cite as: *Met. Ions Life Sci.* 12 (2013) 69–118

1 Introduction

Magnesium is the second most abundant cellular cation after potassium. Total magnesium ion concentrations ranging between 16 to 20 mM have been consistenly measured within mammalian cells by various techniques [1]. These Mg^{2+} levels regulate numerous cellular functions and enzymes, including ion channels, metabolic cycles, and signaling pathways. Despite significant progress, our understanding of how cells regulate Mg^{2+} homeostasis still remains incomplete for conceptual and methodological reasons. The slow turn-over rate for Mg^{2+} movement across the plasma membrane or other biological membranes in the absence of metabolic and hormonal stimuli, the absolute abundance of total and free Mg^{2+} within the cell, and the limited changes in free $[Mg^{2+}]$ have fostered the assumption that cellular Mg^{2+} concentration does not undergo significant changes over time as its level is more than adequate for acting as a cofactor for various cellular enzymes. As a consequence,

there has been limited or not interest in developing techniques and methodologies able to rapidly and accurately measure changes in cellular Mg^{2+} content.

In the last twenty-five years, however, an increasing number of experimental and clinical observations have challenged this assumption. More than one thousand entries in the literature indicate the occurrence of major fluxes of Mg^{2+} in either direction across the plasma membrane of mammalian cells following metabolic or hormonal stimuli, and point at Mg^{2+} as a key regulatory cation for a variety of cellular functions. In turn, the movement of Mg^{2+} across the cell membrane has resulted in appreciable changes in total and free Mg^{2+} level within the cell and specific cellular organelles. Genetic and electrophysiological approaches have identified several Mg^{2+} transport mechanisms that operate in the plasma membrane or in the membrane of cellular organelles such as mitochondria and Golgi. The increased interest in Mg^{2+} as a regulator of biological functions has advanced the development of new methodologies able to detect and measure changes in cellular Mg^{2+} content under specific physiologic and pathologic conditions in both animal models and human patients.

2 Cellular Mg^{2+} Distribution

Determinations of cellular Mg^{2+} content by electron probe X-rays microanalysis (EPXMA), ^{31}P NMR, selective Mg^{2+}-electrode, ^{13}C NMR citrate/isocitrate ratio or fluorescent indicators (Table I in ref. [1], and [3]) consistently indicate that total Mg^{2+} concentration ranges between 16 to 20 mM within mammalian cells [1,2], equivalent Mg^{2+} concentrations being localized within nucleus, mitochondria, and endo-(sarco)-plasmic reticulum. The presence of such a high Mg^{2+} concentration within the organelles has been explained with the tendency of Mg^{2+} to bind to phospholipids, proteins, nucleic acids, chromatin and nucleotides within the organelles. Consequently, between 0.8 to 1.2 mM, or 15% to 22% of the cellular Mg^{2+} content, is free within the cytoplasm and the lumen of organelles such as cardiac and liver mitochondria [4,5]. These values are not too different from the level of Mg^{2+} present as free in the extracellular space [1–3].

Similar free Mg^{2+} concentrations are postulated to be present within the nucleus and the lumen of the endo-(sarco)-plasmic reticulum although no direct determinations have been successfully carried out due to technical limitations. In the case of the nucleus, this assumption is based upon the porous structure of the nuclear envelope, which does not support the presence of an ionic gradient between cytoplasm and intranuclear environment. In the case of the endoplasmic (sarcoplasmic) reticulum, the luminal free [Mg^{2+}] cannot be reliably measured because of the competing effect of elevated millimolar Ca^{2+} concentration present within the organelle [6], and the high affinity of this cation (~50 μM) as compared to the affinity of Mg^{2+} (~1.5 mM) for Mag-Fura and Mag-Indo fluorescent dyes [7]. Cytoplasmic Mg^{2+} represents the last large and well detectable cellular Mg^{2+} pool. The majority of this Mg^{2+} pool (~4–5 mM) forms a complex with ATP, phosphonucleotides, and phosphometabolites [8]. Because of its abundance (~5 mM) and Mg^{2+}-binding affinity (K_d ~78 μM), ATP represents the largest metabolic pool able to bind Mg^{2+} within the

cytoplasm or the mitochondrial matrix [9], and maintain cytosolic *free* [Mg^{2+}] between 0.5–1 mM within these compartments [1]. Hence, it would appear that there is a very limited chemical gradient for Mg^{2+} between intracellular and extracellular environment, and between cytoplasm and the lumen of cellular organelles.

In cells devoid of cellular compartmentation (e.g., erythrocytes), Mg^{2+} buffering depends exclusively on ATP, phosphonucleotides and phosphometabolites, proteins, and metabolic pools. Three kinetically distinct Mg^{2+} binding pools have been observed in erythrocytes [10]: one low-capacity, high-affinity pool represented by cell proteins, including hemoglobin, and two pools that correspond reasonably well to ATP and 2,3-diphosphoglycerate (2,3-DPG) content, respectively [11]. Clearly, small changes in binding distribution can occur based upon the oxygenated or not oxygenated state of hemoglobin within the erythrocytes [12].

As for Mg^{2+} binding by other cellular proteins little information is available. Aside from hemoglobin [12], consensus sequence for Mg^{2+} binding has been observed in calmodulin [13], troponin C [14], parvalbumin [15], and S100 protein [16]. It is presently undefined whether other cytosolic or intra-organelle proteins bind Mg^{2+} and contribute to the total Mg^{2+} concentrations measured within mitochondria or specific regions of endoplasmic or sarcoplasmic reticulum. Bogucka and Wojtczak [17] have suggested the presence of two proteins in the inter-membraneous space of the mitochondrion binding Mg^{2+} with high affinity/low capacity and high capacity/low affinity, respectively. However, neither of these proteins has been identified. The presence of Mg^{2+}-binding sites has been reported for other cellular proteins, but we lack information about the actual role of these proteins in binding Mg^{2+} under physiological or pathological conditions. We also lack evidence for a consistent Mg^{2+}-binding sequence in these proteins. The basic assumption has been that asparagine and glutamate residues are the most likely amino acids able to form coordination bonds with Mg^{2+}. Lastly, the physiological relevance of Mg^{2+} binding by proteins has been challenged by the observation that parvalbumin *null* mice do not present hypomagnesemia or detectable changes in tissue Mg^{2+} homeostasis [18].

Finally, Mg^{2+} concentration in plasma and extracellular fluid is approximately 1.2–1.4 mM, one third of which is bound to proteins (e.g., albumin) or other biochemical moieties [19]. The comparison between this concentration and those reported within the cell supports the notion that chemical free [Mg^{2+}] across any mammalian biological membrane is at, or near *zero trans* condition. Because the electrochemical equilibrium potential for cellular free [Mg^{2+}] is ~50 mM under resting conditions [20], it is evident that mechanisms must operate in the cell membrane to maintain cytosolic *free* Mg^{2+} and total cellular Mg^{2+} content within the measured levels.

3 Mg^{2+} Transport Mechanisms

Many mammalian cells cultured in the presence of low or virtual *zero* $[Mg^{2+}]_0$ do not present a significant decrease in total or free Mg^{2+} content despite the large Mg^{2+} gradient imposed across the cell membrane [2,21]. Turnover rates ranging from

1 hour in adipocytes to several days in lymphocytes have been observed and attributed to structural and functional cellular differences [21]. Discrepancies can also be observed in the same cell types depending on experimental conditions or modality of isolation (e.g., *in situ versus* in culture, or freshly isolated). For example, ^{28}Mg equilibrium in cardiac ventricular myocytes can vary from 3 hours in the whole animal to 72–80 hours in dispersed cells incubated at 37 °C, to even longer periods of time for cells maintained at 20 °C [22–24], consistent with the notion that hormonal or humoral factors can influence Mg^{2+} transport, and the transport mechanisms have a specific Q10. Similar differences have been observed in freshly isolated [25] *versus* cultured [26] lymphocytes.

Clearly, the occurrence of slow Mg^{2+} turn-over in various cells has generated the erroneous impression that cellular Mg^{2+} content does not change, or changes at such a slow pace that it has no physiological relevance. This impression has been challenged and completely reversed by a large body of experimental evidence documenting the occurrence of large fluxes of Mg^{2+} across the plasma membrane of mammalian cells within minutes from the application of a metabolic or hormonal stimulus [21,27–29]. For example, lymphocytes [25,30], erythrocytes [31], cardiac myocytes [32,33], and liver cells [34–36] are several of the mammalian cells able to extrude a significant percentage (between 10% to 20%) of their total cellular Mg^{2+} content in less than 10 min from the application of an adrenergic stimulus. The flux amplitude and its rate support the presence and operation of powerful Mg^{2+} transport mechanisms in the cell membrane (see [29] for a list of cells). Surprisingly, these fluxes while large, have resulted in relatively small changes in free $[Mg^{2+}]_i$ in the majority of cells examined, suggesting that the operation of these Mg^{2+} transport mechanisms is tightly coupled with the ability of the cell to rapidly buffering the amount of Mg^{2+} extruded or accumulated [37,38].

These Mg^{2+} transport mechanisms can be cursorily divided into channels, which allow Mg^{2+} accumulation, and exchange mechanisms, which allow Mg^{2+} extrusion. The majority of the Mg^{2+} entry mechanisms are located at the cell membrane level. Two of the entry mechanisms favor Mg^{2+} accumulation into mitochondria and Golgi cisternae, respectively. Most of these entry mechanisms show modest selectivity for Mg^{2+} over other divalent cations. No information is available about the mechanisms favoring Mg^{2+} entry in the endoplasmic or sarcoplasmic reticulum, or Mg^{2+} extrusion across the cell membrane or the membrane of cellular organelles.

3.1 Channels

Mg^{2+} entry through channels was first observed in prokaryotes [39,40] and protozoan [41]. More recently, Mg^{2+} entry through channels or channel-like features has been observed in eukaryotic cells. The characterization of these Mg^{2+} channels, however, is far from being complete, and information about their regulation is still fragmentary, limiting our understanding of their relative contribution in regulating Mg^{2+} entry in specific cells or physio-pathological conditions.

3.1.1 TRPM Channels

TRPM7 [42] and TRPM6 [43] were the first Mg^{2+} channels identified in mammalian cells. While TRPM7 is ubiquitous and more in control of Mg^{2+} homeostasis in individual cells, TRMP6 is localized in the colon and the distal convolute tubule of the nephron, a distribution that emphasizes the role of this channel in controlling whole body Mg^{2+} homeostasis through intestinal absorption and renal resorption.

These two channels share several similarities in terms of structure and operation. Yet, they differ in various aspects ranging from location to hormonal modulation.

3.1.1.1 TRPM7:

Fleig's group was the first to report the involvement of TRPM7 in Mg^{2+} accumulation and cell growth [42]. Originally identified as LTRPC7, or long TRP channel 7, owing to the presence of a long amino acid extension outside the channel sequence [44], this channel was already known as CHAK1 (channel kinase 1) [45] because of the presence of an α-kinase domain at its C-terminus [45], and its functional homology to eEF2-kinase [46]. Shortly after the original report [42], Runnels et al. [47] confirmed that TRPM7 combines a channel structure with an α-kinase domain at the C-terminus.

Located at the locus 15q21 of the human chromosome 15, TRPM7 is formed by 1865 amino acids arranged in 10 trans-membrane domains with both the C- and N-termini internalized. Ubiquitously expressed, the channel would carry preferentially Mg^{2+} and Ca^{2+} [42], but also divalent cations such as Ni^{2+} and Zn^{2+} [48,49]. The functional structure of the protein is supposed to be a tetramer, but it is unclear whether the channel is a homotetramer or a hetero-tetramer with varying stoichiometry combination of TRPM7 and TRPM6 monomers. Voets and colleagues [50], for example, reported the functional expression of TRPM6 channels in HEK-293 cells with electrophysiological properties similar to those of TRPM7. In contrast, Chubanov et al. [51] reported no electrical conductance through TRPM6 when this channel is expressed by itself in HEK-293 cells or in *X. laevis* oocytes, and suggested that TRPM7 needs to be co-expressed with TRPM6 for the latter to be incorporated into channel complexes in the cell membrane. Although the association of TRPM6 and TRPM7 channel proteins in a functional structure has been confirmed by Schmitz et al. [52], the functional characterization of TRPM6/TRPM7 chimeras has remained controversial [53] until Yue's group demonstrated that (i) TRPM6 and TRPM7 do form a heterotetramer, and (ii) pure TRPM6, pure TRPM7, and TRPM6/TRPM7 chimeras constitute three distinct ion channel entities with different divalent cation permeability, pH sensitivity, and unique single channel conductance [54,55]. This group also reported that the activities of TRPM6, TRPM7, and TRPM6/TRPM7 can be differentiated by using 2-2-aminoethoxydiphenyl-borate (2-APB), which markedly increases Mg^{2+} and Ca^{2+} entry through TRPM6 [54]. These results support the notion that TRPM6 can form functional homotetrameric channels but also heterotetrameric channels with TRPM7 [56]. As the tissue distribution of these channels is

still incomplete, it is possible that homotetrameric TRPM6, homotetrameric TRPM7, and heterotetrameric TRPM6/TRPM7 channels play different roles in diverse tissues under physiological or pathological conditions.

Recently, our understanding of TRPM7 regulation has registered some progress. Protons markedly enhance TRPM7 inward current by competing with Ca^{2+} and Mg^{2+} for binding sites within the channel pore, releasing the blockade of inward monovalent currents by divalent cations [48,49]. At physiological pH, Ca^{2+} or Mg^{2+} bind to TRPM7 and inhibit monovalent cation currents. At higher H^+ concentrations, instead, the protons decrease the affinity of TRPM7 for Ca^{2+} and Mg^{2+}, allowing monovalent cations to permeate the channel [57]. Another level of regulation is provided by PIP2. Initially reported by Clapham's group [58], this regulation has been confirmed by other groups [59–61], which have established that addition of exogenous PIP2 decreases TRPM7 run-down whereas activation of phospholipase C (PLC) by phenylephrine accelerates it [60]. TRPM7 activity is also modulated by ATP [61] and non-hydrolyzable GTP analogs [60], most likely through the formation of $Mg(ATP)^{2-}$ [61] and accelerated PLC-mediated channel run-down, respectively [60]. Bradykinin or angiotensin-II, which also activates PLC, exhibit a similar PIP2-mediated modulation of TRPM7 [59,62]. Activation of TRPM7, however, only occurs in the presence of a physiological cellular $[Mg^{2+}]_i$, any reduction in this concentration resulting in inactivation of TRPM7 activity via PIP2 depletion [59]. All together, these results suggest that PLC-activation accelerates TRPM7 'run-down' via PIP2 depletion. Alternatively, PIP2 depletion would play a feed-back regulation on TRPM7 activation by PLC [59].

A functional TRPM7 is required for a sustained phosphoinositide-3-kinase (PI3K)-mediated signaling in lymphocytes to the point that TRPM7-deficient cells rapidly down-regulate their rate of growth as a result of signaling deactivation downstream PI3-Kinase even in the presence of physiological $[Mg^{2+}]_0$ [63]. Furthermore, TRPM7 regulates the transition of lymphocytes from quiescent to proliferative metabolic state [64]. In contrast, TRPM7-deficient cells upregulate p27, exit cell cycle, and enter quiescence [64]. Because TRPM7 is widely expressed in immuno-competent cells, these results suggest that TRPM7 is essential to regulate rapid cell proliferation and possibly malignancy development.

TRPM7 has originally been identified based on the α-kinase activity present at its C-terminus [45], which phosphorylates serine and threonine residues within an α-helix [65]. Initially, this kinase domain was considered to be essential to modulate TRPM7 activity and gating [58]. Subsequent studies, however, indicated that TRPM7 channels lacking the kinase domain could still be activated by internal Mg^{2+} depletion [66]. Presently, it is undefined which signaling components induce channel opening and/or modulate the α-kinase domain. It is however, clear that kinase autophosphorylation plays a significant role in target recognition by this domain [67]. Massive autophosphorylation of the TRPM7 kinase domain in a region rich in serine and threonine residues located immediately upstream the kinase catalytic domain increases the rate of substrate phosphorylation [68]. Deletion of this region does not affect the intrinsic catalytic activity of the kinase but prevents substrate phosphorylation, supporting the role of this region in substrate recognition [68].

This Ser/Thr region is poorly conserved in TRPM6 in terms of amino acid sequence. However, the kinase domain of TRPM6 appears to require a similar autophosphorylation of its Ser/Thr residues for proper recognition and efficient phosphorylation of the substrates [68].

One obvious consequence of generating TRPM7 lacking the kinase domain is the inability of TRPM7 to properly phosphorylate and activate downstream cellular targets. In support of this notion, homozygous TRPM7-deficient mice carrying the deletion of the kinase domain ($TRPM7^{\Delta Kinase}$) presented early embryonic lethality [69,70]. The heterozygous mice, instead, were viable but presented defective intestinal Mg^{2+} absorption and hypomagnesemia. Cells derived from these heterozygous mice presented reduced TRPM7 currents with an increased sensitivity to inhibition by Mg^{2+} [70]. Embryonic stem cells lacking the TRPM7 kinase domain showed arrest in proliferation and could be rescued by Mg^{2+} supplementation, validating the report by Scharenberg's group [64]. The relevance of the kinase domain in mediating TRPM7 signaling is confirmed by a recent publication by Perraud et al. [70]. These authors reported a role of TRPM7 in regulating the rate of protein synthesis based upon Mg^{2+} availability, and observed that phosphorylation of Thr^{56} residue on eEF2, which inhibits the protein activity, increases under hypomagnesemia. These authors also indicated that Mg^{2+} regulation requires an active and viable kinase domain in the TRPM7 protein [70]. The regulation of eEF2 by TRPM7 is indirect, occurring through eEF2 cognate kinase (eEF2-k), which becomes phosphorylated by TRPM7 kinase on Ser^{77} [70].

At the substrate level, myosin IIA heavy chain [69,71], calpain [72], and annexin I [73,74] have been identified as targets phosphorylated by the TRPM7 kinase domain. Thus, it appears that TRPM7 plays an important role in regulating cell adhesion, contractility or inflammation in different cells, in addition to its role in Mg^{2+} homeostasis. For example, TRPM7 regulates neuronal function and survival under hypoxia or ischemia-reperfusion conditions. Because it can transport either Ca^{2+} or Mg^{2+}, TRPM7 exhibits an ambivalent role based upon the permeating cation. Following activation by reactive oxygen/nitrogen species and prolonged oxygen and glucose deprivation, TRPM7 favors Ca^{2+} fluxes that result in a toxic event for neurons [75]. In contrast, Mg^{2+} permeation enhances anti-apoptotic and cell survival mechanisms, preventing anoxic death of the neurons [67,76]. Following 1 hour occlusion of middle cerebral artery, TRPM7 expression in ipsilateral hippocampus is enhanced, with deleterious consequences for the neurons [77]. Pretreatment of neurons with nerve growth factor activated the TrkA pathway and counteracted both the increase in TRPM7 expression and its harmful consequences [77]. Cell death can be prevented by blocking TRPM7 current via 5-lipoxygenase inhibitors [78]. This block occurs without changes in protein expression and cell membrane concentration [78]. TRPM7 also promotes the specific secretion of acetylcholine at the synaptic level by favoring the fusion of cholinergic vesicles with the pre-synaptic membrane of parasympathetic fibers without any effect on dense core vesicle secretion [79].

The effect of TRPM7 on cell proliferation and differentiation as observed in endothelial cells [76] also extends to osteoblasts [80,81]. Expression of TRPM7

increases during osteoblast differentiation, suggesting a role of cellular Mg^{2+} on cell differentiation. Culturing osteoblasts in low extracellular Mg^{2+} or Ca^{2+} significantly reduces their differentiation [82]. Matrix mineralization is also reduced under these conditions while expression of collagen type I, predominant in the extracellular matrix, increases [80,81]. Osteoblastic differentiation and extracellular matrix mineralization are affected to a comparable extent by TRPM7 silencing during the differentiation stage, further connecting cellular Mg^{2+} homeostasis with TRPM7 expression and activity. Expression of the osteoblastic transcription factor Runx2 was also reduced in cells maintained in the presence of low $[Mg^{2+}]_0$, or by TRPM7 silencing [80]. All together, these results indicate that cellular Mg^{2+} and Ca^{2+} homeostasis via TRPM7 are important for osteoblastic differentiation. It remains to be determined to which extent Mg deficiency in the general population, which is more common than anticipated, associates with altered osteoblastic differentiation and inadequate bone formation and osteoporosis development.

Data by the Clapham's group, however, have casted some doubts about the effective role of TRPM7 in regulating Mg^{2+} entry and homeostasis [82]. This group has observed altered embryonic development and tissue specific deletion of the channel in T cell lineage in TRPM7 *null* mouse, with disrupted thymopoiesis and progressive depletion of thymic medullary cells in the absence of significant changes in acute Mg^{2+} accumulation or total Mg^{2+} content in the T cells. Absence of TRPM7, however, significantly dysregulated the synthesis of several growth factors altering thymic epithelial cells differentiation [82]. Hence, it appears that TRPM7 is the first TRP channel with an essential, non-redundant role in embryogenesis and thymopoiesis. It is still unclear, however, how TRPM7 absence alters T cells differentiation.

3.1.1.2 TRPM6:

The TRPM6 channel is uniquely localized in the colon and the renal distal convolute tubule, two epithelia highly impermeable to salt re-absorption. This specific localization supports the specific role of this channel in controlling whole-body Mg^{2+} homeostasis by regulating intestinal Mg^{2+} absorption and renal Mg^{2+} reabsorption.

The *TRPM6* gene was originally identified by genetic analysis as the site of various mutations responsible for Hypomagnesemia with Secondary Hypocalcemia (HSH, OMIM 602014). A rare autosomal recessive disease, HSH is characterized by Mg^{2+} and Ca^{2+} wasting, a serum Mg^{2+} level around 0.5–0.6 mmol/L, or half the physiological level despite massive intravenous and oral Mg^{2+} administration [43]. Because the primary defect is at the level of the TRPM6 channels expressed in the intestine [43], any excess in Mg^{2+} supplementation is rapidly filtered at the glomerular level and results in increased passive renal absorption via paracellin-1 (see Section 3.1.2). Transcellular absorption via apical TRPM6 channels in the renal epithelium, however, remains depressed and unable to restore physiological serum Mg^{2+} level [43].

Experimental evidence suggests that TRPM6 also forms a functional tetramer at the plasma membrane level [54]. Several point mutations in the TRPM6 amino acid sequence have been identified [83], which result in the expression of a truncated and non-functional channel [83]. The missense mutation $S^{141}L$, for example, which occurs at the N-terminus of the channel, prevents the correct assembly of TRPM6 as a homotetramer, or a heterotetramer with TRPM7 [83]. The missense mutation $P^{1017}R$, instead, occurs in the pore region of the channel, but affects negatively and more significantly TRPM7 when it is co-expressed with TRPM6 [83]. TRPM6 *null* mice have also been developed [84]. Aside for low Mg^{2+} level in plasma (~0.67 *versus* 0.75 mM), heterozygous *Trpm6*$^{+/-}$ mice present normal electrolyte levels, whereas the majority of the homozygous *Trpm6*$^{-/-}$ mice die by embryonic day 12.5 [84,85]. Few animals survive to term, and the majority of them present exencephaly, spina bifida occulta, and other significant neural tube defects. Administration of a high Mg diet to dams allows for offspring survival to weaning [86], althought this aspect has not been confirmed in a more recent study [87].

Similarly to TRPM7, TRPM6 presents an α-kinase domain at the C-terminus with functional homology to eEF2-kinase, whereby the protein was originally termed CHAK2 (channel kinase 2) [46]. This kinase domain phosphorylates serine and threonine residues located within an α-helix [45,46,65]. Owing to their dual function as a channel and a kinase, TRPM6 and TRPM7 are referred to as *chanzymes*. As for TRPM7, removal of the kinase domain does not abolish TRPM6 activity but modulates the extent to which the channel is regulated by intracellular free Mg^{2+} or $Mg(ATP)^{2-}$ complex [51–54,86], and affects the ability of the chanzyme to phosphorylate downstream targets. At variance of what was reported for TRPM7, no specific substrate phosphorylated by TRPM6 kinase has been identified, with the exception of TRPM7 itself. While the TRPM6 kinase domain can phosphorylate residues on the TRPM7 channel within a heterotetramer structure, TRPM7 kinase cannot phosphorylate residues on TRPM6 [52]. Hence, it remains to be clarified whether the TRPM6 kinase domain phosphorylates substrates similar to, or different from those associated with the TRPM7 kinase domain.

At variance with TRPM7, TRPM6 appears to be sensitive to changes in estrogen level and dietary Mg^{2+} intake. Estrogens (17β-estradiol) selectively up-regulate TRPM6 mRNA in both colon and kidney, leaving unaffected TRPM7 mRNA in other tissues [87,88]. In the absence of estrogen, the repressor of estrogen receptor activity (REA) binds to the 6th, 7th, and 8th β-sheets of the TRPM6 kinase domain in a phosphorylation-dependent manner, and inhibits its activity [90]. Estrogen administration rapidly dissociates REA binding, resulting in increased TRPM6 activity [90]. Dietary Mg^{2+} restriction also up-regulates TRPM6 mRNA in both colon and kidney, but it has no effect on TRPM7 mRNA [88,89]. In contrast, exposure to Mg^{2+}-enriched diet up-regulates TRPM6 mRNA only in the colon, consistent with increased intestinal absorption [88]. These changes in TRPM6 expression and Mg^{2+} levels do not occur in mice exhibiting normal or high erythrocyte and plasma Mg^{2+} levels [89]. Therefore, it is becoming progressively apparent that

genetic factors control TRPM6 expression and activity, and that dietary Mg^{2+} restriction promotes a compensatory increase in Mg^{2+} absorption and reabsorption by enhancing TRPM6 expression in intestine and kidney, respectively [88,89].

Cellular ATP decreases TRPM6 current [51–54], as observed also for TRPM7. The site of inhibition resides in the conserved ATP-binding motif GxG(A)xxG within the α-kinase domain [89]. Full deletion of the kinase domain and point mutations within the ATP-binding motif ($G^{1955}D$) completely abolish the inhibitory effect of ATP. The effect of ATP, however, is independent of α-kinase autophosphorylation activity [86].

TRPM6 activity is modulated by cellular signaling molecules. Over-expression of RACK1 (receptor for activated protein kinase C) directly binds to the α-kinase domain of TRPM6, and possibly TRPM7 due to the >84% homology between the two kinase domains [90]. RACK1 binding site is located in the region corresponding to the 6th, 7th, and 8th β sheets, the same sheets involved in REA regulation [87]. Following RACK1 binding, TRPM6 (and possibly TRPM7) activity is inhibited. Channel activity is not suppressed when RACK1 is co-expressed with the α-kinase deleted TRPM6 mutant. The inhibitory effect of RACK1 depends on the autophosphorylation of threonine 1851 (T^{1851}), which is localized at the end of the 4th α-helix adjacent to the RACK1 binding site. Mutating T^{1851} to alanine ($T^{1851}A$) or aspartate ($T^{1851}D$) decreases TRPM6 autophosphorylation but does not affect RACK1 binding. The inhibitory effect of RACK1 on channel activity is abolished by $T^{1851}A$ mutation, while it is unaffected by $T^{1851}D$ mutation [90]. The latter mutation renders the kinase autophosphorylation directly proportional to the Mg^{2+} concentration, with a steady increase in the 0.1 to 1 mM range. The $T^{1851}A$ mutant, instead, is less sensitive to intracellular Mg^{2+} concentrations as compared to the wild-type (IC_{50} ~0.7 *versus* 0.5 mM, respectively). Activation of protein kinase C (PKC) by phorbol-myristate acetate (PMA) prevents the inhibitory effect of RACK1 on channel activity [90] whereas the PKC inhibitor chelerythrine restores the inhibition [90]. All together, these results suggest a competing effect of PKC for RACK1.

The epidermal growth factor (EGF) also acts as an autocrine/paracrine magnesiotropic hormone [91]. Following the receptor engagement in the basolateral domain of the distal convolute tubule, EGF activates TRPM6 at the cellular apical domain and promotes cellular Mg^{2+} accumulation. Point mutation in the pro-EGF sequence ($P^{1070}L$) retains EGF to the apical membrane of the cell and inhibits Mg^{2+} accumulation, resulting in Mg^{2+} wasting (isolated recessive renal hypomagnesemia or IRH syndrome, OMIM 611718). The axis EGF/TRPM6/Mg^{2+} reabsorption becomes altered in cancer patients undergoing treatment with antibodies anti-EGFR [92,93] as the block of the EGF receptor prevents TRPM6 activation and results in renal Mg^{2+} wasting [92,93]. EGF promotes TRPM6 activity and/or expression via ERK1/2 phosphorylation [94] and adaptin protein-1 (AP-1) signaling [95]. The process is prevented by antagonists for integrin $\alpha_v\beta3$ and MEK1/MEK2 activity, or siRNA for TRPM6 [94]. It is undefined whether this signaling axis releases RACK1-mediated inhibition of TRPM6 activity through PKC activation [90].

As TRPM6 is located in the apical domain of the intestinal and renal epithelium, it is unresolved how apically accumulated Mg^{2+} is transported across the cytoplasm to be delivered to the basolateral domain and be extruded into the blood stream. The general consensus is that baso-lateral Mg^{2+} extrusion occurs via a Na^{+}/Mg^{2+} exchanger (see Section 3.2.1). Uncertain is also as to whether Mg^{2+} is transported through the cytoplasm bound to proteins or diffuses freely. Parvalbumin and calbindin-D_{28k}, two proteins abundantly present within the cells of the distal convolute tubule of the nephron, could mediate trans-cellular Mg^{2+} transport, accelerating the delivery rate of the cation to the basolateral domain. However, no detectable defects in Mg^{2+} excretion or homeostasis are observed in parvalbumin *null* mice [17], raising some doubts on whether parvalbumin does play a role in transporting Mg^{2+} under physiological conditions, or other proteins can compensate for its absence in the *null* model.

3.1.2 Claudins

Genetic analysis of patients affected by Familial Hypomagnesaemia with Hypercalciuria and Nephrocalcinosis (FHHNC, OMIM 248250) identified paracellin-1 as the first mammalian protein able to transport Mg^{2+} [96]. FHHNC is characterized by massive renal Mg^{2+} and Ca^{2+} wasting that leads rapidly and irreversibly to renal failure [96] as symptoms and renal deterioration are not ameliorated by Mg^{2+} supplementation [96]. The gene responsible for the disease was termed *Paracellin-1* (*PCLN-1*) [96], which encodes for paracellin-1 (PCLN-1), a protein now renamed claudin-16. This protein is in fact a member of the claudin family [97], a group of tight junction proteins with 4 trans-membrane spans coordinated by 2 extracellular loops, and with both C- and N-termini on the cytoplasm side. More than 20 mutations affecting trafficking or permeability of claudin-16 have been currently identified [98].

Claudin-16 mediates paracellular Ca^{2+} and Mg^{2+} fluxes throughout the nephron. Yet, the modality by which these fluxes are generated is still controversial. Data obtained in LLC-PK1 (a renal cell line of porcine origin) indicate that claudin-16 mediates paracellular Na^{+} permeation which, in turn, generates a positive potential within the lumen of the nephron that acts as driving force for Mg^{2+} and Ca^{2+} reabsorption [99]. Data in MDCK cells, instead, point to an increase in Mg^{2+} and a decrease in Na^{+} permeability [100]. It is unclear whether these discrepancies reflect a different *modus operandi* in cell lines of differing origin, or depend on the experimental conditions utilized in the two studies. Either study, however, support the evidence that *PCLN-1* expression is modulated by $[Mg^{2+}]_0$ [101].

To properly function, claudin-16 has to be delivered to the tight junction where it interacts with the scaffolding protein ZO-1 [102]. Claudin-16.ZO-1 association and dissociation are regulated via PKA-mediated phosphorylation of Ser^{217} within claudin-16 sequence [102]. Activation of the Calcium Sensing Receptor (CaSR) dephosphorylates this residue [103], whereby claudin-16 dissociates from ZO-1 and accumulates within the lysosomal compartment [100]. Mutations of Ser^{217} accelerate claudin-16 turn-over and modulate its function. Mutation of Thr^{233} ($T^{233}R$) also

impairs the claudin-16/ZO-1 interaction, and favors claudin-16 accumulation into lysosomes [100,102].

Recent evidence indicates the involvement of claudin-19 isoform in mediating Mg^{2+} and Ca^{2+} reabsorption [104]. Claudin-19 forms a head-to-head complex with claudin-16 at the level of the tight junction, increasing cation selectivity. While claudin-16 function as a channel does not appear to depend on its association with claudin-19, claudin-19 is necessary to recruit claudin-16 and form a co-polymer at the tight junction level of the nephron and to switch channel selectivity from anion to cation [104]. The heteromeric association between claudin-16 and claudin-19 is affected by point mutations in claudin-16 ($L^{145}P$, $L^{151}F$, $G^{191}R$, $A^{209}T$, and $F^{232}C$) and claudin-19 ($L^{90}P$ and $G^{123}R$). Each of these mutations abolishes the physiological synergism between the two proteins, and results in FHHNC development.

3.1.3 MagT1

Human epithelial cells up-regulate MagT1 encoding gene following exposure to low $[Mg^{2+}]_0$ [105]. This protein has an estimated molecular weight of 38 KDa and 5 trans-membrane domains in its immature form. Following the cleavage of the first trans-membrane segment located near the C-terminus, the mature protein contains only 4 trans-membrane spans. At variance of SLC41 (Section 3.3.1) and Mrs2 (discussed in the next Section), MagT1 does not present any significant degree of homology to prokaryotic Mg^{2+} transporters, but it exhibits some similarities with the oligosaccharide transferase complex OST3/OST6 that regulates protein glycosylation in the endoplasmic reticulum of yeast [106]. The murine orthologue of MagT1 is highly expressed in liver, heart, kidney, and colon, with detectable levels in lung, brain, and spleen [105]. For the most part, MagT1 levels in these tissues are consistent with the mRNA levels, the only exception being the liver in which a low protein level is detected [105]. MagT1 appears to be highly specific for Mg^{2+} ($K_m = 0.23$ mM), and the Mg^{2+}-elicited currents are inhibited by Ni^{2+}, Zn^{2+}, and Mn^{2+} but not Ca^{2+}, although the inhibiting concentrations of any of these cations are >0.2 mM, thus exceeding the physiological concentration present in extra-cellular fluids. Nitrendipine at a concentration of ~10 μM, but not nifedipine, inhibits MagT1-mediated Mg^{2+} current [105]. Limited information is available about N33, a second member of the MagT family. Although able to transport Mg^{2+}, N33 exhibits a much lower specificity for Mg^{2+} than MagT1. In addition, N33 can also transport Fe^{2+}, Mn^{2+}, and Cu^{2+} [105].

MagT1 appears to possess channel-like characteristics and high selectivity for Mg^{2+}, suggesting that this transporter is essential to regulate Mg^{2+} homeostasis in mammalian cells. This hypothesis is supported by the observation that knocking out of MagT1 and its human homolog TUSC3 in HEK-293 cells markedly reduces cellular Mg^{2+} content [106]. Either MagT1 or TUSC3 can complement the yeast Mg^{2+} transporter ALR1 [106]. Exposure of HEK-293 cells to low $[Mg^{2+}]_0$ for 1–2 days increases the mRNA level of MagT1 but not TUSC3, whereas incubation in high $[Mg^{2+}]_0$ does not affect the expression of either protein [106].

3.1.4 Mrs2

Mrs2 was identified during a screening aimed at isolating nuclear genes suppressing RNA splicing defects in yeast mitochondrial introns [107]. Mrs2-deficient yeasts present: (i) a splicing phenotype, (ii) a significant reduction in cytochromes content, and (iii) a deficit in mitochondria respiration to the point that the yeasts become unable to grow on non-fermentable substrates [108].

Structurally, Mrs2 shows short regions of homology to the bacterial transporter CorA [109], and a similar membrane topology with 2 trans-membrane domains. Mutant yeasts lacking Mrs2 present a decrease in total mitochondrial and matrix free Mg^{2+} content [110], and can be rescued by mitochondrial targeted CorA. In contrast, Mrs2 over-expression results in a marked increase in matrix free Mg^{2+} [110]. Hence, suggestion is there for an essential role of Mrs2 in regulating mitochondrial Mg^{2+} homeostasis acting as a channel modulated by mitochondrial membrane potential ($\Delta\psi$). Inhibitors of F0-F1-ATPase or ANT also modulate Mrs2 activity, decreasing the amplitude of Mg^{2+} influx. Highly conserved motifs in the coiled-coil middle region of the channel are essential to gate it and form a functional unit. Knocking out Mrs in HEK-293 cells impairs mitochondrial complex I expression, reduces the level of mitochondrial Mg^{2+}, affects cell morphology and promotes apoptosis [111], to the point that cell viability is completely lost within 2 weeks [111]. It is unclear whether the decrease in mitochondrial Mg^{2+} depends on the absence of Mrs2, or is related to complex I absence, which affects mitochondrial $\Delta\psi$ and consequently Mg^{2+} retention within the organelle [112].

A single Mrs2 orthologue is expressed in mammalian cells, in which it mediates mitochondrial Mg^{2+} entry as the yeast homologue [113]. Under conditions in which Mrs2p is absent or not functional, an alternative but much slower mitochondrial Mg^{2+} entry mechanism becomes operative in restoring or maintaining Mg^{2+} homeostasis, ensuring the survival of Mrs2-deficient yeast. The identity, abundance, and regulation of this alternative transporter in mitochondria are presently unknown. All together, these data suggest that Mrs2 is essential but not indispensable to regulate mitochondrial Mg^{2+} level which, in turn, plays an essential role in modulating mitochondrial dehydrogenases and oxygen consumption [114,115].

3.1.5 MMgTs

This gene family comprehends two proteins termed MMgT1 and MMgT2 (for membrane Mg^{2+} transporter 1 and 2) [116]. The chromosomal locations of these proteins are XA5 for MMgT1 and 11B2 for MMgT2 in the mouse, and Xq36, and 10q23, respectively, in the rat. The human MMgT1 orthologue is located on Xq26.3 [117]. MMgT1 and MMgT2 are located in the Golgi complex and post-Golgi vesicles, in which they contribute to regulate Mg^{2+}-dependent enzymes involved in protein assembly and glycosylation [117]. The localization in the Golgi system, however, does not exclude that these proteins may be transported to the cell membrane or to other destinations downstream the Golgi network, where they can play a

role in modulating Mg^{2+} homeostasis. These proteins are formed by 131 (MMgT1) and 123 (MMgT2) amino acids, assembled into two trans-membrane domains in a wide variety of tissues.

The small size of these proteins suggests that they form homo- and/or hetero-oligomeric channels to favor Mg^{2+} permeation. MMgT-mediated Mg^{2+} uptake exhibits a $K_m \simeq 1.5$ mM for MMgT1, and $\simeq 0.6$ mM for MMgT2, and these values do not vary with voltage. Both MMgT1 and MMgT2 are not specific for Mg^{2+} as they can transport other cations with some slight differences in cation permeation. In addition to Mg^{2+}, MMgT1 transports Sr^{2+}, Fe^{2+}, Co^{2+}, and Cu^{2+}, while MMgT2 transports Sr^{2+}, Co^{2+}, Cu^{2+}, Ba^{2+}, and Mn^{2+} [116].

Limited information is currently available about the specifics of MMgT1 expression and operation, which can be summarized as follows: (i) Mg^{2+}-generated currents in MMgT1 are inhibited by Mn^{2+} (~0.2 mM) but not Gd^{3+} or Ni^{2+} [116], and (ii) MMgT1 mRNA increases ~2.5 fold in the kidney cortex of mice on low-Mg^{2+} diet and ~3.5 fold in MDCT epithelial cells culture in low Mg^{2+} medium whereas MMgT2 mRNA increases ~1.5-fold in kidney cortex and ~3-fold in MDCT cells under similar experimental conditions [116]. These changes in expression are not specific for these transport mechanism as similar increases have been observed for other Mg^{2+} entry mechanisms.

The audience interested in a more detailed description of the intrinsic characteristics of the various Mg^{2+} entry mechanisms described in this section is referred to recent reviews by Touyz et al. [62], Schmitz et al. [117], Bindels et al. [118], and Quamme [119].

3.2 Exchangers

While Mg^{2+} entry is mediated by channels or channel-like mechanisms, Mg^{2+} extrusion is mediated by two exchange mechanisms. The specific electrochemical requirements favoring Mg^{2+} extrusion indicate that these mechanisms operate as a Na^+-dependent and Na^+-independent Mg^{2+} exchanger, respectively. Most of the information about the operation, abundance and tissue specificity of these mechanisms is largely circumstantially or indirectly based upon experimental conditions or pharmacological inhibition.

3.2.1 Na^+-Dependent Exchanger (Na^+/Mg^{2+} Exchanger)

Günther, Vormann, and Forster provided the first evidence for the presence and operation of a Mg^{2+} transport mechanism in erythrocytes [120], and indicated that this transport mechanism elicits Mg^{2+} extrusion in a Na^+-dependent, amiloride-inhibited manner [121]. The operation of such a mechanism has been subsequently confirmed by other groups in a large variety of mammalian cells and tissues [12,122–126] (see also [29] for a list). Several laboratories [25,33,127,128] including ours

[32,34,129–134] have provided compelling evidence that this Na^+-dependent, amiloride-inhibited Mg^{2+} extrusion mechanisms is specifically activated by cAMP, irrespective of the modality by which the cAMP level is increased. Stimulation of β-adrenergic, glucagon, or PGE2 receptors, or administration of forskolin or cell-permeant cAMP analogs all results in Mg^{2+} extrusion via cAMP-mediated phosphorylation of the Na^+-dependent mechanism [127–134]. Inhibition of adenylyl cyclase by Rp-cAMP or PKA by PKI, instead, blocks Mg^{2+} extrusion irrespective of the receptor or the modality utilized to increase cAMP level [25].

To mediate Mg^{2+} extrusion this exchanger requires a physiological concentration of Na^+ in the extracellular milieu [129,131], suggesting that the mechanism occurs through a Na^+/Mg^{2+} exchanger. A recent report [135] infers that this Na^+/Mg^{2+} exchanger is the SLC41 transporter identified by Wabakken et al. [136] and discussed in detail in one of the next sections. However, no detailed information about membrane abundance, proximity to and interaction with other cellular transporters, and stoichiometry of this transporter is currently available. Günther, Vormann, and Forster suggested that this exchanger operates on electroneutral basis ($2Na^+_{in}$:1 Mg^{2+}_{out}) at least in chicken or turkey erythrocytes [120,121]. Data obtained in mammalian erythrocytes including human red blood cells have confuted this hypothesis, supporting an electrogenic operation ($1Na^+_{in}$:1 Mg^{2+}_{out}) [123–125]. The reason behind this discrepancy is not apparent, although it is possible that experimental models (i.e., cell isolation *versus* cultured cells), incubation medium composition, or modality of cellular Mg^{2+} loading can all contribute. Recently, we have reported that Mg^{2+} extrusion via the Na^+/Mg^{2+} exchanger is coupled to the outward movement of Cl^- ions [137]. In the absence of Cl^-, the exchanger switches from electrogenic ($1Na^+_{in}$:1 Mg^{2+}_{out}) to electroneutral ($2Na^+_{in}$:1 Mg^{2+}_{out}) [137]. Interestingly, only inhibitors of the Na^+/Mg^{2+} exchanger block Cl^- extrusion while specific inhibitors of Cl^- transporters (e.g., flufenamic acid, DNDS, or DIDS) are ineffective. A role of cellular Cl^- in stimulating the Na^+/Mg^{2+} exchanger in erythrocytes has been reported by Ebel and Günther [138]. Moreover, Rasgado-Flores and collaborators have observed Cl^- transport following reverse activation of the Na^+/Mg^{2+} exchanger in dialyzed squid axon [139]. Hence, the extrusion of Cl^- can be interpreted as an attempt to equilibrate charge movement across the hepatocyte or the axon membrane. However, it is unclear whether Cl^- extrusion occurs through the Na^+/Mg^{2+} exchanger directly, or through Cl^- channels present in the hepatocyte membrane [140] and activated by the exchanger.

Irrespective of the stoichiometry of exchange and the experimental model utilized, the results obtained by the various groups consistently indicate a K_m for Na^+ between 15 to 20 mM [141–143]. In terms of pharmacological inhibition, amiloride, imipramine, and quinidine represent the three most commonly utilized inhibitors of the Na^+-dependent Mg^{2+} extrusion [120,127,144]. Because of their limited specificity, however, it is unclear as to whether they inhibit the Na^+/Mg^{2+} exchanger directly, or indirectly by blocking other transport mechanisms including Na^+ and K^+ channels, and altering the cell membrane potential and driving force for Mg^{2+} transport across the plasma membrane.

Using a hybridoma screening procedure, the group of Schweigel, Vormann, and Martens has generated inhibiting monoclonal antibodies against the Na^+/Mg^{2+} exchanger present in porcine red blood cells. Western Blot analysis using these

antibodies has evidenced a ~70 KDa protein band [145]. This is the first time that information about the molecular size of the elusive Na^+/Mg^{2+} exchanger has been obtained. Hence, these antibodies could represent an ideal tool to identify and recognize this transporter in mammalian tissue.

3.2.2 Na^+-Independent Exchanger

In the absence of extracellular Na^+ to support the operation of the Na^+/Mg^{2+} exchanger, or in the presence of amiloride, imipramine or quinidine, which block the exchanger, Mg^{2+} extrusion occurs via an alternative, Na^+-independent mechanism. The specificity of this mechanism, however, is not defined. Cations such as Ca^{2+} or Mn^{2+}, and anions such as HCO_3^-, Cl^-, or choline [146,147], have all been observed to promote Mg^{2+} extrusion through this mechanism. Hence, it is unclear whether we are in the presence of a transporter that can operate as an antiporter for cations or a sinporter for cations and anions based upon the experimental conditions. Ebel and collaborators [147] have suggested that this Na^+-independent Mg^{2+} extrusion mechanism is the choline transporter based upon its inhibitability by cinchona alkaloids [147]. It is also unclear whether the Na^+-independent pathway is activated by hormonal stimulation. Stimulation of liver cells by epinephrine, a mixed adrenergic agonist, elicits an extrusion of Mg^{2+} that is equivalent to the sum of the amounts of Mg^{2+} mobilized by the separate stimulation of α_1- and β-adrenergic receptors [131,132,148]. The stimulation of α_1-adrenergic receptors by phenylephrine requires the presence of physiological concentrations of both Na^+ and Ca^{2+} in the extracellular medium to elicit Mg^{2+} extrusion from liver cells [131,132]. Since phenylephrine stimulation would activate Ca^{2+}-CaM signaling and capacitative Ca^{2+} entry to induce Mg^{2+} extrusion [132], it might be possible that the Ca^{2+}-CaM signaling pathway represents an alternative modality of activation of the Na^+/Mg^{2+} exchanger. Alternatively, it is possible that it activates a different Mg^{2+} extrusion mechanism identifiable with the Na^+-independent mechanism.

It is also controversial whether ATP is required for the operation of the Na^+-independent and Na^+-dependent mechanisms. Reports by Günther and collaborators [149,150] indicate a dependence of the Na^+/Mg^{2+} exchanger on the presence of a physiological concentration of cellular ATP to the point that cellular Mg^{2+} efflux is reduced under conditions that markedly decrease cellular ATP level [149,150]. In the case of red blood cells, Mg^{2+} homeostasis and transport are affected by changes in both ATP and 2,3-diphosphoglycerate levels [11]. The absence of a regulatory effect of ATP on Mg^{2+} extrusion has been confirmed in purified liver plasma membrane vesicles [143].

3.2.3 Mg^{2+}/H^+ Exchange

This exchange mechanism, originally identified in *A. thaliana* and termed AtMHX, is ubiquitous in plants [151]. It presents 11 putative trans-membrane domains, it is exclusively localized in the plant vacuolar membrane, and it electrogenically

exchanges Mg^{2+} or Zn^{2+} for protons. Following ectopic over-expression of the transporter, tobacco plants become able to grow in the presence of elevated concentrations of Mg^{2+} (or Zn^{2+}) [151]. Presently, no corresponding gene and encoded protein has been identified in mammalian cells, although some experimental evidence suggests direct or indirect exchange of Mg^{2+} for H^+ under well defined conditions [152]. An enhanced extrusion of cellular Mg^{2+} has been reported to occur in cells incubated in the presence of an acidic extracellular environment, which imposes an inwardly oriented H^+ gradient, as long as extracellular Na^+ is present [152,153]. Amiloride derivates, which inhibit the Na^+/H^+ exchanger with high affinity, are ineffective at blocking Mg^{2+} extrusion under these experimental conditions [154], thus excluding the involvement of the Na^+/H^+ exchanger in mediating Mg^{2+} extrusion either directly or indirectly through a coupling of this exchanger with the Na^+/Mg^{2+} antiporter.

3.3 Carriers

Several novel Mg^{2+} transport mechanisms of murine or human origin have been identified as a result of exposure to low Mg^{2+} in the diet (diet restriction) or in the culture medium (medium restriction). The *modus operandi* of these transport mechanisms is plagued by limited information. For practical reasons, these transport mechanisms are non-descriptively classified here as carriers.

3.3.1 SLC41 (Solute Carrier Family 41)

This family of Mg^{2+} transport mechanisms includes three members (A1, A2, and A3), all distantly related to prokaryotic MgtE channel [155]. Because no study has addressed function and structure of the SLC41A3 isoform, all the available information provided here refers to the SLC41A1 and A2 isoforms.

SLC41A1 was the first member of this family to be identified [136]. The hydrophobic profile of this protein (~56 kDa Mr) predicts the presence of 10 transmembrane domains, two of which presenting a discrete level of homology with MgtE [136]. Northern blot analysis indicates a broad distribution of the *SLC41A1* gene, but its abundance varies markedly among tissues, the highest expression being in heart and testis and the lowest being in hematopoietic tissues and cells [136]. The expression of this gene is modest under basal conditions, but becomes markedly up-regulated in the renal cortex of mice fed low Mg^{2+} diet for several days [156]. Functional expression of mouse SLC41A1 in *X. laevis* oocyte indicates that this protein can transport Mg^{2+} but also Fe^{2+}, Cu^{2+}, Zn^{2+}, and Cd^{2+}. In contrast, Ca^{2+} is not transported nor does it inhibit Mg^{2+} transport [156]. The initial observation suggested the presence of a Mg^{2+}-generated current, which would be consistent with SLC41A1 operating as a channel [156], or alternatively as an electrogenic antiporter. A recent report [157], however, strongly suggests that SLC41A1 operates as

a carrier in promoting Mg^{2+} efflux. This hypothesis is supported by another recent report [135] indicating this transporter as the putative Na^+/Mg^{2+} exchanger previously described.

Incubation of HEK-293 cells in Mg^{2+}-free media resulted in a significant reduction of total Mg^{2+} content and free cellular Mg^{2+} concentration ($[Mg^{2+}]_i$,), the amplitude of Mg^{2+} loss depending on the number of SLC41A1 molecules expressed in the membrane and the induction time. Lastly, the changes in $[Mg^{2+}]_i$ were sensitive to the experimental temperature but insensitive to the Mg^{2+} channel blocker CoHexamine [157]. Kolisek and collaborators also suggested that SLC41A1 forms high-molecular-weight complexes within the cell membrane with molecular masses ranging between 720 and 1236 kDa [157]. Addition of SDS resulted in the progressive degradation of the complexes in a step-wise manner until a protein band of ~56 kDa is obtained, which corresponds to the molecular mass of the SLC41A1 monomer [157]. It is presently undefined whether the SLC41A1 monomer aggregates to form large multimeric complexes or interacts with auxiliary proteins. The reason for the absence of Mg^{2+}-generated currents in this study as compared to the original observation by Goytain and Quamme [156] is also not clear. One possibility could be that the murine [156] and human orthologs [157] operate differently although they are expected to operate in a similar manner based upon their high degree (>90%) of homology. Yet, the possibility that point mutations can dramatically alter SLC41A1 ion specificity and modality of function cannot be completely dismissed. Another point of difference is that while Goytain and Quamme did not report a dependency of SLC41A1 operation on Na^+ or other cations or anions following expression in *X. laevis* oocytes [156], Kolisek and collaborators reported a marked Cl^- conductance following expression in HEK-293 cells, which was abolished by DIDS [157]. Whether this reflects the operation of additional transport mechanisms or the presence of structural differences in the cell membrane of HEK-293 cells [157] as compared to *X. laevis* oocyte [156] are possibilities that need further investigation.

A second isoform labeled SLC41A2 has been identified in both humans and mice. SLC41A2 transports Mg^{2+} as well as other divalent cations albeit with a different selectivity and inhibition profile than SLC41A1 [158]. In addition to Mg^{2+}, SLC41A2 can carry Ba^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, and Mn^{2+} but not Ca^{2+}, Cu^{2+} or Zn^{2+}. At variance of SLC41A1, Mg^{2+} transport via SLC41A2 is inhibited by Ca^{2+} [158]. Both SLC41A1 and SLC41A2 generate Mg^{2+} currents in *X. laevis* oocyte, and the ionic uptake is voltage-dependent with an apparent affinity of 0.75 mM and 0.31 mM, respectively [156,158]. SLC41A2 is also widely expressed in mammalian tissues, but its expression is not affected by low Mg^{2+} diet [158]. At the structural level, SLC41A2 shares >70% homology with SLC41A1 and it is supposed to present 10 trans-membrane domains as well. A recent study by Scharenberg's group, however, suggests a structural arrangement in 2 spans of five trans-membrane motifs each linked together by a supplementary spanning motif [159]. Hydrophobicity analysis indicates that the C- and N- termini are located on different sites of the cell membrane [159], a configuration that will be consistent with a total of 11 trans-membrane segments.

3.3.2 ACDP2

The human *ACDP* gene family was identified by Wang and collaborators [160] as a possible candidate of the urofacial syndrome. Mapped to the 10q23-10q24 chromosome, this gene family comprises 4 isoforms differentially located in human tissues. ACDP1 is essentially restricted to the brain. ACDP2 is more widely expressed, but still retains the highest expression in the brain while being absent in skeletal muscles. ACDP3 and ACDP4 are both ubiquitous, but have the highest expression in the heart [161]. The murine distribution of ACDP isoforms is very similar to that observed for the human orthologues [162]. Termed ancient conserved domain protein because all isoforms share one domain phylogenetically conserved from bacteria to man [160], these proteins are >50% homologous to the CorC transporter, which together with CorB and CorD plays a role in Mg^{2+} efflux in prokaryotes [163]. Over-expression of ACDP2 in *X. laevis* oocytes indicates that this protein can transport a variety of divalent cations including Mg^{2+}, Co^{2+}, Mn^{2+}, Sr^{2+}, Ba^{2+}, Cu^{2+}, and Fe^{2+}, whereas Zn^{2+} inhibits its activity [164]. Mg^{2+} transport via ACDP2 is voltage-dependent, occurs with a K_m of ~0.5 mM, and does not require the presence of extracellular Na^+ or Cl^- [164]. Similarly to SLC41A1, *ACDP2* gene becomes over-expressed following exposure to Mg^{2+}-deficient diet [164].

3.3.3 NIPA

Located in the SPG6 locus of chromosome 15q11-q13, the *NIPA1* gene is so called for 'non-imprinted in Prader-Willi/Angelman syndrome, a disease characterized by a complex developmental disorder that affects numerous organs and systems [165]. Located among a set of approximately 30 genes linked to the disease [165], *NIPA1* has also been implicated in autosomal dominant hereditary spastic paraplegia (HSP, OMIM 182600). The human and mouse genomes contain four members of the NIPA family, termed NIPA1 through NIPA4, with an overall similarity of ~40%. Homology between human and mice proteins is ~98%. NIPA1 [166] and NIPA2 [167] can both operate as Mg^{2+} transporters. Presenting a sequence of 323 (NIPA1) and 359 amino acids (NIPA2) arranged to form 9 and 8 trans-membrane spans, respectively, these two proteins transport Mg^{2+} in a saturable fashion, with different K_m and specificity. NIPA1 has a K_m ~0.66 mM for Mg^{2+} [166], but can also transport Sr^{2+}, Fe^{2+} or Co^{2+}, albeit to a lesser extent [166]. NIPA2, instead, is highly specific for Mg^{2+} with K_m ~0.31 mM [167]. Neither NIPA3 nor NIPA4 transports Mg^{2+}; NIPA3 transports Sr^{2+}, Ba^{2+}, Fe^{2+}, and Cu^{2+} while NIPA4 transport Sr^{2+} and Ba^{2+}.

The insurgence of autosomal dominant HSP is based on specific point mutations in NIPA1 (i.e., $G^{100}R$ or $T^{45}R$) [168]. Both glycine and threonine residues are conserved among ortholog NIPA1 channels in different species. There are no similar consensus sites in NIPA2, NIPA3, and NIPA4 paralogs, implying that the folding of these proteins might be different. In HSP patients, NIPA2 appears to be normal but it cannot functionally replace NIPA1 to ameliorate HSP symptoms, nor can NIPA3 or NIPA4 substitute for the defective NIPA1. This is surprising for NIPA2 as the encoding

gene is part of the 30 genes cluster associated with the Prader-Willi/Angelman syndrome together with *NIPA1*. Presently, there is no indication as to whether the Prader-Willi/Angelman syndrome presents alteration in Mg^{2+} homeostasis.

3.3.4 Huntingtin

Huntingtin-interacting protein 14 (HIP14) and its related protein HIP14-like (HIP14L) are significantly up-regulated (~3-fold) by low extracellular Mg^{2+} [169]. Formed by ~532 amino acids arranged in 6 trans-membrane spans, HIP14 presents 69% homology to HIP14L and a strong sequence similarity to the ankyrin repeat protein Akr1p [170]. HIP14 also possesses a cytoplasmic DHHC cysteine-rich domain. Defined by an Asp-His-His-Cys sequence motif this domain confers palmitoyl-acyltransferase activity to the protein, and gives it the ability to palmitoylate membrane components whereby modulating their structure. Mg^{2+} accumulation via HIP14 and HIP14L appears to be electrogenic, voltage-dependent, and saturable, with K_m ~0.87 and ~0.74 mM, respectively [169]. Inhibition of palmitoylation activity by 2-Br-palmitate, or deletion of the DHHC domain decreases HIP14-mediated Mg^{2+} accumulation by ~50%, suggesting that palmitoylation is not required for basal Mg^{2+} transport.

The widespread tissue distribution and intracellular localization of HIP14 (nuclear and perinuclear regions, Golgi complex, mitochondria, microtubules, endosomes, clathrin-coated and non-coated vesicles, and plasma membrane [171]) implicates this protein in numerous cellular processes including transcriptional regulation, mitochondrial bioenergetics, structural scaffolding, vesicle trafficking, endocytosis, and dendrite formation [171]. Golgi and post-Golgi vesicles, however, appear to be the primary location of HIP14 [169,171]. Hence, it can be hypothesized that Mg^{2+} accumulation via this protein is associated with the role HIP14 plays in the physiological functioning of the cellular compartments in which the protein is located. The neuropathological manifestation of Huntington disease is characterized by progressive neurodegenerative disorders, cognitive deficits, and choreic movements. All these manifestations are linked to the abnormal expansion of glutamine residues from less than 34 to more than 37 at the 18th amino acid position [170]. Presently, the mechanism responsible for the insurgence of these defects is unknown [170]. Similarly unknown is whether the poly-glutamine expansion alters Mg^{2+} transport, and whether perturbation of Mg^{2+} homeostasis plays any role in the insurgence of the neuronal defects typical of Huntington disease.

3.4 Mg^{2+} Transport in Purified Plasma Membrane Vesicles

Several laboratories including ours have used plasma membrane vesicles to better characterize how different Mg^{2+} extrusion mechanisms operate in particular cell types. The plasma membrane model presents several advantages including: (i) a

well defined ionic extra- and intra-vesicular milieu composition to determine the modality of operation of the various Mg^{2+} transporters under rigorous experimental conditions, and (ii) the ability to investigate the operation of different Mg^{2+} extrusion mechanisms in the absence of Mg^{2+} buffering by ATP, proteins or other cytosolic components, and partitioning within intracellular organelles. By purifying total liver plasma membrane or cardiac sarcolemmal vesicles as well as specific subpopulations enriched in basolateral or apical domains, our laboratory has been able to provide a better understanding of the selective location and specificity of operation of the Na^+-dependent and Na^+-independent Mg^{2+} extrusion mechanisms in liver cells and cardiac myocytes.

In the hepatocyte, the Na^+-dependent extrusion mechanism is specifically located in the basolateral domain [143], is selectively activated by Na^+ [143,172], and is inhibited only by imipramine [172], and not by amiloride and amiloride derivates [172]. Moreover, the operation of the exchanger is completely abolished by pretreatment of basolateral vesicles with alkaline phosphatase, but it can be restored by loading the vesicles with ATP and PKA-catalytic subunit [133,134], leaning further support to the notion that the Na^+/Mg^{2+} exchanger is activated upon phosphorylation by cAMP. As this exchanger continues to operate in the presence of *zero trans* Mg^{2+} across the plasma membrane (i.e., 20 mM Mg^{2+} inside and outside the vesicles) [143], indication is there that Mg^{2+} extrusion strictly depends on the Na^+ transmembrane gradient, with a $K_m \leq 20$ mM [143], in good agreement with kinetic data obtained in isolated hepatocytes [130] and other cell types [141]. Experiments based on TPP^+ distribution have confirmed the electrogenicity of this exchange mechanism in plasma membrane vesicles, supporting a $1Na^+_{in}:1Mg^{2+}_{out}$ exchange ratio under the majority of experimental conditions tested [133,143,172]. Upon removal of intravesicular Cl^-, the stoichiometry ratio of the exchanger switches from electrogenic to electroneutral (i.e. $2Na^+_{in}:1Mg^{2+}_{out}$) [137]. Interestingly, in the presence of intravesicular Cl^-, a Cl^- extrusion is observed in concomitance with the extrusion of Mg^{2+} and the accumulation of external Na^+ into the vesicles [137]. This Cl^- extrusion is not inhibited by inhibitors of anion transport (e.g., DNDS, DIDS, or niflumic acid), of the Na/K/Cl cotransporter NKCC1 (e.g., bumetanide or furosemide) [137], excluding that Cl^- movement occurs via one of these mechanisms. The only agent able to block Cl^- extrusion is imipramine [137], which specifically blocks the operation of the Na^+/Mg^{2+} exchanger in the basolateral domain of the hepatocyte [172]. Hence, it would appear that Cl^- is extruded via the Na^+/Mg^{2+} exchanger or, alternatively, via Cl^- channels for partial charge compensation [137]. The possibility that Cl^- is extruded via the Na^+/Mg^{2+} exchanger has been suggested by Rasgado-Flores et al. [139] in dialyzed squid axons, and it would be in good agreement with the observation by Ebel and Günther that intracellular Cl^- has a stimulatory role on the activity of the Na^+/Mg^{2+} antiport in red blood cells [138].

Experiments carried out in liver plasma membrane vesicles enriched in apical domain indicate the presence of two apparently distinct and unidirectional Mg^{2+} transport mechanisms, which extrude intravesicular Mg^{2+} for extravesicular Na^+ and Ca^{2+}, respectively [172]. The apical Na^+-dependent Mg^{2+} transporter presents several similarities to the basolateral transporter: (i) its K_m for Na^+ is comparable

at ~15–20 mM; (ii) it selectively uses Na^+ over other monovalent cations; (iii) it electrogenically exchanges $1Na^+_{in}$:$1Mg^{2+}_{out}$ [172]. This apical exchanger can be pharmacologically distinguished from the basolateral one due to its specific inhibition by amiloride [172], although it retains a significant level of inhibition by imipramine. In contrast, imipramine only blocks the basolateral antiport [172]. The apical exchanger can also be distinguished from the basolateral antiport based on its inability to operate in reverse mode [172] and the non-requirement for cAMP-mediated phosphorylation to become active [133]. Based on preliminary observation, it would appear that this apical antiport does not transport Cl^- as part of its operation (A. M. P. Romani, personal observation).

The apical domain of the hepatocyte also possesses a Ca^{2+}-dependent Mg^{2+} extrusion mechanism [133]. Specifically located in this domain, this exchanger is activated by micromolar Ca^{2+} concentrations ($K_m \geq 50$ μM), and is insensitive to alkaline phosphatase pre-treatment [133,134]. The Mg^{2+} extrusion through this antiport occurs on electroneutral basis (i.e., $1Ca^{2+}_{in}$:1 Mg^{2+}_{out}) [133]. This exchanger is not Ca^{2+}-specific, as Mg^{2+} extrusion can occur following the extravesicular addition of micromolar concentrations of various divalent cations ($Ca^{2+} >> Co^{2+} = Mn^{2+} > Sr^{2+} >> Ba^{2+} > Cu^{2+} >> Cd^{2+}$) [172]. Similarly to the apical Na^+/Mg^{2+} antiport, the Ca^{2+}-dependent mechanism is inhibited by amiloride or imipramine [172]. This observation raises the question as to whether we are in the presence of two distinct apical mechanisms, modulated by Na^+ and cations, respectively. Several lines of evidence, however, do not fully support this possibility. First, the co-addition of Na^+ and Ca^{2+} to a purified subpopulation of apical plasma membrane vesicles does not appear to enlarge Mg^{2+} extrusion to a significant extent (A. M. P. Romani, personal observation). Second, amiloride inhibits both Na^+- and Ca^{2+}-dependent Mg^{2+} extrusion processes to a comparable extent at a similar concentration [172]. Third, alkaline phosphatase treatment does not affect the Mg^{2+} extrusion elicited by either exchanger in apical liver plasma membrane vesicles [133]. Fourth, neither of these exchangers can operate in reverse at variance of the basolateral Na^+/Mg^{2+} antiport. Taken together, these observations suggest the operation of a non-selective exchange mechanism able to utilize monovalent or divalent cations to promote Mg^{2+} extrusion. At the present time, the physiological implication for the operation of such an exchanger in the apical domain of the hepatocyte is not clear.

The operation of functionally similar Na^+- and Ca^{2+}-dependent Mg^{2+} extrusion mechanisms has also been observed in cardiac sarcolemma vesicles [134]. As in the case of liver plasma membrane vesicles, cardiac sarcolemma vesicles do not require intravesicular ATP to support the operation of Mg^{2+} transporters [134], and pretreatment of the vesicles with alkaline phosphatase specifically inhibits the reversible Na^+-dependent Mg^{2+} extrusion mechanism but not the Ca^{2+}-dependent Mg^{2+} extrusion pathway [134]. For technical reasons, it is presently undefined whether cardiac myocytes also possess two distinct Na^+-dependent Mg^{2+}-extrusion mechanisms in the sarcolemma, and whether the Ca^{2+}/Mg^{2+} exchanger in sarcolemmal vesicles can also utilize Na^+ to promote Mg^{2+} extrusion.

The operation of specific Mg^{2+} transport mechanisms has been observed in plasma membrane vesicles from brush border cells of rabbit ileum [173] and from rat

duodenum and jejunum [174]. In these models, however, Mg^{2+} accumulation rather than extrusion has been observed. By using membrane vesicles from rabbit ileum and cell permeant and non-permeant Mag-Fura, Juttner and Ebel have observed the operation of a saturable Mg^{2+} uptake mechanism when the intracellular Na^+ concentration is higher than the extracellular concentration [173]. The process becomes inoperative when the Na^+ gradient is reversed (i.e., $[Na^+]_i < [Na^+]_o$), the vesicles are in *zero trans* condition for Na^+, or external Na^+ is removed. At variance with the Na^+-Mg^{2+} antiporter operating in liver plasma membrane vesicles, the pathway in ileum vesicles is not reversible and appears to be electroneutral. Yet, it possesses a K_m for Na^+ of 16 mM, a value similar to the K_m calculated in liver plasma membranes [143], in smooth muscle cells from guinea pig tenia caecum [141], and in chicken erythrocytes [175]. Another similarity with the transporter operating in basolateral liver plasma membranes is the lack of inhibition by amiloride analogs [173]. In good agreement with other reports [138], the transporter characterized by Juttner and Ebel is modulated by intravesicular anions, especially Cl^- and SCN^-, and is markedly stimulated by antagonists of anion transport (e.g., H_2-DIDS) [173].

The main difference between plasma membrane vesicles from duodenum and jejunum [174,175] is that a single Mg^{2+} uptake mechanism operates in the duodenum with a K_m ~0.8 mM, whereas two transporters operate in the jejunum with K_m values of 0.15 mM and 2.4 mM, respectively. In both these experimental models, Mg^{2+} but not Ca^{2+} accumulation is reduced in the presence of alkaline phosphatase inhibitors [176], suggesting that Ca^{2+} and Mg^{2+} are transported via distinct pathways. This hypothesis is further supported by the observation that Mg^{2+} accumulation is inhibited by amiloride but not by Ca^{2+} channel antagonists. Consistent with the report by Juttner and Ebel [173], Mg^{2+} accumulation is stimulated by an intravesicular electronegative potential or an alkaline pH_o [174]. The effect of external pH, however, is lost when $[Mg^{2+}]_0 > 1$ mM [174]. Under the latter condition (i.e. $[Mg^{2+}]_0 > 1$ mM), Mg^{2+} accumulation is enhanced by the presence of Na^+ or K^+ but it is inhibited by the presence of divalent cations ($Co^{2+} > Mn^{2+} > Ca^{2+} > Ni^{2+} > Ba^{2+} > Sr^{2+}$) in the extravesicular space [174]. The molecular aspects of these processes, however, have not been elucidated and remain highly speculative.

4 Regulation of Mg^{2+} Transport and Homeostasis

The majority of mammalian cells retains their basal Mg^{2+} content virtually unchanged under resting conditions even when a major trans-membrane gradient is artificially imposed [1–3]. At the same time, compelling evidence supports the notion that different hormones induce the movement of large amounts of Mg^{2+} in either direction across eukaryotic cell membranes. As a result of these movements, changes in serum, total and – to a lesser extent – free Mg^{2+} content have been observed. These changes have resulted in detectable variations in Mg^{2+} level within organelles, especially mitochondria, with significant repercussions on cellular

bioenergetics. Hence, a picture is slowly emerging, which relates changes in total Mg^{2+} content to energetic substrate utilization (e.g., glucose), cell cycle progression [64] or meaningful changes in Mg^{2+} content within discrete portions of the cell or cellular organelles with consequent modulation of the activity of specific enzymes located therein.

4.1 Mg^{2+} Extrusion

Hormones like catecholamine or glucagon induce Mg^{2+} extrusion from various cell types or perfused tissues. The majority of these hormones have in common their ability to increase cellular cAMP level. While the Mg^{2+} extrusion elicited by these hormones depletes to a varying extent the Mg^{2+} pools present within the cytoplasm and the cellular compartments, the physical outward transport of Mg^{2+} across the cell membrane primarily occurs via the Na^+/Mg^{2+} exchanger previously described. Magnesium extrusion has also been observed following metabolic treatments that decrease cellular ATP content, the main Mg^{2+} buffering component. Interestingly, several of the hormones that induce Mg^{2+} extrusion from liver cells also elicit glucose output from the hepatocyte. Conversely, hormones that promote glycogen synthesis stimulate Mg^{2+} accumulation rather than extrusion (discussed in Section 4.2). Hence, it would appear that at least in liver cells Mg^{2+} extrusion is functionally associated with glucose transport and utilization.

4.1.1 Cyclic AMP-Dependent Mg^{2+} Extrusion

Elliot and Rizack were the first to report Mg^{2+} transport across the plasma membrane of adipocytes stimulated by adrenocorticotrophic hormone [177], but the authors did not elucidate the modality of transport or the mechanism involved. The first extensive characterization of hormone-induced Mg^{2+} transport was provided by Maguire and colleagues in S49 lymphoma cells and primary lymphocytes stimulated by β-adrenergic receptor agonist or PGE1 [178–181]. Maguire and Erdos [182] also provided the first observation that protein kinase C (PKC) activation enhances Mg^{2+} influx in S49 cells at variance of β-adrenergic receptor stimulation, which inhibits the process. Observation carried out in S49 cells lacking protein kinase A (PKA) or adenylyl cyclase (AC), however, indicated that the inhibitory effect of β-adrenergic agonists was not mediated by cAMP [183,184]. At variance of what was reported for primary lymphocytes [25], Mg^{2+} transport in S49 cells appears to be independent of extracellular Na^+ concentration or membrane potential (Maguire, unpublished observation). Further, Mg^{2+} turnover in S49 requires more than 40 hours as compared to the much faster Ca^{2+} turnover, which is completed in less than 3 hours [184].

These initial observations were followed by a long series of reports all supporting the notion that β-adrenergic agonists and other hormones control Mg^{2+} homeostasis in mammalian cells. In the majority of eukaryotic cells, hormones or agents that increase cellular cAMP level elicit a significant extrusion of Mg^{2+} into the extracellular space or the circulation [32–34]. This effect has been observed in cardiac ventricular myocytes [32,33,129,185], liver cells [34,35,130–132], red blood cells [31], lymphocytes [30], and Ehrlich ascites cells [186] among other cells (see [27] for a more comprehensive list), as well as in anesthetized animals [187,188]. In all these cell types, Mg^{2+} extrusion is a fast process that reaches the maximum within 8 min from the application of the stimulus irrespective of the hormone (catecholamine, isoproterenol, glucagon, PGE1, or arachidonic acid) [30–35,185,186] or agent (i.e., forskolin or cell permeant cyclic AMP analogs) [30–34,130–132] utilized to increase cellular cAMP level. The key role of cAMP in modulating Mg^{2+} extrusion is further corroborated by the observation that pre-treatment of cells with hormones or agents that decrease cAMP production (e.g., carbachol [30–34,130–132], insulin [189]) or prevent PKA activation (e.g., Rp-cAMP [25]) completely prevents cellular Mg^{2+} mobilization. In an open perfusion system, the amount of Mg^{2+} extruded from the organ (i.e. heart or liver) returns towards baseline level within 8 min from the application of the agonist irrespective of its dose or persistence in the perfusate [32,34]. This temporally limited extrusion suggests that Mg^{2+} is rapidly mobilized from a well defined cellular pool(s) that is (are) rapidly depleted. This notion is supported by the observation that sub-maximal doses of agonist sequentially infused within a few minutes from each other elicit Mg^{2+} extrusions of progressively decreasing amplitudes [30].

Under all these conditions, limited changes in cytosolic free $[Mg^{2+}]_i$ have been observed [37,190], suggesting that Mg^{2+} is rapidly released from binding and buffering sites within the cytoplasm or cellular organelle(s) and extruded across the cell membrane. Irrespective of the hormone utilized, cAMP-mediated Mg^{2+} extrusion occurs via the putative Na^+/Mg^{2+} exchanger described previously. In fact, either the removal of extra-cellular Na^+ [142] or the cell pre-treatment with non-selective Na^+ transport inhibitors like amiloride or imipramine [33,142], abolishes the Mg^{2+} extrusion almost completely. Under either inhibitory condition the reduced Mg^{2+} extrusion across the cell membrane originates a more sustained rise in cytosolic free $[Mg^{2+}]_i$ [37,190], suggestive of the concept that blocking the Na^+-dependent transport mechanism prevents Mg^{2+} from being extruded across the cell membrane but not from being released from binding/buffering sites such as ATP or proteins, and/or from cellular organelles (i.e., mitochondria and endoplasmic reticulum) into the cytoplasm. Two corollaries of this observation are that: (i) cAMP operates on at least two different levels (i.e., cellular organelle(s) and plasma membrane) to mobilize Mg^{2+} from the cell, and (ii) only Mg^{2+} transport across the cell membrane is Na^+-dependent whereas the mobilization from cellular organelle(s) is largely Na^+-independent. Alternatively, it has to be postulated that cytosolic Na^+ concentration, which ranges between 15 to 20 mM in most cell types) is more than sufficient to favor Mg^{2+} transport across the membrane of cellular organelles.

4.1.2 Cyclic AMP-Independent Mg^{2+} Extrusion

In 1989, Jakob and collaborators reported that phenylephrine administration also promotes Mg^{2+} extrusion from liver cells via α_1-adrenergic stimulation [36]. In addition to confirming this observation, our laboratory has provided the first evidence that co-stimulation of α_1- and β-adrenergic receptor are additive and complementary processes to induce Mg^{2+} extrusion from liver cells [131,148]. This event is of particular relevance especially when the two classes of adrenergic receptors are stimulated by mix-adrenergic agonists such as epinephrine or norepinephrine [131,148]. Pre-infusion of insulin only abolishes β-adrenergic receptor mediated Mg^{2+} extrusion from liver cells, leaving unaffected the mobilization of Mg^{2+} mediated via α_1-adrenergic receptors [148]. The inhibitory effect of insulin persists even in cells treated with cell-permeant cAMP analogs [148]. A similar inhibitory effect of insulin on β-adrenergic receptor mediated, cAMP-modulated, Mg^{2+} extrusion has been observed in cardiac myocytes [189]. These results have been attributed to an inhibitory effect of insulin on β-adrenergic receptor activation [191], and a stimulatory effect of the hormone on the cytosolic phosphodiesterase that degrades cAMP [192]. Experimental evidence also suggests a direct modulating effect of insulin on the Na^+/Mg^{2+} exchanger, at least in erythrocytes [193].

Fagan and Romani [131,132] further investigated the modality of Mg^{2+} extrusion following α_1-adrenergic receptor stimulation in liver cells. Their results indicate that phenylephrine-induced Mg^{2+} extrusion strictly depends on the activation of capacitative Ca^{2+} entry [132]. Inhibition of IP3-induced Ca^{2+} release from the endoplasmic reticulum, chelating of cytosolic Ca^{2+}, or inhibition of Ca^{2+} entry at the plasma membrane level all result in the complete inhibition of Mg^{2+} extrusion from the hepatocyte [132]. The scant information available about possible binding of Mg^{2+} by cellular proteins prevented the authors from ascertaining whether Mg^{2+} extruded from the hepatocyte was mobilized from the ER, or displaced from cytosolic binding sites following the massive entry of Ca^{2+} across the cell membrane ([132] and refs. therein). Interestingly, extracellular Na^+ and Ca^{2+} are both required for the phenylephrine-induced Mg^{2+} extrusion to occur [132]. In the absence of extracellular Ca^{2+}, in fact, the amplitude of Mg^{2+} extrusion is decreased by ~15% to 20% whereas extracellular Na^+ is responsible for the remaining 80%–85% of the extrusion. It is presently unclear whether Mg^{2+} extrusion occurs via the Ca^{2+}-activated, Na^+-dependent mechanism observed in the apical domain of the hepatocyte, or whether Na^+ is required to maintain membrane potential and facilitate Ca^{2+} entry across the hepatocyte cell membrane. In the absence of receptor activation, thapsigargin administration can mimic phenylephrine stimulation and elicit Mg^{2+} extrusion from the hepatocyte, even in the absence of extracellular Ca^{2+} [132], although to a lesser extent. Hence, it would appear that an optimal level of cytosolic Ca^{2+} has to be attained in order for Mg^{2+} extrusion to occur via displacement from cellular binding sites or via a Ca^{2+}-calmodulin-activated mechanism [132]. Interestingly, the group of Schweyen has provided evidence that in yeast Mg^{2+} deprivation accelerates Ca^{2+} accumulation. In turn, this translates into a more rapid activation of Ca^{2+}-mediated signaling [194].

4.1.3 Mg^{2+} Homeostasis and Glucose

The presence of redundant Mg^{2+} extrusion mechanisms or modalities of activation of a common Mg^{2+} extrusion pathway raises the question: What is the physiological significance of Mg^{2+} mobilization in mammalian cells?

The general answer is that Mg^{2+} extrusion can have a different significance in different cells due to the physiological differentiation and function of the various cell types. In the case of cardiac myocytes, an increase in extracellular Mg^{2+} level has been associated with a modulating effect on the open probability of the L-type Ca^{2+}-channels [195] and a temporary decrease in sino-atrial node action potential [185]. In the case of liver cells, instead, Mg^{2+} transport appears to be associated with a regulatory role on glucose transport and utilization. Catecholamine [131,148] or glucagon [131], and adrenergic agonists like isoproterenol or phenylephrine [131,148], which elicit Mg^{2+} extrusion from liver cells, all activate glycogenolysis and promote release of hepatic glucose into the blood-stream within a similar time frame [131]. Interestingly, the presence of amiloride or imipramine inhibits both Mg^{2+} extrusion and hepatic glucose output [131]. The converse is also true. Inhibition of glucose transporter activity by phlorethin results in a qualitatively similar inhibition of Mg^{2+} extrusion from liver cells [131]. The presence of a close functional 'link' between glucose and Mg^{2+} homeostasis is corroborated by the observation that overnight starvation completely depletes hepatic glycogen and glucose, and concomitantly decreases to a significant extent (minus 15%) total hepatic Mg^{2+} content as a consequence of pro-glycemic hormones (i.e., catecholamine and glucagon) activation [196]. Noteworthy, this decrease in hepatic Mg^{2+} content is equivalent in amplitude to that elicited via *in vitro* stimulation of perfused livers by the same hormones [196], or that observed to occur in livers of type-I diabetic animals [197], which are markedly depleted in cellular glycogen. This functional link between glucose and Mg^{2+} homeostasis can also be observed under conditions in which glucose accumulation and glycogen synthesis are stimulated by insulin administration to cardiac ventricular myocytes [189] or pancreatic β cells [198]. In both experimental models, the amount of Mg^{2+} accumulated within the cells is directly proportional to the amplitude of glucose accumulation. Conversely, decreasing extracellular Mg^{2+} concentration directly reduces the amount of glucose accumulated within the cells [131,189]. A role of Mg^{2+} in regulating glucose homeostasis is underlined by the observation that several glycolytic enzymes, including hexokinase, phosphofructokinase, phosphoglycerate mutase, phosphoglycerate kinase, enolase and pyruvate kinase, show activation at low, and inhibition at high Mg^{2+} concentrations [199,200].

4.1.4 Mg^{2+} Homeostasis and ATP

Mg^{2+} extrusion also occurs following exposure to various agents or conditions that markedly decrease cellular ATP content and production including cyanide [153,201], mitochondrial uncouplers [38,112], fructose [202], ethanol [203], or

hypoxia [204]. All these agents, in fact, decrease ATP content by preventing the mitochondrial electron chain from generating ATP (cyanide or uncouplers), by acting as an ATP trap (fructose), or by altering the redox state of pyridine nucleotide within the cytoplasm and the mitochondrion (ethanol). Because ATP represents the major Mg^{2+} buffering component within the cell [8,9], a decrease in its content or its degradation into ADP or AMP results in an increased dissociation of Mg^{2+} from the binding and an increase in cytosolic free $[Mg^{2+}]_i$. Ultimately, such an increase in cytosolic Mg^{2+} level originates in a detectable Mg^{2+} extrusion from the cell [153,201–204].

Such an extrusion can be observed in erythrocytes, which possess limited cellular buffering capacity for Mg^{2+} and no compartmentation [205], as well as in cells that possess additional Mg^{2+} buffering due to the presence of proteins or cellular organelles in addition to ATP and phosphonucleotides [153,201–204]. In several cases, such as fructose addition [202], the changes in cytosolic $[Mg^{2+}]_i$ can elicit glycogenolysis via activation of glycogen phosphorylase and glucose utilization to restore cellular ATP levels [202]. The majority of these experimental conditions promote a modest increase in cytosolic free $[Mg^{2+}]_i$, which is considerably lower than the increase expected to occur based upon the corresponding decrease in ATP level. This observation strongly supports the notion that the majority of Mg^{2+} released from ATP and other binding sites is extruded from the cell. Furthermore, because the ATP level decreases following changes in pyrimidine nucleotide ratio or mitochondria poisoning, it would appear that not phosphorylation but the rise in cytosolic Mg^{2+}, even if modest, is sufficient to activate Mg^{2+} extrusion and limit the rise in cytosolic free Mg^{2+} concentration to approximately 100–200 μM at the most [201]. Hence, it can be presumed that such an increase is sufficient to activate enzymes and metabolic reactions controlled by Mg^{2+}.

On the other hand, cellular ATP regulates Mg^{2+} extrusion in ways other than acting as a buffering component. Evidence for an additional role of ATP has been provided by experiments in giant squid axon [206], mammalian hepatocytes [207] or erythrocytes [11]. In squid axon, the Na^+-dependent Mg^{2+} extrusion requires a physiological level of ATP to operate, and as the level of ATP decreases so does the amplitude of Mg^{2+} extrusion [207]. In erythrocytes and hepatocytes, instead, ATP appears to regulate the Na^+-independent Mg^{2+} extrusion process [11,207]. The modality by which ATP regulates the Mg^{2+} extrusion process is unclear, but it appears to be unrelated to the operation of an ATPase mechanism. This notion is supported by the observation that a decrease in cellular ATP level as it occurs for example under diabetic or alcoholic conditions paradoxically results in an increased extrusion of Mg^{2+} via the Na^+-dependent mechanism in a manner directly proportional to the decrease in ATP level [197,203]. Because ATP predominantly acts as a ligand for Mg^{2+} both in the cytoplasm and the mitochondrial matrix [8,9], any decrease in ATP level will result in an increase in free Mg^{2+} and the consequent extrusion of Mg^{2+} from the cell.

4.2 Mg^{2+} Accumulation

The identification of several Mg^{2+} entry mechanisms strongly support the hypothesis that cellular Mg^{2+} is dynamically maintained through the operation of entry and exit mechanisms that are differentially regulated by hormones and metabolic conditions. A striking difference is there, however, between the Mg^{2+} exit and the Mg^{2+} entry mechanisms. In the case of Mg^{2+} extrusion mechanisms we have a good understanding of the signaling activating their operation but we lack any information about the structure of the mechanisms themselves. In the case of Mg^{2+} entry mechanisms, instead, we do have structural information about several of these mechanisms but for the most part we lack detailed information about their individual activation by hormones or second messenger, and their possible cooperation under specific conditions.

4.2.1 Role of Protein Kinase C

Experimental evidence indicates that mammalian cells can accumulate large amounts of Mg^{2+} as a result of hormonal stimulation. Administration of hormones like carbachol, vasopressin, angiotensin-II, or insulin to various cell types results in the inhibition of cAMP-mediated Mg^{2+} extrusion and/or the reversal of Mg^{2+} extrusion into Mg^{2+} accumulation [32,189]. The list of cells that respond to hormonal stimulation by accumulating Mg^{2+} is quite long (see [29] for a list), and includes cardiac myocytes [32,189], smooth muscle cells [208], hepatocytes [34,209], platelets [210], lymphocytes [211], fibroblasts [212], and pancreatic β cells [198]. In addition to inhibiting cAMP production, several of the hormones indicated above activate protein kinase C (PKC) as part of their cellular signaling. Evidence supporting a role of PKC in mediating Mg^{2+} accumulation has been provided by several laboratories. Maguire and collaborators have reported that administration of phorbol-myristate acetate (PMA), which directly activates PKC, elicits a marked accumulation of Mg^{2+} in S49 lymphoma cells [180]. A similar effect of PMA has been reported in thymocytes [213], cardiac myocytes [209] and hepatocytes [209].

Furthermore, our group has reported that down-regulation of PKC by exposure to a large dose of PMA for 3 hours completely abolishes the ability of cardiac and liver cells to accumulate Mg^{2+} while leaving unaffected the responsiveness of these cells to adrenergic agonists [209]. A similar inhibition of Mg^{2+} accumulation has been observed following treatment of cells with the PKC inhibitors calphostin [208] or staurosporine [214]. Alteration in PKC distribution and activity associated with a defective accumulation of Mg^{2+} have been observed in arterial smooth muscle cells [215] and hepatocytes [216] isolated from animals exposed to alcohol, or in liver cells of diabetic animals [217].

Protein kinase C activation is only part of the integral response of hormones like angiotensin-II or vasopressin. The interaction of these hormones with their

receptor, in fact, activates phospholipase C which, in turn hydrolyses PIP2 to generate diacyl-glycerol (DAG) and IP3. These two molecules would then activate PKC and IP3 receptor in the ER, respectively. Activation of IP3 receptor results in a marked but transient increase in cytosolic Ca^{2+} followed by a more sustained Ca^{2+} entry across the plasma membrane through store-operated channels (SOC). Thus, Ca^{2+} signaling is an integral component of the cellular response elicited by these hormones. Yet, the contribution of Ca^{2+} increase and signaling in mediating Mg^{2+} accumulation is poorly defined. Liver cells loaded with Bapta-AM, which chelates cytosolic Ca^{2+}, are unable to extrude and accumulate Mg^{2+} following stimulation by phenylephrine and PMA, respectively [130]. Administration of thapsigargin, which inhibits the SRCA pumps and increases cytosolic Ca^{2+} by favoring its release from the endoplasmic reticulum, also prevents Mg^{2+} accumulation [130] and actually induces a Mg^{2+} extrusion from the liver cell if applied for more than 3-5 min [130,131]. Because of the different time-scale and amplitude of the changes in cellular Ca^{2+} and Mg^{2+} content [130], it is difficult to properly correlate these variations. Cytosolic free Ca^{2+} transiently increases several orders of magnitude above its resting level. In contrast, cytosolic free Mg^{2+}, which is already in the millimolar or sub-millimolar range, increases by 10% to 15% [37] at the most, although in absolute terms this amount far exceeds the overall change in cytosolic Ca^{2+} mass.

An unresolved point of inconsistency in the role of Ca^{2+} and PKC signaling in regulating Mg^{2+} accumulation is provided by the reports that the administration of phenylephrine, which activates PKC signaling in addition to inositol 1,4,5-trisphosphate and Ca^{2+} signaling, does not elicit Mg^{2+} accumulation but induces a Mg^{2+} extrusion from liver cells [132]. These results raise the question as to what modulates the different cellular response to the administration of phenylephrine or vasopressin. One possibility could be that different PKC isoforms are activated under one condition but not the other. For example, hepatocytes possess 3 classical and at least 2 novel PKC isoforms [217]. Thus, it is possible that one isoform (or class of isoforms) is involved in mediating Mg^{2+} accumulation while another isoform (or class of isoforms) is involved in modulating Mg^{2+} extrusion. Consistent with this hypothesis, recent data from our laboratory suggests that PKCε is essential for Mg^{2+} accumulation to occur [216]. Under conditions in which the expression of this isoform is inhibited by antisense, or its translocation to the cell membrane is prevented, for example, by ethanol administration, no Mg^{2+} accumulation is observed in liver cells [216]. Interestingly, this PKC isoform has the highest affinity for Mg^{2+} among all PKC isoenzymes, with a K_m ~1 mM [218], close to the physiological free $[Mg^{2+}]_i$ measured in the cytoplasm of the hepatocyte [37,219] and other mammalian cells as well [208]. Although the mechanism ultimately responsible for the accumulation of Mg^{2+} within the hepatocyte has not been identified, it is worth considering the recent observation by Bindels and collaborators that in the absence of PKC activation or following RACK1 over-expression, RACK1 can bind to TRPM6, and possibly TRPM7, at the level of the kinase domain and inhibit the channel activity [118].

4.2.2 Role of MAPKs

Several lines of evidence indicate that additional signaling pathways (e.g., MAPKs) are involved in determining differing cellular responses under seemingly similar stimulatory conditions. Reports from Altura and collaborators in arterial muscle cells [220], Touyz's laboratory in vascular smooth muscle cells [221], and our group in liver cells [222] indicate that pharmacological inhibition of ERK1/2 and p38 MAPKs abolishes PKC-mediated Mg^{2+} accumulation [222]. In addition, inhibition of MAPKs signaling hampers Mg^{2+} accumulation and affects cyclin activity in vascular smooth muscle cells [221], preventing the cells from progressing in the cell cycle [221]. This effect may occur via changes in nuclear functions directly regulated by Mg^{2+}, as proposed by Rubin [223], and/or changes in nuclear signaling by ERK2, which depends on Mg^{2+} level to properly dimerize, translocate, and activate specific nuclear targets [224]. The role of ERK1/2 in regulating Mg^{2+} homeostasis is further emphasized by the evidence that increased ERK1/2 phosphorylation and TRPM6 expression have been observed following EGF administration to renal epithelial cells [94,95]. The role of MAPKs in Mg^{2+} homeostasis, however, is far from clear as ERK1/2 appears to be involved in mediating also Mg^{2+} extrusion [220,225].

4.2.3 Role of the Epidermal Growth Factor

EGF is also implicated in regulating Mg^{2+} accumulation, at least in kidney cells. The administration of EGF controls TRPM6 channel expression and operation in the apical domain of renal epithelial cells to promote Mg^{2+} accumulation [94,95,226]. Point mutations in the EGF sequence limit TRPM6 functioning and Mg^{2+} accumulation within the cells [227]. The modulation of TRPM6 expression appears to occur via ERK1/2 signaling coupled to activating protein-1 (AP-1) [95]. Indirect evidence that EGF regulates Mg^{2+} homeostasis is provided by the observation that antibodies against EGF used in several forms of colon cancer [92,93,228] induce Mg^{2+} wasting and hypomagnesemia.

5 Serum Mg^{2+} Level and Mg^{2+}-Sensing Mechanism

Humans and many mammals present a circulating Mg^{2+} level of ~1.2–1.4 mEq/L (~0.8 mM) [19,229]. Clinical and experimental evidence indicates that serum Mg^{2+} level decreases in humans and animals in several chronic diseases [197]. Yet, there is a remarkable lack of information as to whether serum Mg^{2+} undergoes circadian fluctuations following hormonal or non-hormonal stimuli (e.g., fasting or exercise). The infusion of catecholamine [229–231] or isoproterenol [177,187,232] results in a marked dose- and time-dependent increase in circulating Mg^{2+} content. This increase is maximal within 20 min from the agent administration [187], remaining

unchanged for up to 2 hours following the removal of the agonist [187]. Considering this time frame of changes, the pre-infusion level of serum Mg^{2+}, the glomerular filtration rate (1.62 mL/min), and the fractional excretion (17%) [233], it is evident that the increase in serum Mg^{2+} level is independent of the hemodynamic changes elicited by the β-adrenergic agonist [187] and renal excretion [233]. Consistent with the whole body distribution of β_2 versus β_1 adrenergic receptors [234,235], the increase in serum Mg^{2+} occurs can be mimicked by specific β_2-adreno-ceptor agonist and inhibited by specific β_2-blocker [187]. The amplitude of the increase in circulating Mg^{2+} level suggests that the adrenergic agonist mobilizes Mg^{2+} from various tissues [187], including bone [188]. The latter hypothesis is supported by the observation that the infusion of carbonic anhydrase inhibitor prevents the increase in serum Mg^{2+} level elicited by isoproterenol administration in anesthetized rats [188]. It is interesting to note that the hormones that increase plasma Mg^{2+} by mobilizing the cation from different organs or tissues are also responsible for increasing Mg^{2+} reabsorption in the Henle's loop, thus preventing a net Mg^{2+} loss.

Presently, no specific Mg^{2+}-sensing mechanism in the circulation has been identified. However, the Ca^{2+}-sensing receptor [236] can detect changes in circulating Mg^{2+} level in a range of concentrations higher than those of Ca^{2+} [237] and consistent with the increase in serum Mg^{2+} levels reported in the literature [187,188]. The observation that in cells of the distal convoluted tubule (MDCT) of the mouse the Ca^{2+}-sensing receptor can be activated by extracellular Ca^{2+} and Mg^{2+} with comparable sensitivity [238] suggests interesting hypotheses in terms of whole body physiology. The activation of this sensor mechanism would inhibit glucagon- or vasopressin-mediated Mg^{2+} accumulation into the cells [239] and favor its urinary elimination, possibly explaining the clinical and experimental evidence that hypomagnesemia and hypocalcemia inhibit hormone-stimulated cAMP-mediated reabsorption of Mg^{2+} and Ca^{2+} along the different segments of the nephron [240]. In addition, the Ca^{2+} sensing receptor would represent a distal regulatory mechanism to restore magnesemia to a physiological level following the increase observed in anesthetized animals infused with adrenergic agonists [187,188]. It is still an open question as to whether this sensing mechanism or associated modulating components are altered under diabetic conditions in which a significant loss of tissue Mg^{2+} content and increased magnesuria are observed.

At variance of hypercalcemia, which is associated with muscle weakness and arrhythmia, an increase in serum Mg^{2+} level appears to be well tolerated under *in vivo* conditions. Rats infused with boluses of Mg^{2+} that increase serum Mg^{2+} level by 50% do not exhibit significant systemic hemodynamic changes but show a marked increase in coronary artery flow [241]. Baboons infused with pharmacological doses of Mg^{2+} sufficient to prevent epinephrine-induced cardiac arrhythmias show a significant attenuation of epinephrine-induced increase in mean arterial pressure and systemic vascular resistance [242]. It would appear, therefore, that an increase in extracellular Mg^{2+} concentration regulates catecholamine release from peripheral and adrenal sources [243] and consequently cardiac contractility [184]. Taken together, these observations suggest that an increase in serum Mg^{2+} level following adrenergic stimulation can: (i) act as a feed-back mechanism to modulate

catecholamine release and activity, and (ii) contribute to improved blood flow and O_2 delivery to the heart and possibly other tissues at a time when an increase in energy production is expected.

The presence of a Mg^{2+} *sensor* at the cell level is also debated. The presence and operation of such a *sensor* mechanism is supported by several lines of evidence. First, prolonged exposure to 0 mM $[Mg^{2+}]_0$ decreases cytosolic *free* Mg^{2+} concentration by approximately 50% in cardiac ventricular myocytes [244], MDKC [245], or MDCT cells [246]. This reduced cytosolic Mg^{2+} level is maintained as long as the cells are incubated in the presence of 0 mM $[Mg^{2+}]_0$, but returns to normal level as soon as $[Mg^{2+}]_0$ is increased in a time-frame that is directly proportional to the extracellular Mg^{2+} concentration utilized [244–246]. The presence of L-type Ca^{2+}-channel inhibitors (e.g., verapamil or nifedipine) or La^{3+} in the extracellular milieu prevents the restoration of Mg^{2+} level [244]. The concomitant absence of significant changes in cytosolic $[Ca^{2+}]_i$ excludes that Ca^{2+} may act as a regulatory mechanism, and suggests a direct effect of these inhibitory agents on the Mg^{2+} entry mechanism [244]. As TRPM7 operation is affected by gadolinium [54], these results anticipate the presence and operation of the Mg^{2+}-specific channels TRPM7 [42] and TRPM6 [43].

A second line of evidence for the presence of a Mg^{2+} sensor in eukaryotic cells is provided by the occurrence of Mg^{2+} extrusion in all the conditions in which cellular ATP decreases as a result of chemical hypoxia [201], or exposure to fructose [202], ethanol [203] or cyanide [153]. Under all these conditions, Mg^{2+} extrusion only occurs when extracellular Na^+ is available to be exchanged for cellular Mg^{2+}. In the absence of external Na^+, almost no Mg^{2+} extrusion occurs [153,203,247], and a significant increase in cytosolic Mg^{2+} can be detected [153,247]. Hence, a scenario can be envisage whereby release of Mg^{2+} from cellular organelles or from binding moieties such as ATP [153,203] results in an increase in cytosolic Mg^{2+} content that is detected by the sensor which, in turn, activates the Mg^{2+} extrusion mechanism. The nature of this sensor is still undefined. Because almost all the metabolic conditions mentioned above are characterized by changes in the ratio between reduced and oxidized pyrimidine nucleotide levels (e.g., ethanol [203]), it is an appealing albeit unproved hypothesis that the concentrations of these nucleotides (or their ratio) act as a Mg^{2+} sensor in eukaryotic cells.

Similarly to Mg^{2+} extrusion, cellular Mg^{2+} accumulation also requires proper ion distribution, especially phosphate [246] and potassium [248], across the cell membrane. The role of potassium is of particular relevance as it suggests that Mg^{2+} is accumulated for charge compensation as the result of changes in membrane potential [249–251]. Especially in polarized epithelia (e.g., nephron and intestine) Mg^{2+} entry mechanisms such as TRPM7 and TRPM6 are located on the apical side, counterbalancing the operation of the Na^+/Mg^{2+} exchanger and the Na^+/K^+-ATPase on the basolateral domain of the cell (see [118] for a review). Whether the effect on K^+ occurs through changes in membrane potential, or indirectly via a reduced operation of the Na^+/K^+-ATPase coupled to the operation in reverse of the Na^+/Mg^{2+} exchanger [252] is topic for future investigation. In the particular case of K^+, it has also to be noted that pathological conditions characterized by a marked decrease in tissue

Mg^{2+} content (e.g., diabetes, [197]) are also characterized by an inability of the tissue to properly transport potassium [253,254]. This effect is the direct result of insulin absence or ineffectiveness coupled to a reduced activity rate of the Na^{+}/K^{+}-ATPase. It remains to be determined as to whether changes in pyrimidine nucleotide levels (or ratio), this time in an opposite direction, promote Mg^{2+} accumulation.

6 Physiological Role of Intracellular Mg^{2+}

One of the conclusions generated by the data presented in the previous sections is that Mg^{2+} acts as an indispensable regulatory cation for enzymes, phosphometabolites, and channels [1,255]. Several glycolytic enzymes, including hexokinase, phosphofructokinase, phosphoglycerate mutase, phosphoglycerate kinase, enolase and pyruvate kinase, show activation at low, and inhibition at high Mg^{2+} concentrations [199,200]. Adenylyl cyclase represents the best example of an enzyme directly regulated by Mg^{2+}. As suggested by Maguire's data (reviewed in [256]), Mg^{2+} exerts this effect by acting at two different sites: one site is on the guanine nucleotide coupling protein, where it regulates agonist affinity as well as the interaction with the catalytic subunit. The second site is on the catalytic subunit and regulates the activity of this subunit.

The regulation of adenylyl cyclase and other cellular enzymes (such as those involved in glucose homeostasis [199,200]) occurs at Mg^{2+} concentrations between 0.5 to 1 mM, which are well within the fluctuations in free $[Mg^{2+}]_i$ measured in the cytoplasm of various cells including hepatocyte [219]. With the exception of the glycolytic enzymes, however, studies attempting to evidence *in vitro* or *in situ* a regulatory role of Mg^{2+} for cytosolic enzymes have been disappointing, mostly because of the underlying assumption that Mg^{2+} would operate as Ca^{2+} in modulating enzyme activity. While Ca^{2+} presents a major concentration gradient between cytoplasm and extracellular space and between cytoplasm and endoplasmic (and sarcoplasmic) reticulum lumen, free Mg^{2+} concentrations in the cytoplasm and the extracellular fluid are very similar, both being in the millimolar or sub-millimolar range. Consequently, an increase or a decrease in cytosolic Mg^{2+} level of an amplitude equivalent to those observed for Ca^{2+} will remain largely undetected by fluorescent or ^{31}P NMR techniques. Heretofore, a role of Mg^{2+} as transient regulator of cytosolic enzymes appears to be unlikely. It has to be noted that even under conditions in which hormonal and non-hormonal stimuli elicit major fluxes of Mg^{2+} across the cell plasma membrane in either direction, massive translocations of Mg^{2+} that increase or decrease total cellular Mg^{2+} content by 1–2 mM (equivalent to 5%–10% of the total cell Mg^{2+} content) result in limited or no changes in cytosolic $[Mg^{2+}]_i$ [189,201]. This disconnection can be explained by assuming that the source or destination of the transported Mg^{2+} is a cellular organelle, or a major binding site, or that Mg^{2+} is rapidly buffered by phosphonucleotides, phospholipids, or G proteins. Therefore, regulation of cellular functions by Mg^{2+} should not be necessarily expected to occur in the cytosol, like for Ca^{2+}, but within organelles

and plasma where Mg^{2+} concentration can rapidly increase or decrease by more than 20% [187,188].

The following pages will highlight what is known about the regulatory effect of extracellular or intracellular Mg^{2+} on cation channels activity at the plasma membrane level, as well as on mitochondria respiration and integrity following changes in Mg^{2+} concentration within the organelle.

6.1 Ca^{2+} and K^+ Channels

White and Hartzell were the first to report a regulatory effect of intracellular free Mg^{2+} on calcium channels [257]. These authors observed that increasing intracellular free $[Mg^{2+}]_i$ from 0.3 to 3.0 mM by internal perfusion in cardiac ventricular myocytes resulted in a small decrease of basal L-type Ca^{2+} channel current (I_{Ca}) while it decreased by more than 50% the cAMP-mediated enhancement in I_{Ca} amplitude [257]. This effect was due to a direct action of Mg^{2+} on the phosphorylated channel or on the dephosphorylation rate of the channel rather than to changes in cAMP concentration or cAMP-dependent phosphorylation [257]. Similar results were reported in guinea pig cardiac myocytes by Agus and Morad who observed a Mg^{2+}-induced block on Ca^{2+} current by direct effect on the inactivation state of the channel [258]. The block persisted in the presence of cAMP, and was not reversed by elevation of extracellular Ca^{2+} concentration or addition of catecholamine [258]. Similar effects of Mg^{2+} on Ca^{2+} channels have been observed in vascular smooth muscle cells and endothelial cells from human placenta [259], in which $MgCl_2$ (but also $MgSO_4$) acts at an extracellular site of the voltage-gated Ca^{2+} channels, and on T-type Ca^{2+}-channels [260]. Recent evidence by Catterall and his group proposes a modulating effect of Mg^{2+} on the EF-hand motif located in the C-terminus of $Ca_v1.2$ channels [261].

Additional Ca^{2+} channels modulated by extracellular Mg^{2+} are the store-operated Ca^{2+} channels (SOC) and the store-operated calcium release-activated Ca^{2+} (CRAC) channels. In the case of SOCs, Mg^{2+} prevents or reverses the vasoconstriction elicited by phenylephrine administration but not that induced by K^+ depolarization [262]. This observation would suggest that Mg^{2+} contributes to regulate both the myogenic tone and the α_1-adrenoceptor-induced, Ca^{2+}-mediated vasoconstriction occurring through SOCs. This effect on the vasculature could be lost to a significant extent under hypertensive conditions, in which a decrease in plasma Mg^{2+} and a vasoconstriction hypertone have been observed.

As for CRACs, the effect of Mg^{2+} is more at the intracellular level [263]. CRAC channels are highly selective for Ca^{2+} under physiological conditions whereas removal of extracellular divalent cations makes them freely permeable to monovalent cations, in particular Na^+. Experimental evidence indicates that intracellular Mg^{2+} can modulate the activity and selectivity of these channels therefore affecting monovalent cation permeability. A report by Prakriya and Lewis [263], however, argues that the channels modulated by intracellular Mg^{2+} are not CRAC channels,

but a different class of channels that open when Mg^{2+} is washed out of the cytosol. These channels have been termed $\underline{Mg}^{2+}$-inhibited cation (MIC) channels, and could be distinguished by CRAC channels based upon modality of inhibition, regulation, ion permeation and selectivity [262]. These results, however, do not exclude the possibility of an inhibitory effect of intracellular Mg^{2+} on CRAC channels.

Potassium channels are also targets for Mg^{2+}. Matsuda [264] has reported that cytosolic Mg^{2+} blocks the outward currents of inwardly rectifying K^+ channels without affecting the inward currents. However, the Mg^{2+} block is achieved at a half-saturating concentration of 1.7 μM, a concentration far from the physiological Mg^{2+} level in the cytoplasm. Hence, it is difficult to envision the occurrence of a similar regulatory effect under normal conditions without invoking Mg^{2+} micro compartmentalization. More realistic would be the occurrence of a regulatory role of intracellular Mg^{2+} on voltage-regulated potassium channels (K_v channels) in vascular smooth muscle cells [265]. In this case, in fact, an increase in intracellular Mg^{2+} – in a range of concentrations consistent with its physiological variations – slows down the kinetic of activation of the K_v channel, causing also inward rectification at positive membrane potentials and a shift in voltage-dependent inactivation [265]. Intracellular Mg^{2+} also modulates large-conductance (BK-type) Ca^{2+}-dependent K^+ channels either by blocking the pore of BK channels in a voltage-dependent manner, or by activating the channels independently of changes in Ca^{2+} and voltage through preferential binding to the channel open conformation at a site different from Ca^{2+} sites. Interestingly, Mg^{2+} may also bind to Ca^{2+} sites and competitively inhibit Ca^{2+}-dependent activation [266].

The inhibitory effect of Mg^{2+} is not restricted to channels in the cell membrane. Experimental evidence by Bednarczyk et al. [267] indicates that Mg^{2+} within the mitochondrial matrix can modulate gating and conductance of mitochondrial K_{ATP} channels, which play a key role in promoting mitochondrial recovery and cell survival under ischemia/reperfusion conditions.

6.2 Mitochondrial Dehydrogenases

Mitochondria represent one of the major cellular Mg^{2+} pools. The concentration of Mg^{2+} within the organelle ranges between 14 to 16 mM [268], and circumstantial evidence from this [269] and other laboratories [247,270,271] suggests that Mg^{2+} can be mobilized from mitochondria under various conditions including hormonal stimulation through a not fully elucidated mechanism. Regulation of mitochondrial Mg^{2+} homeostasis has been analyzed in detail in several recent reviews [16,20,268], and we direct the interested reader to them for further information. In this section, we will focus on the role of intra- and extra-mitochondrial Mg^{2+} in modulating the activity of specific proteins within the organelle.

It is commonly accepted that changes in matrix Ca^{2+} can affect the activity rate of mitochondrial dehydrogenases and consequently the respiration rate [272,273]. Experimental evidence supports a similar role for Mg^{2+} as the activity of several

mitochondrial dehydrogenases has been observed to increase within minutes from the application of hormonal or metabolic stimuli in the absence of a detectable increase in mitochondrial Ca^{2+} [274,275]. In particular, the results indicate that a decrease in mitochondrial Mg^{2+} increases several fold the activity of succinate and glutamate dehydrogenases while leaving unaffected the activity of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase [114,115]. This evidence would support the concept that changes in matrix Mg^{2+} content (in combination with, or in alternative to changes in mitochondrial Ca^{2+}) can control mitochondrial respiration, at least under well defined conditions. In this respect, mitochondrial Mg^{2+} content appears to change quite significantly during transition from state 3 to state 4 [276], affecting the amplitude of mitochondria respiration. In addition, data from our laboratory [269], from Zhang and Melvin [271], and Kubota et al. [247] all suggest that catecholamine stimulation can mobilize mitochondrial Mg^{2+} via a direct effect of cAMP on mitochondria. Hence, catecholamine administration will enhance mitochondrial respiration via cAMP-mediated modulation of mitochondrial Mg^{2+}, which, in turn, will directly stimulate succinate and glutamate dehydrogenases while sensitizing other dehydrogenases to changes in mitochondrial Ca^{2+} concentrations.

Additional mitochondrial function modulated by changes in Mg^{2+} within the organelle are anion channels present in the mitochondrial membrane [277] as well as the opening of the permeability transition pore [278]. The mitochondrial inner membrane anion channel (IMAC) transports various anions, and is involved in regulating the organelle volume in conjunction with the K^+/H^+ antiporter. Although its fine regulation is not fully elucidated as yet, experimental evidence suggests that matrix Mg^{2+} and protons maintain the channel in its closed state [277]. Kinetic studies by Beavis and Powers support a main role of Mg^{2+} in maintaining the channel in a conformation that would allow fine modulation by small changes in pH and proton distribution under physiological conditions [277]. The end results will be the maintenance of an optimal proton gradient and $\Delta\psi$ across the mitochondrial membrane, essential to retain proper organelle function and intra-mitochondrial Mg^{2+} content [112].

Perturbance of mitochondrial $\Delta\psi$, Ca^{2+} content or ATP level all result in the opening of the permeability transition pore (PTP) in the inner mitochondrial membrane [278] and the rapid re-equilibration of intra-mitochondrial ions and solutes down their concentration gradient. While it is well established that an increase in mitochondrial Ca^{2+} content facilitates PTP opening, an increase in mitochondrial Mg^{2+} antagonizes it. This effect can be appreciated well in yeasts, which do not possess a canonical PTP [279]. Creatine kinase also regulates PTP opening by tightly associating to the mitochondrial membrane and remaining in an active state [280]. Both the binding and activity state of the protein are Mg^{2+}-dependent, and removal of Mg^{2+} from the extra-mitochondrial environment results in a decline in creatine kinase activity and PTP opening [280].

Hence, it appears that Mg^{2+} regulates volume, ion composition, and ATP production within the mitochondrion, modulating the metabolic interaction between the organelle and the hosting cell.

6.3 Reticular Glucose 6-Phosphatase

The endoplasmic reticulum (ER) represents another major Mg^{2+} pool within the cell, with a total concentration estimated to be between 14 to 18 mM [1]. Yet, no information is available about the modality by which Mg^{2+} ions enter and exit the organelle and how it is buffered within the ER lumen. Limited information is also available about any major role of luminal Mg^{2+} on reticular functions other than protein synthesis [223].

Work by Volpe and collaborators [281,282], Gusev and Niggli [283], and Laver and Honen [284] suggests that cytosolic and perhaps luminal Mg^{2+} concentrations have a major effect in limiting Ca^{2+} uptake into the ER/SR and its release from the organelle via IP3 [282] and ryanodine receptor (RyR) [284]. While a direct effect of Mg^{2+} on RyR opening has been observed [283,284], it is unclear whether a similar effect takes place on the IP_3 receptor.

Recently, our laboratory has reported that cytosolic Mg^{2+} can have a regulatory role on the activity of reticular glucose 6-phosphatase (G6Pase) in liver cells [285]. This effect is biphasic, with an optimal stimulatory effect at ~0.5 mM $[Mg^{2+}]_i$ and an inhibitory effect at higher Mg^{2+} concentrations [285]. The Mg^{2+} effect appears to be at the level of the glucose 6-phosphate (G6Pi) transport component of the G6Pase enzymatic complex in that it is abolished by EDTA (as Mg^{2+} chelating agent) or taurocholic acid, which permeabilizes the ER membrane allowing for the direct delivery of G6Pi to the catalytic site of the G6Pase within the ER lumen bypassing the transport mechanism [285]. This effect of Mg^{2+} on G6Pase hydrolysis rate also occurs in purified microsomes [286] isolated from livers of animals exposed for 2 weeks to a Mg^{2+}-deficient diet [286]. Also in microsomes, the G6Pi hydrolysis rate is dynamically decreased by addition of Mg^{2+} at a concentration similar to that reported to be present in the hepatocyte cytoplasm, or increased by EDTA addition [286]. It is presently undetermined whether Mg^{2+} exerts a similar modulating effect on other reticular enzymatic activities.

6.4 Cell pH and Volume

Cells exposed to cyanide [153], fructose [202], hypoxia, [201,204], ethanol [203], or choline chloride [130] undergo a marked cellular acidification, decrease in cellular ATP content, and a large Mg^{2+} extrusion. This extrusion is the consequence of a decrease in buffering capacity (ATP loss) and binding affinity within the cytoplasm. Recently, Yamaguchi and Ishikawa [287] reported that a cytosolic $[Mg^{2+}]_i$ of ~1 mM (a physiological Mg^{2+} concentration measured in the cytosol of various cells [219,255]), inhibits by ~50% the current generated by the electrogenic Na^+-HCO_3^- cotransporter NBCe1-B. Increasing the *free* Mg^{2+} concentration to 3 mM completely abolishes NBCe1-B current. This regulatory effect is exerted by Mg^{2+} and not

$Mg(ATP)^{2-}$, and occurs at the N-terminus of the transporter [287]. It is still unresolved whether Mg^{2+} binds the N-terminus of the transporter directly or exerts its effects via an intermediate, Mg^{2+}-modulated regulatory protein [287].

On the other hand, increasing cellular Mg^{2+} content has a stimulatory role on the expression of aquaporin 3 in CaCo-3 cells [288]. This isoform of aquaporin is highly expressed in the gastrointestinal tract, in which it absorbs water, glycerol, and urea. The effect of Mg^{2+} on aquaporin mRNA expression appears to involve cAMP/PKA/CREB signaling, as well as MEK1/2 and MSK1 [288], suggesting the occurrence of both short- and long-term regulation on the protein activity and expression. As aquaporin 3 is highly expressed in brain, erythrocytes, kidney, and skin, in addition to the gastrointestinal tract, the occurrence of a modulating effect of Mg^{2+} on aquaporin 3 expression in these tissues may be highly relevant for various physiological and pathological conditions including brain swelling following traumatic injury. It remains to be determined whether Mg^{2+} exerts a similar regulatory role on other aquaporin isoforms.

Taken together, these two sets of information emphasize a role of Mg^{2+} in regulating directly pH, volume, and cation concentration, especially Na^{+} within the cell, and indirectly fatty acid metabolism via aquaporin 3-mediated glycerol accumulation.

6.5 Cell Cycle

Cell cycle [221,289,290], cell proliferation [291], and cell differentiation [292–294] have all been associated with the maintenance of an optimal Mg^{2+} level. Under conditions in which cellular Mg^{2+} accessibility is restricted or reduced, cell proliferation and cell cycle progression are markedly impaired as is cell differentiation [292–294]. The mechanisms by which a decrease in cellular Mg^{2+} content affects these cellular processes revolve around defective MAPKs [221] and p27 [290] signaling, increased oxidative stress level [292], and decreased $Mg(ATP)^{2-}$ levels [223,294]. Because the cellular $Mg(ATP)^{2-}$ level is at a level optimal for protein synthesis [223], any alteration in this metabolic parameter will have major repercussion on the proper functioning of the cell. In addition, extracellular Mg^{2+} levels regulate integrin signaling, *de facto* modulating the interaction among cells and between cells and extracellular matrix [295]. All together, these observations support the notion that an optimal Mg^{2+} level is essential to guarantee cell cycle progression and retention of proper cell morphology and function, and prevent the undesired progression towards cell death or neoplastic destiny [296].

7 Conclusions

In the last few years, our understanding of the mechanisms regulating cellular and whole body Mg^{2+} homeostasis has advanced significantly. Although in terms of overall understanding the field still lags behind the knowledge available for other

ions such as Ca^{2+}, H^+, K^+ or Na^+, the identification of Mg^{2+} channels and transport mechanisms in the membrane of cells and cellular organelles, and a better comprehension of the various signaling pathways and conditions regulating Mg^{2+} transport are providing new tools to address essential questions about the relevance of Mg^{2+} for various cell functions under physiological and pathological conditions.

Abbreviations

$[Mg^{2+}]_0$	extracellular Mg^{2+} concentration
$[Mg^{2+}]_i$	cytosolic free Mg^{2+} concentration
2,3-DPG	2,3-diphosphoglycerate
2-APB	2-2-aminoethyldiphenyl-borate
AC	adenylyl cyclase
ACDP2	ancient conversed domain protein 2
ADP	adenosine 5'-diphosphate
AMP	adenosine monophosphate
AP-1	adaptin protein 1
ATP	adenosine 5'-triphosphate
Bapta-AM	1,2-bis-(o-Aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid, tetraacetoxymethyl ester
BK channel	big potassium channel
CaM	calmodulin
cAMP	cyclic-adenosine monophosphate
CaSR	calcium-sensing receptor
CHAK	channel kinase
CRAC channel	calcium release-activated Ca^{2+} channel
CREB	cAMP response element binding
DAG	diacyl glycerol
DHHC	Asp-His-His-Cys motif
DIDS	4,4'-diisothiocyano-2,2'-stilbenedisulfonic acid
DNDS	4,4'-dinitrostilbene-2,2'-disulfonic acid
EDTA	ethylenediamine-N,N,N',N'-tetraacetate
eEF2-k	eEF2 cognate kinase
EGF	epidermal growth factor
ER	endoplasmic reticulum
ERK	extracellular responsive kinase
EXPMA	electron probe X-ray microanalysis
FHHNC	familial hypomagenesemia with hypercalciuria and nephrocalcinosis
G6Pase	glucose 6-phosphatase
G6Pi	glucose 6-phosphate
GTP	guanosine 5'-triphosphate
HIP	huntingtin-interacting protein
HSH	hypomagnesemia with secondary hypocalemia

HSP	hereditary spastic paraplegia
IC_{50}	inhibitory dose achieving 50% inhibition
IMAC	inner membrane anion channel
IP3	1,4,5, inositol-trisphophate
IRH	isolated recessive renal hypomagnesemia
Mag-Fura	5-oxazolecarboxylic acid, 2-[5-[2-[(acetyloxy)methoxy]-2-oxoethoxy]-6-[bis[2-[(acetyloxy)methoxy]-2-oxoethyl]amino]-2-benzofuranyl]-(acetyloxy)methyl ester
MagT1	magnesium transporter 1
MAPK	mitogen-activated protein kinase 1
MDCT cells	mouse distal convoluted tubule cells
MDKC cells	Madin-Darby canine kidney cells
MEK	MAP (erk1/2) kinase kinase
MIC channel	Mg^{2+}-inhibited cation channel
MMgT	membrane Mg^{2+} transporter
MSK	musculoskeletal
NIPA	non-imprinted in Prader-Willi/Angelman syndrome
OMIM	Online Mendelian Inheritance in Man
OST	oligosaccharide transferase
PCLN-1	paracellin-1 = claudin 16
PG1	polygalacturonase 1
PI3K	phosphoinositide-3-kinase
PIP2	phosphatidyl-inositol bisphohate
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PMA	phorbol-myristate acetate
PTP	permeability transition pore
Q10	Q10 temperature coefficient
RACK	receptor for activated protein kinase C
REA	repressor of estrogen receptor activity
RyR	ryanodine receptor
SCN	sodium cyanide
SDS	sodium dodecyl sulfate
siRNA	short inhibitory RNA molecule
SLC41	solute carrier family 41
SOC	store-operated channel
SR	sarcoplasmic reticulum
SERCA	sarcoplasmic-endoplasmic reticulum calcium pump
TRPM6	transient receptor potential melastatin subfamily, isoform 6
TRPM7	transient receptor potential melastatin subfamily, isoform 7

Acknowledgments This work was supported by grant AA-11593.

References

1. A. Romani, A. Scarpa, *Arch. Biochem. Biophys*. **1992**, *298*, 1–12.
2. F. I. Wolf, A. Torsello, S. Fasanella, A. Cittadini, *Mol. Asp. Med*. **2003**, *24*, 11–26.
3. F. I. Wolf, A. Cittadini, *Mol. Asp. Med*. **2003**, *24*, 3–9.
4. D. W. Jung, L. Apel, G. P. Brierley, *Biochemistry* **1990**, *29*, 4121–4128.
5. G. A. Rutter, N. J. Osbaldeston, J. G. McCormack, R. M. Denton, *Biochem. J*. **1990**, *271*, 627–634.
6. A. V. Somlyo, G. McClellan, H. Gonzalez-Serratos, A. P. Somlyo, *J. Biol. Chem*. **1985**, *260*, 6801–6807.
7. A. M. Hofer, T. E. Machen, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 2598–2602.
8. A. Scarpa, F. J. Brinley, *Fed. Proc*. **1981**, *40*, 2646–252.
9. D. Lüthi, D. Günzel, J.A. McGuigan, *Exp. Physiol*. **1999**, *84*, 231–252.
10. P. W. Flatman, V. L. Lew, *J. Physiol*. **1981**, *315*, 421–446.
11. T. Günther, J. Vormann, J. A. McGuigan, *Biochem. Mol. Biol. Int*. **1995**, *37*, 871–875.
12. J. E. Raftos, V. L. Lew, P. W. Flatman, *Eur. J. Biochem*. **1999**, *263*, 635–645.
13. S. Oki, M. Ikura, M. Zhang, *Biochemistry* **1997**, *36*, 4309–4316.
14. S. Wang, S. E. George, J. P. Davis, J. D. Johnson, *Biochemistry* **1998**, *37*, 14539–14544.
15. D. Allouche, J. Parello, Y. H. Sanejouand, *J. Mol. Biol*. **1999**, *285*, 857–873.
16. Y. Ogoma, H. Kobayashi, T. Fujii, Y. Kondo, A. Hachimori, T. Shimizu, M. Hatano, *Int. J. Biol. Macromol*. **1992**, *14*, 279–286.
17. K. Bogucka, L. Wojtczak, *Biochem. Biophys. Res. Commun*. **1971**, *44*, 1330–1337.
18. H. Belge, P. Gailly, B. Schwaller, J. Loffing, H. Debaix, E. Riveira-Munoz, R. Beauwens, J. P. Devogelaer, J. G. Hoenderop, R. J. Bindels, O. Devuyst, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 14849–14854.
19. Geigy Scientific Tables. Ed C. Lentner, Ciba-Geigy, Basel, Switzerland, 1984.
20. P. W. Flatman, *J. Membr. Biol*. **1984**, *80*, 1–14.
21. A. Romani, *Arch. Biochem. Biophys*. **2007**, *458*, 90–102.
22. P. I. Polimeni, E. Page, in *Recent Advances in Study in Cardiac Cells and Metabolism*, Ed N. S. Dhalle, Volume IV, University Park Press, Baltimore, 1974, pp. 217–232.
23. T. A. Rogers, F. L. Haven, P. E. Mahan, *J. Natl. Cancer. Inst*. **1960**, *25*, 887–888.
24. T. A. Rogers, *J. Cell. Comp. Physiol*. **1961**, *57*, 119–121.
25. F. I. Wolf, A. Di Francesco, V. Covacci, A. Cittadini, *Arch. Biochem. Biophys*. **1997**, *344*, 397–403.
26. M. E. Maguire, J. J. Erdos, *J. Biol. Chem*. **1978**, *253*, 6633–6636.
27. R. D. Grubbs, M. E. Maguire, *Magnesium* **1987**, *6*, 113–127.
28. A. M. Romani, M. E. Maguire, *Biometals* **2002**, *15*, 271–283.
29. A. Romani, A. Scarpa, *Front. Biosc*i. **2000**, *5*, D720–D734.
30. T. Günther, J. Vormann, *Magnes. Trace Elem*. **1990**, *9*, 279–282.
31. T. Matsuura, Y. Kanayama, T. Inoue, T. Takeda, I. Morishima, *Biochim. Biophys. Acta* **1993**, *1220*, 31–36.
32. A. Romani, A. Scarpa, *Nature* **1990**, *346*, 841–844.
33. J. Vormann, T. Günther, *Magnesium* **1987**, *6*, 220–224.
34. A. Romani, A. Scarpa, *FEBS Lett*. **1990**, *269*, 37–40.
35. T. Günther, J. Vormann, V. Hollriegl, *Magnes. Bull*. **1991**, *13*, 122–124.
36. A. Jakob, J. Becker, G. Schottli, G. Fritzsch, *FEBS Lett*. **1989**, *246*, 127–130.
37. M. Fatholahi, K. Lanoue, A. Romani, A. Scarpa, *Arch. Biochem. Biophys*. **2000**, *374*, 395–401.
38. T. Kubota, Y. Shindo, K. Tokuno, H. Komatsu, H. Ogawa, S. Kudo, Y. Kitamura, K. Suzuki, K. Oka, *Biochim. Biophys. Acta* **2005**, *1744*, 19–28.
39. D. G. Kehres, C. H. Lawyer, M. E. Maguire, *Microb. Comp. Genomics* **1998**, *3*, 151–159.

40. M. B. Moncrief, M. E. Maguire, *J. Biol. Inorg. Chem*. **1999**, *4*, 523–527.
41. R. R. Preston, *Science* **1990**, *250*, 285–288.
42. M. J. Nadler, M. C. Hermosura, K. Inabe, A.-L. Perraud, Q. Zhu, A. J. Stokes, T. Kurosaki, J. P. Kinet, R. Penner, A. M. Scharenberg, A. Fleig, *Nature* **2001**, *411*, 590–595.
43. K. P. Schlingmann, S. Weber, M. Peters, N. L. Niemann, H. Vitzthum, K. Klingel, M. Kratz. E. Haddad, E. Ristoff, D. Dinour, M. Syrrou, S. Nielsen, M. Sassen, S. Waldegger, H. W. Seyberth, M. Konrad, *Nat. Genet*. **2002**, *31*, 166–170.
44. H. Yamaguchi, M. Matsushita, A. Nairn, J. Kuriyan, *Mol. Cell*. **2001**, *7*, 1047–1057.
45. L. V. Ryazanova, K. S. Pavur, A. N. Petrov, M. V. Dorovkov, A. G. Ryazanov, *Mol. Biol.* (Moskow) **2001**, *35*, 321–332.
46. A. G. Ryazanov, *FEBS Lett*. **2002**, *514*, 26–29.
47. L. W. Runnels, L. Yue, D. E. Clapham, *Science* **2001**, *291*, 1043–1047.
48. B. F. Bessac, A. Fleig, *J. Physiol*. **2007**, *582*, 1073–1086.
49. M. K. Monteilh-Zoller, M. C. Hermosura, M. J. Nadler, A. M. Scharenberg, R. Penner, A. Fleig, *J. Gen. Physiol*. **2003**, *121*, 49–60.
50. T. Voels, B. Nilliues, S. Hoefs, A. W. van der Kemp, G. Droogmans, R. J. Bindels, J. G. Hoenderop, *J. Biol. Chem*. **2004**, *279*, 19–25.
51. V. Chubanov, S. Waldegger, Y. Mederos, M. Schnitzler, H. Vitzthum, M.C. Sassen, H.W. Seyberth, M. Konrad, T. Gudermann, *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 2894–2899.
52. C. Schmitz, M. V. Dorovkov, X. Zhao, B. J. Davenport, A. G. Ryazanov, A.-L. Perraud, *J. Biol. Chem*. **2005**, *280*, 37763–37771.
53. V. Chubanov, T. Gudermann, K. P. Schlingmann, *Pflugers. Arch*. **2005**, *451*, 228–234.
54. M. Li, J. Jiang, L. Yue, *J. Gen. Physiol*. **2006**, *127*, 525–537.
55. M. Li, J. Du, J. Jiang, W. Ratzan, L. T. Su, L.W. Runnels, L. Yue, *J. Biol. Chem*. **2007**, *282*, 25817–25830.
56. A. Gwanyanya, B. Amuzescu, S. I. Zakharov, R. Macianskiene, K .R. Sipido, V. M. Bolotina, J. Vereecke, K. Mubagwa, *J. Physiol*. **2004**, *559*, 761–776.
57. J. Jiang, M. Li, L. Yue, *J. Gen. Physiol*. **2005**, *126*, 137–150.
58. L. W. Runnels, L. Yue, D. E. Clapham, *Nat. Cell. Biol*. **2002**, *4*, 329–336.
59. M. Langeslag, K. Clark, W. H. Moolenaar, F. N. van Leeuwen, K. Jalink, *J. Biol. Chem*. **2007**, *282*, 232–239.
60. R. Macianskiene, A. Gwanyanya, J. Vereecke, K. Mubagwa, *Cell. Physiol. Biochem*. **2008**, *22*, 109–118.
61. A. Gwanyanya, K. R. Sipido, J. Vereecke, K. Mubagwa, *Am. J. Physiol*. **2006**, *291*, C627–C635.
62. R. M. Touyz, Y. He, A. C. I. Montezano, G. Yao, V. Chubanov, T. Gudermann, G. E. Callera, *Am. J. Physiol*. **2006**, *290*, R73–R78.
63. J. Sahni, A. M. Scharenberg, *Cell. Metab*. **2008**, *8*, 84–93.
64. J. Sahni, R. Tamura, I. R. Sweet, A. M. Scharenberg, *Cell Cycle* **2010**, y, 3565–3574.
65. J. Middelbeek, K. Clark, H. V. Venselaar, M. A. Huynen, F. N. van Leeuwen, *Cell. Mol. Life Sci*. **2010**, *67*, 875–890.
66. C. Schmitz, A.-L. Perraud, C. O. Johnson, K. Inabe, M. K. Smith, R. Penner, T. Kurosaki, A. Fleig, A. M. Scharenberg, *Cell* **2003**, *114*, 191–200.
67. K. Clark, M. Langeslag, B. van Leeuwen, L. Ran, A. G. Ryazanov, C. G. Figdor, W. H. Moolenaar, K. Jalink, F. N. van Leeuwen, *EMBO J*. **2006**, *25*, 290–301.
68. L. V. Ryazanova, L. J. Rondon, S. Zierler, Z. Hu, J. Galli, T. P. Yamaguchi, A. Mazur, A. Fleig, A. G. Ryazanov, *Nature Commun*. **2010**, *1*, 109.
69. T. E. Woudenberg-Vrenken, A. Sukinta, A. W. van der Kemp, R. J. Bindels, *Nephron Physiol*. **2011**, *117*, 11–19.
70. A.-L. Perraud, X. Zhao, A. G. Ryazanov, C. Schmitz, *Cell. Signal*. **2011**, *23*, 587–593.
71. K. Clark, J. Middelbeek, E. Lasonder, N. G. Dulyaninova, N. A. Morrice, A. G. Ryazanov, A. R. Bresnick, C. G. Figdor, F. N. van Leeuwen, *J. Mol. Biol*. **2008**, *378*, 790–803.
72. L.-T. Su, M.A. Agapito, M. Li, W. Simpson, A. Huttenlocher, R. Habas, L. Yue, L. W. Runnels, *J. Biol. Chem*. **2006**, *281*, 11260–11270.

73. M. V. Dorovkov, A. G. Ryazanov, *J. Biol. Chem*. **2004**, *279*, 50643–50646.
74. T. M. Paravicini, A. Yogi, A. Mazur, R. M. Touyz, *Hypertension* **2009**, *53*, 423–429.
75. M. Aarts, K. Iihara, W. L. Wei, Z. G. Xiong, M. Arundine, W. Cerwinski, J. F. MacDonald, M. Tymianski, *Cell* **2003**, *115*, 863–877.
76. W. L. Wei, H. S. Sun, M. E. Olah, X. Sun, E. Czerwinska, W. Czerwinski, Y. Mori, B. A. Orser, Z. G. Xiong, M. F. Jackson, M. Tymianski, J. F. MacDonald, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 16323–16328.
77. H. Jiang, S. L. Tian, Y. Zeng, L. L. Li, J. Shi, *Brain Res. Bull*. **2008**, *76*, 124–130.
78. H.-S. Chen, J. Xie, Z. Zhang, L.-T. Su, L. Yue, L. W. Runnels, *PLoS One* **2010**, *5*, e11161.
79. S. Brauchi, G. Krapivinsky, L. Krapivinsky, D. E. Clapham, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 8304–8308.
80. E. Abed, R. Moreau, *Cell, Prolif*. **2007**, *40*, 849–865.
81. E. Abed, C. Martineau, R. Moreau, *Calcif. Tissue Int*. **2011**, *88*, 246–253.
82. J. Jin, B. N. Desai, B. Navarro, A. Donovan, N. C. Andrews, D. E. Clapham, *Science* **2008**, *322*, 756–760.
83. R. Y. Walder, D. Landau, P. Meyer, H. Shalev, M. Tsolia, Z. Borochowitz, M. B. Boettger, G. E. Beck, R. K. Englehardt, R. Carmi, V. C. Sheffield, *Nat. Genet*. **2002**, *31*, 171–174.
84. R. Y. Walder, B. Yang, J.B. Stokes, P. A. Kirby, X. Cao, P. Shi, C. C. Searby, R. F. Husted, V. C. Sheffield, *Human Mol. Gen*. **2009**, *18*, 4367–4375.
85. T. E. Woudenberg-Vrenken, A. Sukinta, A. W. van der Kemp, R. J. Bindels, J. G. Hoenderop, *Nephron. Physiol*. **2011**, *117*, 11–19.
86. S. Thébault, G. Cao, H. Venselaar, Q. Xi, R. J. Bindels, J. G. Hoenderop, *J. Biol. Chem*. **2008**, *283*, 19999–20007.
87. G. Cao, J. van der Wijst, A. van der Kemp, F. van Zeeland, R. J. Bindels, J. G. Hoenderop, *J. Biol. Chem*. **2009**, *284*, 14788–14795.
88. W. M. Groenestege, J. G. Hoenderop, L. van den Heuvel, N. Knoers, R. J. Bindels, *J. Am. Soc. Nephrol*. **2006**, *17*, 1035–1043.
89. L. J. Rondón, W. M. Groenestege, Y. Rayssiguier, A. Mazur, *Am. J. Physiol*. **2008**, *294*, R2001–R2007.
90. G. Cao, S. Thébault, J. van der Wijst, A. van der Kemp, E. Lasonder, R. J. Bindels, J. G. Hoenderop, *Curr. Biol*. **2008**, *18*, 168–176.
91. W. M. Groenestege, S. Thébault, J. van der Wijst, D. van den Berg, R. Janssen, S. Tejpar, L. P. van den Heuvel, E. van Cutsem, J. G. Hoenderop, N. V. Knoers, R. J. Bindels, *J. Clin. Invest*. **2007**, *117*, 2260–2267.
92. D. Cunningham, Y. Humblet, S. Siena, D. Khayat, H. Bleiberg, A. Santoro, D. Bets, M. Mueser, A. Harstrick, C. Verslype, I. Chau, E. van Cutsem, *N. Engl. J. Med*. **2004**, *351*, 337–345.
93. H. Dimke, J. van der Wjist, T. R. Alexander, I. M. Mejier, G. M. Mulder, H. van Goor, S. Tejpar, J. G. Hoenderop, R. J. Bindels, *J. Am. Soc. Nephrol*. **2010**, *21*, 1309–1316.
94. A. Ikari, C. Okude, H. Sawada, Y. Yamazaki, J. Sugatani, M. Miwa, *Biochem. Biophys. Res. Commun*. **2008**, *369*, 1129–1133.
95. A. Ikari, A. Sanada, C. Okude, H. Sawada, Y. Yamazaki, J. Sugatani, M. Miwa, *J. Cell. Physiol*. **2010**, *222*, 481–487.
96. D. B. Simon, Y. Lu, K. A. Choate, H. Velazquez, E. Al-Sabban, M. Praga, G. Casari, A. Bettinelli, G. Colussi, J. Rodrigues-Soriano, D. McCredie, D. Milford, S. Sanjad, R. P. Lifton, *Science* **1999**, *285*, 103–1106.
97. M. Lal-Nag, P. J. Morin, *Genome Biol*, **2009**, *10*, 235.
98. P. J. Kausalya, S. Amasheh, D. Gunzel, H. Wurps, D. Muller, M. Fromm, W. Hunziker, *J. Clin. Invest*. **2006**, *116*, 878–891.
99. J. Hou, D. L. Paul, D. A. Goodenough, *J. Cell. Sci*. **2005**, *118*, 5109–118.
100. A. Ikari, S. Matsumoto, H. Harada, K. Takagi, H. Hayashi, Y. Suzuki, M. Degawa, M. Miwa, *J. Cell. Sci*. **2006**, *119*, 1781–1789.
101. E. Efrati, J. Arsentiev-Rozenfeld, I. Zelikovic, *Am. J. Physiol*. **2005**, *288*, F272–F283.

102. D. Muller, P. J. Kausalya, F. Claverie-Martin, I. C. Meij, P. Eggert, V. Garcia-Nieto, W. Hunziker, *Am. J. Hum. Genet*. **2003**, *73*, 1293–1301.
103. M. A. Khan, A. D. Conigrave, *Br. J. Pharmacol*. **2010**, *159*, 1039–1050.
104. J. Hou, A. Renigunta, A. S. Gomes, M. Hou, D. L. Paul, S. Waldegger, D. A. Goodenough, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 15350–15355.
105. A. Goytain, G. A. Quamme, *BMC Genomics* **2005**, *6*, 48.
106. T. Shibatani, L. L. David, A. L. McCormack, K. Frueh, W. R. Skach, *Biochemistry* **2005**, *44*, 5982–5992.
107. H. Zhou, D. E. Clapham, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 15750–15755.
108. G. Wiesenberger, M. Waldherr, R. J. Schweyen, *J. Biol. Chem*. **1992**, *267*, 6963–6969.
109. D. M. Bui, J. Gregan, E. Jarosch, A. Ragnini, R. J. Schweyen, *J. Biol. Chem*. **1999**, *274*, 20438–20443.
110. M. Kolisek, G. Zsurka, J. Samaj, J. Weghuber, R. J. Schweyen, M. Schweigel, *EMBO J*. **2003**, *22*, 1235–1244.
111. M. Piskacek, L. Zotova, G. Zsurka, R. J. Schweyen, *J. Cell. Mol. Med*. **2009**, *13*, 693–700.
112. K. E. Akerman, *J. Bioenerg. Biomembr*. **1981**, *13*, 133–139.
113. G. Zsurka, J. Gregan, R. J. Schweyen, *Genomics* **2001**, *72*, 158–168.
114. A. Panov, A. Scarpa, *Biochemistry* **1996**, *35*, 427–432.
115. A. Panov, A. Scarpa, *Biochemistry* **1996**, *35*, 12849–12856.
116. A. Goytain, G.A. Quamme, *Am. J. Physiol*. **2008**, *294*, C495–502.
117. C. Schmitz, F. Deason, A.-L. Perraud, *Magnes. Res*. **2007**, *20*, 6–18.
118. R. T. Alexander, J. G. Hoenderop, R. J. Bindels, *J. Am. Soc. Nephrol*. **2008**, *19*, 1451–1458.
119. G. A. Quamme, *Am. J. Physiol*. **2010**, *298*, 407–429.
120. T. Günther, J. Vormann, R. Forster, *Biochem. Biophys. Res. Commun*. **1984**, *119*, 124–131.
121. T. Günther, J. Vormann, *Biochem. Biophys.Res. Commun*. **1985**, *130*, 540–545.
122. J.-C. Feray, R. Garay, *Biochim. Biophys. Acta* **1986**, *856*, 76–84.
123. P. W. Flatman, L. M. Smith, *J. Physiol*. **1990**, *431*, 11–25.
124. W. Xu, J. S. Willis, *J. Membr. Biol*. **1994**, *141*, 277–287.
125. H. Ludi, H. J. Schatzmann, *J. Physiol*. **1987**, *390*, 367–382.
126. J. Vormann, K. Magdorf, T. Günther, U. Wahn, *Eur. J. Clin. Chem. Clin. Biochem*. **1994**, *32*, 833–836.
127. T. Günther, J. Vormann, *FEBS Lett*. **1992**, *297*, 132–134.
128. F. I. Wolf, A. Di Francesco, V. Covacci, D. Corda, A. Cittadini, *Arch. Biochem. Biophys*. **1996**, *331*, 194–200.
129. A. Romani, C. Marfella, A. Scarpa, *Circ. Res*. **1993**, *72*, 1139–1148.
130. A. Romani, C. Marfella, A. Scarpa, *J. Biol. Chem*. **1993**, *268*, 15489–15495.
131. T. E. Fagan, A. Romani, *Am. J. Physiol*. **2000**, *279*, G943–G950.
132. T. E. Fagan, A. Romani, *Am. J. Physiol*. **2001**, *280*, G1145–G1156.
133. C. Cefaratti, C. Ruse, *Mol. Cell. Biochem*. **2007**, *297*, 209–214.
134. C. Cefaratti, A. Romani, *Mol. Cell. Biochem*. **2007**, *303*, 63–72.
135. M. Kolisek, A. Nestler, J. Vormann, M. Schweigel-Rontgen, *Am. J. Physiol.* **2012**, *302*, C318-326
136. T. Wabakken, E. Rian, M. Kveine, H. C. Aasheim, *Biochem. Biophys. Res. Commun.* **2003**, *306*, 718–724.
137. C. Cefaratti, A. Romani, *Mol. Cell. Biochem*. **2011**, *351*, 133–142.
138. H. Ebel, T. Günther, *FEBS Lett*. **2003**, *543*, 103–107.
139. H. Rasgado-Flores, H. Gonzalez-Serratos, J. DeSantiago, *Am. J. Physiol*. **1994**, *266*, C1112–C1117.
140. E. C. Aromataris, M. L. Roberts, G. J. Barritt, G. Y. Rychkov, *J. Physiol.* **2006**, *573*, 611–625.
141. M. Tashiro, M. Konishi, *Biophys. J*. **1997**, *73*, 3371–3384.
142. T. Günther, *Magnes. Bull*. **1996**, *18*, 2–6.
143. C. Cefaratti, A. Romani, A. Scarpa, *Am. J. Physiol*. **1998**, *275*, C995–C1008.
144. J.-C. Feray, R. Garay, *Naunyn-Schmied. Arch. Pharmacol*. **1988**, *338*, 332–337.

145. M. Schweigel, J. Vormann, H. Martens, *Am. J. Physiol*. **2000**, *278*, G400–G408.
146. T. Günther, *Miner. Electrolyte Metab*. **1993**, *19*, 259–265.
147. H. Ebel, M. Hollstein, T. Günther, *Biochim. Biophys. Acta* **2002**, *1559*, 135–144.
148. D. Keenan, A. Romani, A. Scarpa, *FEBS Lett*. **1996**, *395*, 241–244.
149. T. Günther, J. Vormann, V. Höllriegl, *Biochim. Biophys. Acta* **1990**, *1023*, 455–461.
150. H. Ebel, M. Hollstein, T. Günther, *Biochim. Biophys. Acta* **2004**, *1667*, 132–140.
151. O. Shaul, D. W. Hilgemann, J. de-Almeida-Engler, M. Van Montagu, D. Inz, G. Galili, *EMBO J*. **1999**, *18*, 3973–3980.
152. T. Günther, J. Vormann, *FEBS Lett*. **1990**, *265*, 55–58.
153. P. Dalal, A. Romani, *Metabolism* **2010**, *59*, 1663–1671.
154. D. Günzel, W. R. Schlue, *J. Physiol*. **1996**, *491*, 595–608.
155. R. L. Smith, L. J. Thompson, M. E. Maguire, *J. Bacteriol.* **1995**, *177*, 1233–1238.
156. A. Goytain, G. A. Quamme, Physiol. *Genomics* **2005**, *21*, 337–342.
157. M. Kolisek, P. Launay, A. Beck, G. Sponder, N. Serafini, M. Brenkus, E. M. Froschauer, H. Martens, A. Fleig, M. Schweigel, *J. Biol. Chem.* **2008**, *283*, 16235–16247.
158. A. Goytain, G. A. Quamme, Biochem. *Biophys. Res. Commun*. **2005**, *330*, 701–705.
159. J. Sahni, B. Nelson, A. M. Scharenberg, *Biochem. J*. **2007**, *401*, 505–513.
160. C. Y. Wang, A. Davoodi-Semiromi, J. D. Shi, P. Yang, Y. Q. Huang, J. A. Agundez, J. M. Moran, B. Ochoa, B. Hawkins-Lee, J.X. She, *Am. J. Med. Genet*. *A* **2003**, *119*, 9–14.
161. C. Y. Wang, J. D. Shi, P. Yang, P. G. Kumar, Q. Z. Li, Q. G. Run, Y. C. Su, H. S. Scott, K. J. Kao, J. X. She, *Gene* **2003**, *306*, 37–44.
162. C. Y. Wang, P. Yang, J. D. Chi, S. Purohit, D. Guo, H. An, J. G. Gu, J. Ling, Z. Dong, J. X. She, *BMC Genomics* **2004**, *5*, 7.
163. D. G. Kehres, M. E. Maguire, *Biometals* **2002**, *15*, 261–270.
164. A. Goytain, G. A. Quamme, *Physiol. Genomics*. **2005**, *22*, 382–389.
165. M. G. Butler, *Am. J. Med. Genet*. **1990**, *35*, 319–332.
166. A. Goytain, R. M. Hines, A. El-Husseini, G. A. Quamme, *J. Biol. Chem*. **2007**, *282*, 8060–8068.
167. A. Goytain, R. M. Hines, G. A. Quamme, *Am. J. Physiol*. **2008**, *295*, C944–C953.
168. S. Rainier, J. H. Chai, D. Tokarz, R. D. Nicholls, J. K. Fink, *Am. J. Hum. Genet*. **2003**, *73*, 967–971.
169. A. Goytain, R. M. Hines, G. A. Quamme, *J. Biol. Chem*. **2008**, *283*, 33365–33374.
170. S.-H. Li, X.-J. Li, *Trends Genet*. **2004**, *20*, 146–152.
171. A. Yanai, K. Huang, R. Kang, R. R. Singaraja, P. Arstikaitis, L. Gan, P. C. Orban, A. Mullard, C. M. Cowan, L. A. Raymond, R. C. Drisdel, W. N. Green, B. Ravikumar, D. C. Rubinsztein, A. El-Husseini, M. R. Hayden, *Nat. Neurosci*. **2006**, *9*, 824–831.
172. C. Cefaratti, A. Romani, A. Scarpa, *J. Biol. Chem*. **2000**, *275*, 3772–3780.
173. R. Juttner, H. Ebel, *Biochim. Biophys. Acta* **1998**, *1370*, 51–63.
174. M. Baillien, M. Cogneau, *Magnesium* **1995**, y, 331–339.
175. H. J. Schatzmann, *Biochim. Biophys. Acta* **1993**, *1148*, 15–18.
176. M. Baillien, H. Wang, M. Cogneau, *Magnesium* **1995**, *8*, 315–329.
177. D. A. Elliot, M. A. Rizack, *J. Biol. Chem*. **1974**, *249*, 3985–3990.
178. S. J. Bird, M. E. Maguire, *J. Biol. Chem*. **1978**, *253*, 8826–8834.
179. J. J. Erdos, M. E. Maguire, *Mol. Pharmacol*. **1980**, *18*, 379–383.
180. J. J. Erdos, M. E. Maguire, *J. Physiol*. **1983**, *337*, 351–371.
181. R. D. Grubbs, C. A. Wetherill, K. Kutschke, M. E. Maguire, *Am. J. Physiol*. **1984**, *248*, C51–C57.
182. M. E. Maguire, J. J. Erdos, *J. Biol. Chem*. **1978**, *253*, 6633–6636.
183. M. E. Maguire, J. J. Erdos, *J. Biol. Chem*. **1980**, *255*, 1030–1035.
184. R. D. Grubbs, S. D. Collins, M. E. Maguire, *J. Biol. Chem*. **1985**, *259*, 12184–12192.
185. F. C. Howarth, J. Waring, B. I. Hustler, J. Singh, *Magnes. Res*. **1994**, *7*, 187–197.
186. F. I. Wolf, A. Di Francesco, A. Cittadini, *Arch. Biochem. Biophys*. **1994**, *308*, 335–341.
187. D. Keenan, A. Romani, A. Scarpa, *Circ. Res*. **1995**, *77*, 973–983.
188. T. Günther, J. Vormann, *Magnes. Bull*. **1992**, *14*, 122–125.

189. A. M. Romani, V. D. Matthews, A. Scarpa, *Circ. Res*. **2000**, *86*, 326–333.
190. T. Amano, T. Matsubara, J. Watanabe, S. Nakayama, N. Hotta, *Brit. J. Pharmacol*. **2000**, *130*, 731–738.
191. V. Karoor, K. Baltensperger, H. Paul, M. C. Czech, C. C. Malbon, *J. Biol. Chem*. **1995**, *270*, 25305–25308.
192. J. A. Smoake, G.-M. M. Moy, B. Fang, S. S. Solomon, *Arch. Biochem. Biophys*. **1995**, *323*, 223–232.
193. A. Ferreira, A. Rivera, J. R. Romero, *J. Cell. Physiol*. **2004**, *199*, 434–440.
194. G. Wiesenberger, K. Steinleitner, R. Malli, W. F. Graier, J. Vormann, R. J. Schweyen, J. A. Stadler, *Eukaryot. Cell* **2007**, *6*, 592–599.
195. M. Wang, J. R. Berlin, *Am. J. Physiol*. **2006**, *291*, C83–C92.
196. L. M. Torres, J. Youngner, A. Romani, *Am. J. Physiol*. **2005**, *288*, G195–G206.
197. T. E. Fagan, C. Cefaratti, A. Romani, *Am. J. Physiol*. **2004**, *286*, E184–E193.
198. J. C. Henquin, T. Tamagawa, M. Nenquin, M. Cogneau, *Nature* **1983**, *301*, 73–74.
199. D. Garfinkel, L. Garfinkel, *Magnesium* **1988**, *7*, 249–261.
200. M. Otto, R. Heinrich, B. Kuhn, G. Jacobasch, *Eur. J. Biochem*. **1974**, *49*, 169–178.
201. A. W. Harman, A. L. Nieminen, J. J. Lemasters, B. Herman, *Biochem. Biophys. Res. Commun*. **1990**, *170*, 477–483.
202. V. Gaussin, P. Gailly, J.-M. Gillis, L. Hue, *Biochem. J*. **1997**, *326*, 823–827.
203. P.A. Tessman, A. Romani, *Am. J. Physiol*. **1998**, *275*, G1106–G1116.
204. A. Gasbarrini, A. B. Borle, H. Farghali, C. Bender, A. Francavilla, D. van Thiel, *J. Biol. Chem*. **1992**, *267*, 6654–6663.
205. J. Hwa, A. Romani, C. Marfella, A. Scarpa, *Biophys. J*. **1993**, *63*, A307.
206. R. Di Polo, L. Beauge, *Biochim. Biophys. Acta* **1988**, *946*, 424–428.
207. T. Günther, V. Höllriegl, *Biochim. Biophys. Acta* **1993**, *1149*, 49–54.
208. R. M. Touyz, E. L. Schiffrin, *J. Biol. Chem*. **1996**, *271*, 24353–24358.
209. A. Romani, C. Marfella, A. Scarpa, *FEBS Lett*. **1992**, *296*, 135–140.
210. D. L. Hwang, C. F. Yen, J. L. Nadler, *J. Clin. Endocrinol. Metab*. **1993**, *76*, 549–553.
211. R. D. Grubbs, M. E. Maguire, *J. Biol. Chem*. **1986**, *261*, 12550–12554.
212. S. Ishijima, M. Tatibana, *J. Biochem*. **1994**, *115*, 730–737.
213. P. Csermely, P. Fodor, J. Somogyi, *Carcinogenesis* **1987**, *8*, 1663–1666.
214. T. Günther, J. Vormann, *Biochim. Biophys. Acta* **1995**, *1234*, 105–110.
215. Z. W. Yang, J. Wang, T. Zheng, B. T. Altura, B. M. Altura, *Stroke* **2001**, *32*, 249–257.
216. L. M. Torres, B. Konopnika, L. N. Berti-Mattera, C. Liedtke, A. Romani, *Alcohol. Clin. Exp. Res*. **2010**, *34*, 1659–1669.
217. E. Y. Tang, P. J. Parker, J. Beattie, M. D. Houslay, *FEBS Lett*. **1993**, *326*, 117–123.
218. Y. Konno, S. Ohno, Y. Akita, H. Kawasaki, K. Suzuki, *J. Biochem*. **1989**, *106*, 673–678.
219. B. E. Corkey, J. Duszynski, T. L. Rich, B. Matchinsky, J. R. Williamson, *J. Biol. Chem*. **1986**, *261*, 2567–2574.
220. Z. Yang, J. Wang, B. T. Altura, B. M. Altura, *Pflugers Arch*. **2000**, *439*, 240–247.
221. R. M. Touyz, G. Yao, *J. Cell. Physiol*. **2003**, *197*, 326–335.
222. L. M. Torres, C. Cefaratti, B. Perry, A. Romani, *Mol. Cell. Biochem*. **2006**, *288*, 191–199.
223. H. Rubin, *Adv. Cancer Res*. **2005**, *93*, 1–58.
224. W. F. Waas, K. N. Dalby, *Biochemistry* **2003**, *42*, 2960–2970.
225. S. J. Kim, H. S. Kang, M. S. Kang, X. Yu, S. Y. Park, I. S. Kim, N. S. Kim, S. Z. Kim, Y. G. Kwak, J. S. Kim, *Biochem. Biophys. Res. Commun*.**2005**, *333*, 1132–1138.
226. S. Thebault, R. T. Alexander, W. M. Tiel Groenestege, J. G. Hoenderop, R. J. Bindels, *J. Am. Soc. Nephrol.* **2009**, *20*, 78–85.
227. J. van der Wjist, J. G. Hoenderop, R. J. Bindels, *Magnes. Res*. **2009**, *22*, 127–132.
228. G. H. Mudge, in *The Pharmacological Basis of Therapeutics*, Eds A. Goodman Gilman, L. S. Godman, T. W. Rall, F. Murad, MacMillian, New York, 1989.
229. H. Joborn, G. Akerstrom, S. Ljunghall, *Clin. Endocrinol*. **1985**, *23*, 219–226.
230. C. Bailly, M. Imbert-Teboul, N. Roinel, C. Amiel, *Am. J. Physiol*. **1990**, *258*, F1224–F1231.

231. Y. Rayssiguier, *Horm. Metab. Res*. **1977**, *9*, 309–314.
232. G. Guideri, *Arch. Intern. Pharmacodyn. Therap*. **1992**, *14*, 122–125.
233. I. M. Shafik, G. A. Quamme, *Am. J. Phyiol*. **1989**, *257*, F974–F977.
234. P. B. Molinoff, *Drugs* **1984**, *28*, 1–14.
235. P. J.Barnes, *Am. J. Respir. Crit. Care Med*. **1995**, *152*, 838–860.
236. E. M. Brown, G. Gamba, D. Riccardi, M. Lombardi, R. Butters, O. Kifor, A. Sun, M. A. Hediger, J. Lytton, S. C. Herbert, *Nature* **1993**, *366*, 575–580.
237. E. F. Nemeth, A. Scarpa, J. Biol. Chem. **1987**, *262*, 5188–5196.
238. B. W. Bapty, L. J. Dai, G. Ritchie, F. Jirik, L. Canaff, G. N. Hendy, G. A. Quamme, *Kidney Int*. **1998**, *53*, 583–592.
239. B. W. Bapty, L. J. Dai, G. Ritchie, L. Canaff, G. N. Hendy, G. A. Quamme, *Am. J. Physiol*. **1998**, *275*, F353–F360.
240. G. A. Quamme, J. H.Dirks, *Am. J. Physiol*. **1980**, *238*, F187–F198.
241. D. J. Dipette, K. Simpson, J. Guntupalli, *Magnesium* **1987**, *6*, 136–149.
242. J. B. Stanbury, *J. Pharmacol. Exp. Ther*. **1984**, *93*, 52–62.
243. M. F. M. James, R. C. Cork, G. M. Harlen, J. F. White, *Magnesium* **1988**, *7*, 37–43.
244. G. A. Quamme, S. W. Rabkin, *Biochem. Biophys. Res. Commun*. **1990**, *167*, 1406–1412.
245. G. A. Quamme, L.-S.Dai, *Am. J. Physiol*. **1990**, *259*, C521–C525.
246. L.-J. Dai, P. A. Friedman, G. A.Quamme, *Kidney Inter*. **1997**, *51*, 1710–1718.
247. T. Kubota, Y. Shindo, K. Tokuno, H. Komatsu, H. Ogawa, S. Kudo, Y. Kitamura, K. Suzuki, K. Oka, *Biochim. Biophys. Acta* **2005**, *1744*, 19–28.
248. L.-J. Dai, P. A. Friedman, G. A. Quamme, *Kidney Inter*. **1991**, *51*, 1008–1017.
249. M. Tashiro, P. Tursun, T. Miyazaki, M. Watanabe, M. Konishi, *Jpn. J. Physiol*. **2002**, *52*, 541–551.
250. M. Schweigel, I. Lang, H. Martens, *Am. J. Physiol*. **1999**, *277*, G976–G982.
251. M. Schweigel, H. Martens, *Am. J. Physiol*. **2003**, *285*, G45–G53.
252. T. Günther, J.Vormann, *Biochim. Biophys. Acta* **1995**, *1234*, 105–110.
253. C. E. Mondon, C. B. Dolkas, J. M. Olefsky, G. M. Reaven, *Diabetes* **1974**, *24*, 225–229.
254. R. Taylor, L. Agius, *Biochem. J*. **1988**, *250*, 625–640.
255. R. D. Grubbs, M. E. Maguire, *Magnesium* **1987**, *6*, 113–127.
256. M. E. Maguire, *Trends Pharmacol. Sci*. **1984**, *5*, 73–77.
257. R. E. White, H. C. Hartzell, *Science* **1988**, *239*, 778–780.
258. Z. S. Agus, M. Morad, *Annu. Rev. Physiol*. **1991**, *53*, 299–307.
259. M. Bara, A. Guiet-Bara, *Magnes. Res*. **2001**, *14*, 11–18.
260. J. Serrano, S. R. Dashti, E. Perez-Reyes, S. W. Jones , *Biophys. J*. **2000**, *79*, 3052–3062.
261. S. Brunet, T. Scheuer, R. Klevit, W. A. Catterall, *J. Gen. Physiol*. **2006**, *126*, 311–323.
262. J. Zhang, G. Wier, M. P. Blaustein, *Am. J. Physiol*. **2002**, *283*, H2692–H2705.
263. M. Prakriya, R. S. Lewis, *J. Gen. Physiol*. **2002**, *119*, 487–507.
264. H. Matsuda, *Annu. Rev. Physiol*. **1991**, *53*, 289–298.
265. P. Tammaro, A. L. Smith, B. L. Crowley, S. V. Smirnov, *Cardiovasc. Res*. **2005**, *65*, 387–396.
266. J. Shi, G. Krishnamoorthy, Y. Wang, L. Hu, N. Chaturvedi, D. Harilal, J. Qin, J. Cui, *Nature* **2002**, *418*, 876–880.
267. P. Bednarczyk, K. Dolowy, A. Szewczyk, *FEBS Lett*. **2005**, 579, 1625–1630.
268. T. Günther, *Magnesium* **1986**, *5*, 53–59.
269. A. Romani, E. Dowell, A. Scarpa, *J. Biol. Chem*. **1991**, *266*, 24376–24384.
270. G. H. Zhang, J. E. Melvin, *J. Biol. Chem*. **1992**, *267*, 20721–20727.
271. G. H. Zhang, J. E. Melvin, *J. Biol. Chem*. **1996**, *271*, 29067–29072.
272. J. G. McCormack, A. Halestrap, R. M. Denton, *Physiol. Rev*. **1990**, *70*, 391–425.
273. R. G. Hansford, *J. Bioenerg. Biomembr*.**1994**, *26*, 495–508.
274. C. S. Moravec, M. Bond, *Am. J. Physiol*. **1991**, *260*, H989–H997.
275. C. S. Moravec, M. Bond, *J. Biol. Chem*. **1992**, *267*, 5310–5316.
276. G. P. Brierley, M. Davis, D. W. Jung, *Arch. Biochem. Biophys*. **1987**, *253*, 322–332.

277. A. D. Beavis, M. Powers, *J. Biol. Chem*. **2004**, *279*, 4045–4050.
278. P. Bernardi, *Physiol. Rev*. **1999**, *79*, 1127–1155.
279. P. C. Bradshaw, D. R. Pfeiffer, *BMC Biochem*. **2006**, *7*, 4.
280. M. Dolder, B. Walzel, O. Speer, U. Schlattner, T. Wallimann, *J. Biol. Chem*. **2003**, *278*, 17760–17766.
281. P. Volpe, B. H. Alderson-Lang, G .A. Nickols, *Am. J. Physiol*. **1990**, *258*, C1077–C1085.
282. P. Volpe, L. Vezú, *Magnes. Res*. **1993**, *6*, 267–274.
283. K. Gusev, E. Niggli, *J. Gen. Physiol*. **2008**, *132*, 721–730.
284. D. R. Laver, B. N. Honen, *J. Gen. Physiol*. **2008**, *132*, 429–446.
285. L. Doleh, A. Romani, *Arch. Biochem. Biophys*. **2007**, *467*, 283–290.
286. A. Barfell, A. Crumbly, A. Romani, *Arch. Biochem. Biophys*. **2011**, *509*, 157–163.
287. S. Yamaguchi, T. Ishikawa, *Biochem. Biophys. Res. Commun*. **2008**, *376*, 100–104.
288. M. Okahira, M. Kubota, K. Iguchi, S. Usui, K. Hirano, *Eur. J. Pharmacol*. **2008**, *588*, 26–32.
289. M. E. Maguire, *Ann. N.Y. Acad. Sci*. **1988**, *551*, 215–217.
290. A. Sgambato, F. I. Wolf, B. Faraglia, A. Cittadini, *J. Cell. Physiol*. **1999**, *180*, 245–254.
291. F. I. Wolf, V. Trapani, M. Simonacci, A. Boninsegna, A. Mazur, J. A. Maier, *Nutr. Cancer* **2009**, *61*, 131–136.
292. V. Covacci, N. Bruzzese, A. Sgambato, A. Di Francesco, M. A. Russo, F. I. Wolf, A. Cittadini, *J. Cell. Biochem*. **1998**, *70*, 313–322.
293. F. I. Wolf, V. Covacci, N. Bruzzese, A. Di Francesco, A. Sachets, D. Cord, A. Cittadini, *J. Cell. Biochem*. **1998**, *71*, 441–448.
294. A. Di Francesco, R. W. Desnoyer, V. Covacci, F. I. Wolf, A. Romani, A. Cittadini, M. Bond, *Arch. Biochem. Biophys*. **1998**, *360*, 149–157.
295. A. Trache, J. P. Trzeciakowski, G. A. Meininger, *J. Mol. Recognit*. **2010**, *23*, 316–321.
296. F. I. Wolf, A. R.Cittadini, J. A. Maier, *Cancer Treat. Rev*. **2009**, *35*, 378–382.