Intracellular magnesium homeostasis

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Abstract

Magnesium (Mg^{2+}) is the fourth most abundant cation in the whole body and the second most abundant cation within the cell. Numerous cellular functions and enzymes, including ion channels, metabolic cycles, and signalling pathways are regulated by Mg^{2+}. Our understanding of how cells regulate Mg^{2+} homeostasis and transport has registered significant progress in recent time. Yet, several aspects of Mg^{2+} homeostasis within cellular organelles, and the nature of the Mg^{2+} extrusion mechanisms at the cell membrane, are still undefined. The present work attempts to provide a comprehensive and updated review of the mechanisms regulating cellular Mg^{2+} homeostasis in eukaryotic cells under physiological conditions and the modifications these mechanisms undergo in various human and animal pathologies.

Introduction

Mammalian cells contain high concentrations of total and free magnesium ion (Mg^{2+}). These concentrations are essential to regulate numerous cellular functions and enzymes, including ion channels, metabolic cycles, and signalling pathways. While the increasing number of observations supports a key regulatory role for Mg^{2+} within the cell, our understanding of how Mg^{2+} homeostasis is regulated at the cellular and subcellular level remains sketchy and incomplete. There are both conceptual and methodological reasons for this limitation. The relative slow turn-over of Mg^{2+} 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 occurrence of significant changes in free [Mg^{2+}] have all contributed for a long time to the assumption that cellular Mg^{2+} concentration does not change significantly, and is consistently at a level adequate for its role as a co-factor for various cellular enzymes and proteins. Consequently, this conceptual assumption has limited the interest to develop techniques and methodologies able to rapidly and accurately quantify changes in cellular Mg^{2+} content. In the last two decades, however, an increasing number of experimental and clinical observations have challenged this assumption. More than 1000 entries in the literature highlight the regulatory role of Mg^{2+} on various cellular functions and cycles, and indicate the occurrence of major fluxes of Mg^{2+} across the plasma membrane of various mammalian cells under a variety of metabolic or hormonal stimuli. In turn, these fluxes have resulted in appreciable changes in cytosolic free [Mg^{2+}] and total Mg^{2+} content within the cell and cellular organelles. Furthermore, genetic and electrophysiological approaches in bacteria, yeast and mammalian cells have identified several Mg^{2+} entry mechanisms that operate either at the cell membrane level or in the membrane of cellular organelles such as mitochondria and Golgi. At the same time, the increased interest in biological functions regulated by Mg^{2+} has stimulated the development of methodological approaches aimed at better detecting and quantifying variations in cellular Mg^{2+} level, and renewed interest in relating alterations in Mg^{2+} homeo-stasis with the onset of specific pathologies and complications in human patients. All these different aspects will be elucidated in the present review to provide a framework as comprehensive as possible to correlate changes in cellular Mg^{2+} homeostasis and content with variations in the function of specific enzymes, which ultimately affect the *modus operandi* of different cellular organelles and cell types.

Cellular Mg^{2+} distribution

Direct and indirect measurement of total cellular Mg^{2+} content by various techniques consistently

indicates that total Mg^{2+} concentration ranges between 17 to 20mM in the majority of mammalian cell types examined (Romani and Scarpa, 1992; Wolf *et al*., 2003). Determinations of total and free Mg^{2+} concentrations 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 Romani and Scarpa, 1992, and in Wolf and Cittadini, 2003) localize major amounts of Mg^{2+} within mitochondria, nucleus, and endo-(sarco)-plasmic reticulum, with total concentrations ranging between 15 to 18 mM in each of these organelles. Binding of Mg^{2+} by phospholipids, proteins, nucleic acids, chromatin and nucleotides has been invoked to explain the persistence of such a high Mg^{2+} concentration within these organelles. Although the specific modality and nature of these bindings have not been fully investigated, experimental evidence indicates that only a small fraction of such a large Mg^{2+} content is actually free in the lumen of these structures. Concentrations of 0.8 and 1.2 mM free [Mg^{2+}] have been measured in the matrix of cardiac and liver mitochondria (Jung *et al*., 1990; Rutter *et al*., 1990). No determinations of free [Mg^{2+}] are available for the nucleus and the endo-(sarco)-plasmic reticulum. The porous structure of the nuclear envelope makes it reasonable to envision that the intranuclear free [Mg^{2+}] is similar to the concentration measured in the cytoplasm. The free [Mg^{2+}] within the endo-(sarco)-plasmic reticulum lumen cannot be reliably determined due to the elevated millimolar concentration of Ca^{2+} inside the organelle (Somlyo *et al*., 1985), and the high affinity of the fluorescent dyes Mag-Fura or Mag-Indo for Ca^{2+} (50mM) as compared to Mg^{2+} (1.5 mM) (Hofer and Machen, 1993). A third considerable pool of Mg^{2+} (~4-5mM) is present in the cytoplasm in the form of a complex with ATP, and other phosphonucleotides and phosphometabolites (Scarpa and Brinley, 1981). Because of its abundance (~ 5mM) and Mg^{2+} binding affinity (K_d ~78μM), ATP constitutes the largest metabolic pool able to bind Mg^{2+} within the cytoplasm and the mitochondria matrix as well (Luthi *et al*., 1999). The binding/buffering capacity of ATP, phosphonucleotides and phosphometabolites, and possibly proteins, maintains cytosolic *free* [Mg^{2+}] between 0.5-1mM, or less than 5% of total cellular Mg^{2+} content in almost all the cells and tissues examined (Table I in Romani and Scarpa, 1992). Similar values have been obtained using fluorescent dyes, ^{31}P-NMR and citrate/isocitrate ratio (Romani and Scarpa, 1992). Overall, these results support the presence of a very limited chemical Mg^{2+} gradient across the cell membrane, and across the membrane of cellular organelles. In erythrocytes, which lack cellular Mg^{2+} compartmentation, Flatman and Lew (1981) have observed three kinetically distinct binding pools for Mg^{2+}: a low capacity, high affinity pool represented by cell proteins, and two pools that correspond reasonably well to ATP and 2,3-diphosphoglycerate (2,3-DPG) respectively (Gunther *et al*., 1995). This model has been further refined by Raftos *et al*., (1999) to take into account Mg^{2+} binding by hemoglobin under oxygenated and not oxygenated conditions.

Limited information is available about the ability of proteins to bind Mg^{2+} within the cell and cellular organelles. Aside from calmodulin (Oki *et al*., 1997), troponin C (Wang *et al*., 1998), parvalbumin (Allouche *et al*., 1999), and S100 protein (Ogoma *et al*., 1992), there is no indication as to whether other cytosolic or intra-organelle proteins can bind substantial amounts of Mg^{2+} and contribute to the elevated concentrations of total Mg^{2+} measured within the mitochondria or discrete regions of the endo-(sarco)-plasmic reticulum. An early report by Bogucka and Wojitczak (1971) has suggested the presence of two proteins able to bind Mg^{2+} with high affinity/low capacity and high capacity/low affinity, respectively, in the intermembraneous space of the mitochondrion. However, no subsequent study has confirmed their presence or identified the proteins. The presence of Mg^{2+} binding sites has been indicated for several other cellular proteins, but no clear information is available as to whether these proteins do bind Mg^{2+} under basal conditions and whether binding changes to a significant extent following hormonal or metabolic stimuli, or under pathological conditions. Moreover, the potential physiological relevance of Mg^{2+} binding by any of the mentioned proteins has been called into question by the observation that parvalbumin *null* mice do not exhibit hypomagnesaemia or significant changes in tissue Mg^{2+} handling and homeostasis (Belge *et al*., 2007).

Taking into account the cellular distribution and assuming a Mg^{2+} concentration in plasma and

extracellular fluid of 1.2-1.4 mM, one-third of which is binding extracellular proteins (e.g. albumin) or other biochemical moieties (Geigy, 1984), it appears that most mammalian cells are at or near *zero trans* condition as far it concerns the chemical free [Mg^{2+}] concentration across the cell membrane or the biomembrane of cellular organelles (e.g. mitochondria). Because the electrochemical equilibrium potential for cellular free [Mg^{2+}] is approximately 50mM in most mammalian cells under resting conditions (Flatman, 1984), 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.

Mg^{2+} transport mechanisms

Eukaryotes retain their cellular Mg^{2+} content virtually unaltered under resting conditions even when exposed to a significant gradient across the cell membrane (e.g. culturing in virtually *zero* extracellular Mg^{2+} content) (Wolf *et al*., 2003; Romani, 2007). Atomic absorbance spectrophotometry determinations and radioisotopic equilibrium indicate Mg^{2+} turnover rates ranging from 1 hour (adipocytes) to several days (lymphocytes) as a result of structural and functional specificity of different tissues and cells (Romani, 2007). Within the same cell types discrepancies can be observed based upon the experimental conditions or the modality of isolation, e.g. cells *in situ* versus freshly isolated cells versus cells in culture. For example, cardiac ventricular myocytes attain ^{28}Mg equilibrium within 3 hours in the whole animal but require 72-80 hours, as dispersed cells incubated at 37°C, or even a longer period of times when exposed to 20°C (Polimeni and Page, 1974; Rogers *et al*., 1960; Rogers, 1961). Lymphocytes also present differences in amplitude (or operation) of Mg^{2+} transport depending on whether they are freshly isolated (Wolf *et al*., 1997) or cultured (Maguire and Erdos, 1978) cells.

For a long time, the slow Mg^{2+} turnover observed in various tissues or cells has contributed to the erroneous idea that Mg^{2+} content in mammalian cells does not change, or changes at such a slow pace that it lacks physiological significance. In the last twenty years, this notion has been completely reverted by a large body of experimental evidence, which indicates that large fluxes of Mg^{2+} can cross the plasma membrane of eukaryotic cells within minutes from the application of metabolic or hormonal stimuli, with relatively small changes in free Mg^{2+} level (Romani, 2007; Grubbs and Maguire, 1987; Romani and Scarpa, 2000; Romani and Maguire, 2002). Lymphocytes (Gunther and Vormann, 1990; Wolf *et al*., 1997), erythrocytes (Matsuura, 1993), cardiac myocytes (Vormann and Gunther, 1987; Romani and Scarpa, 1990a) and liver cells (Jakob *et al*., 1989; Romani and Scarpa, 1990b; Gunther *et al*., 1991) are just a few examples of the mammalian cells that have been reported to extrude 10% to 20% of their total cellular Mg^{2+} content in less than 10min from the application of adrenergic or metabolic stimuli. The amplitude and rapidity of these fluxes presuppose the presence and operation of powerful transport mechanisms able to move large amounts of Mg^{2+} in and out of mammalian cells following various stimuli (for a list of experimental models and conditions see Romani and Scarpa, 2000). In addition, the operation of Mg^{2+} entry mechanisms appears to be tightly coupled with the ability of the cell to rapidly and efficiently buffering the magnesium ions accumulated, as suggested by the limited changes in cytosolic free [Mg^{2+}]i in the large majority of conditions tested (Fatholahi *et al*., 2000; Kubota *et al*., 2005).

The Mg^{2+} transport mechanisms operating at the level of the cell membrane or in the membrane of cellular organelles are represented by channels and exchangers. While channels are predominantly involved in Mg^{2+} accumulation, exchangers mediate essentially Mg^{2+} extrusion. The majority of recently identified Mg^{2+} entry mechanisms operate in the cell membrane but two of them have been located in the mitochondrial membrane and the Golgi system, respectively. For the most part, these entry mechanisms present a modest selectivity for Mg^{2+}, and do not appear to be involved in Mg^{2+} extrusion. No information is currently available about the mechanisms that contribute to maintain an elevated Mg^{2+} concentration within the lumen of the endoplasmic (sarcoplasmic) reticulum.

What it follows is a general description of our current knowledge about the channels and exchangers involved in Mg^{2+} transport across biological membranes (summarized in Table 1).

Channels

Magnesium ions enter the cell via channels or channel-like mechanisms. Channels able to transport Mg^{2+} into the cell have been originally described in prokaryotes (Kehres *et al*., 1998; Moncrief and Maguire, 1999), including protozoans (Preston, 1990), but recently several Mg^{2+} entry mechanisms with channels or channels-like features have been identified in eukaryotic cells. Some of these mechanisms exhibit a relatively high specificity for Mg^{2+} but they can permeate other divalent cations as well. Whereas the majority of these channels are located in the cell membrane or perhaps translocate between early endosomal vesicles and the cell membrane, other are specifically located in the mitochondrial membrane or in the Golgi cysternae. Because the identification and characterization of these eukaryotic Mg^{2+} transporting channels is far from being complete, information relative to their regulation is still largely fragmentary. The abundance of mechanisms favouring Mg^{2+} entry into the cell also raises the question as to what extent the different mechanisms cooperate to modulate Mg^{2+} entry or exert an absolute (or relative predominance) in specific cells under well defined conditions.

TRPM Channels

TRPM7 (Nadler *et al*., 2001) and TRPM6 (Schlingmann *et al*., 2002) were the first channels identified as being able to transport Mg^{2+} into mammalian cells. While Fleig and her group (Nadler *et al*., 2001) reported a preferential Mg^{2+} permeation through the LTRPC7 channel (i.e. TRPM7 based on the current nomenclature), genetic analysis (Schlingmann *et al*., 2002) indicated, more or less at the same time, that TRPM6, another member of the melastatin subfamily of TRP channels, exhibits a selective Mg^{2+} permeation. At variance of the ubiquitous nature of TRPM7, TRMP6 is specifically localized in the colon and the distal convolute tubule of the nephron, a distribution that strongly emphases the role of TRPM6 in controlling whole body Mg^{2+} homeostasis via intestinal absorption and renal resorption. In contrast, it would appear that TRPM7 is more in control of Mg^{2+} homeostasis in individual cells.

The original observations have led to a flurry of studies aimed at better understanding the role, regulation and interaction of these channels with other cellular components involved to a varying degree in Mg^{2+} homeostasis, and presently more than 190 publications relative to TRPM7 and 110 publications relative to TRPM6 can be found in the literature. Although sharing several similarities in terms of structure and operation, these two channels differ in various aspects ranging from location to hormonal modulation.

• *TRPM7*

The key role of TRPM7 in transporting Mg^{2+} into cells and modulating cell growth was first evidenced by Nadler *et al*., (2001). At the time, the channel was identified as LTRPC7 or long TRP channel 7 owing to the presence of a particularly long extension outside the channel segment (Yamaguchi *et al*., 2001). Due to the presence of an alpha-kinase domain at the C-terminus (Ryazanova *et al*., 2001) and its functional homology to eEF2-kinase (Ryazanov, 2002), this protein was already known as CHAK1 (channel kinase 1) (Ryazanova *et al*., 2001). Shortly after the observation of Nadler *et al*., (2001), Runnels *et al*., (2001) evidenced the peculiar structure of TRPM7, combining a channel structure with an alpha-kinase domain at the C-terminus. Although originally investigated for a possible role in Ca^{2+} signalling in lymphocyte, it became rapidly apparent that the channel would preferentially carry Mg^{2+} and Ca^{2+} (Nadler *et al*., 2001) but also trace amounts of divalent cations such as Ni^{2+} and Zn^{2+} (Bessac and Fleig, 2007; Monteilh-Zoller *et al*., 2003).

Located on the human chromosome 15 at the locus 15q21, the protein is formed by 1865 amino acids arranged to possibly form 10 transmembrane domains, with both the C- and N-termini internalized. The protein is ubiquitously expressed albeit to a varying extent in all mammalian cells tested so far. The functional structure is supposed to be a tetramer but disagreement exists as to whether it is formed by 4 identical monomers or by a combination of TRPM7 and TRPM6 (see following section) arranged with a varying stoichiometry in different portion of the cell membrane or in different cell types. Voets and colleagues reported the functional expression of TRPM6 channels in HEK-293 cells with electrophysiological properties similar to those of TRPM7 (Voets *et al*., 2004). In contrast, Chubanov *et al*., (2004) reported the absence of functional currents through TRPM6

Table 1. Mg^{2+} transporters in eukaryotes

	Family	Members	Apparent K_m	Type of Transporter	Reference
Entry Mechanisms					
Cell Membrane	TRPM	TRPM6	~0.7 mM	Channel	Schlingmann *et al*., 2002
		TRPM7	~0.7 mM	Channel	Nadler *et al*., 2001
	Claudins	Claudin 16 (PCLN-1)	~0.7mM	Channel	Simon *et al*., 1999
		Claudin-19	~0.7mM	Channel	Hou *et al*., 2009
	MagT1	MagT1	0.2 mM	Channel	Goytain and Quamme, 2005a Zou and Clapham, 2009
	SLC41	SLC41A1	0.7 - 3 mM	Carrier	Goytain and Quamme, 2005b
		SLC41A2	0.7 - 3 mM	Carrier	Goytain and Quamme, 2005c Sahni *et al*., 2007
	ACDP	ACDP1	~0.7 mM	Carrier	Goytain and Quamme, 2005d
		ACDP2	~0.5 mM	Carrier	Goytain and Quamme, 2005d
	NIPA	NIPA1 (SPG6)	0.7 mM	Carrier	Goytain *et al*., 2007
		NIPA2	0.7 mM	Carrier	Goytain *et al*., 2008a
	Huntingtin	Huntingtin1 (HIP14)	0.87 mM	Carrier	Goytain *et al*., 2008b
		HIP14L	0.74 mM	Carrier	Goytain *et al*., 2008b
Mitochondria	Mrs2	Mrs2/AtMrs2, Lpe10	~1.5 mM	Channel	Koliske *et al*., 2003
Golgi	MMgt	MMgT1	1.5 mM	Channel	Goytain and Quamme, 2008
		MMgT2	0.6 mM	Channel	Goytain and Quamme, 2008

	Family	Members	Apparent K_m	Type of Transporter	Reference
Exit Mechanisms					
Cell Membrane	Na^+/Mg^{2+} exchanger	ND	15-20 mM	Antiport	Gunther and Vormann, 1984 Tashiro and Konishi, 1997 Cefaratti *et al*., 1998
	Na^+- independent	ND (choline?)	~20mM	Exchanger (?)	Ebel *et al*., 2002
	SLC41	SLC41A1	~0.7 mM	Carrier	Kolisek *et al*., 2008
	H^+/Mg^{2+} exchanger	AtMHX[#]	~15mM	Exchanger	Shaul *et al*., 1999

Identified only in plants and yeast and not in mammalian/human cells.

when the channel is expressed by itself in either HEK-293 cells or *X. Laevis* oocytes, and suggested that TRPM7 co-expression was required for TRPM6 to incorporate into channel complexes at the plasma membrane level. Schmitz *et al*., (2005) subsequently confirmed the association of TRPM6 and TRPM7 channel proteins to obtain a functional structure. Yet, a detailed functional characterization of the TRPM6/7 chimeric channel remained undefined (Chubanov *et al*., 2005) until Yue and collaborators addressed the issue in two elegant electro-physiology studies (Li *et al*., 2006; Li *et al*., 2007). In these studies, the authors demonstrate that TRPM6 and TRPM7 can indeed form a chimeric heterotetramer, and that TRPM6, TRPM7, and TRPM6/7 constitute three distinct ion channels with different divalent cation permeability, pH sensitivity, and unique single channel conductance. In addition, these authors reported that 2-APB can differentially regulate the channel activities of TRPM6, TRPM7, and TRPM6/7, markedly increasing Mg^{2+} and Ca^{2+} entry through TRPM6 (Li *et al*., 2006). Based on these results, it would then appear that TRPM6 can form either functional homotetrameric channels or hetero-tetrameric TRPM6/7 channels (Gwanyanya *et al*., 2004). A corollary of this observation would be that TRPM6, TRPM7, and TRPM6/7 channels may play different roles under various physiological or pathological conditions in different tissues. A detailed mapping of the distribution of homomeric TRPM7 *versus* heteromeric TRPM6/7 channels in various tissues, however, is still lacking, leaving their relative role largely undefined.

More recently, some light has been shed on the modality of TRPM7 regulation. At the direct channel level, TRPM7 inward current is markedly enhanced by protons, which compete with Ca^{2+} and Mg^{2+} for binding sites, most likely at the level of the channel pore, thereby releasing the blockade of divalent cations on inward mono-valent currents (Bessac and Fleig, 2007; Monteilh-Zoller *et al*., 2003). Not only extracellular protons significantly increased monovalent cation permeability, but higher proton concentrations are required to induce 50% of maximal increase in TRPM7 currents under conditions in which extracellular Ca^{2+} and Mg^{2+} concentrations are increased. Following the increase in extracellular H^+ concentration, in fact, the apparent affinity for Ca^{2+} and Mg^{2+} is significantly diminished. This set of observation suggests that at physiological pH, Ca^{2+} and Mg^{2+} bind to TRPM7 and inhibit the monovalent cation currents. At higher H^+ concentrations, instead, the affinity of TRPM7 for Ca^{2+} and Mg^{2+} is decreased, thus allowing monovalent cations to permeate the channel (Jiang *et al*., 2005). Another level of regulation appears to be provided by PIP2, as initially reported by Clapham and his collaborators (Runnels *et al*., 2002). This observation was not confirmed by Fleig's group, which instead reported a regulatory role by cAMP (Takezawa *et al*., 2004). More recently, however, Langeslag *et al*., (2007) have observed that the depletion in PIP2 level resulting from PLC-activation counteracts TRPM7 activation. It would therefore appear that either PLC-activation accelerates TRPM7 'rundown' via PIP2 depletion or PIP2 depletion plays a feedback regulatory role on the channel activation by PLC (Langeslag *et al*., 2007). Additional evidence for a regulatory role by PIP2 on TRPM7 has been provided by Mubagwa's group (Gwanyanya *et al*., 2006; Macianskiene *et al*., 2008). This group, in fact, has reported that inhibition of phospholipase C or addition of exogenous PIP2 decreases the run-down of the channel whereas the extracellular addition of phenylephrine accelerates it (Macianskiene *et al*., 2008). In addition, this group has observed that both ATP (Gwanyanya *et al*., 2006) and non-hydrolysable GTP analogs modulate the channel activity, most likely by forming MgATP and by accelerating the channel run-down via phospholipase-C activation, respectively (Macianskiene *et al*., 2008). The regulatory role of PIP2 on TRPM7 is further emphasized by the experimental evidence that agonists like bradykinin or angiotensin-II, which activate phospholipase-C coupled receptors via Gq signalling (Touyz *et al*., 2006; Langeslag *et al*., 2007) can modulate the channel activity via PIP2 metabolism. Interestingly, TRPM7 activation only takes place in the presence of a physiological cellular $[Mg^{2+}]_i$, whereas reducing this concentration below its physiological level with EDTA-AM results in a PLC-mediated inactivation of TRPM7 activity, most likely via PIP2 depletion (Langeslag *et al*., 2007).

The interaction between TRPM7 and phosphatidyl-inositol metabolites is further supported by the observation that TRPM7 is required for a sustained phosphoinositide-3-

kinase signalling in lymphocytes (Sahni and Scharenberg, 2008). In the presence of a physiological concentration of extracellular Mg^{2+}, TRPM7-deficient cells rapidly down-regulate their rate of growth as a result of a signalling deactivation downstream PI3-Kinase (Sahni and Scharenberg, 2008), the phenotype being rescued by supplementing the culture medium with Mg^{2+} (Sahni and Scharenberg, 2008).

A structural peculiarity of TRPM7 is the presence of an alpha-kinase at the C-terminus, which specifically phosphorylates serine and threonines located in an alpha-helix (Middelbeek *et al*., 2010). Initial experimental evidence (Runnels *et al*., 2002) invoked an essential role of this kinase domain in modulating channel activation and gating. Subsequent studies, however, failed to fully support this initial claim, as they indicated that TRPM7 channels lacking the kinase domain could still be activated by internal Mg^{2+} depletion (Schmitz *et al*., 2003). One consequence of lacking the kinase domain, however, is the inability of properly phosphorylating and consequently activating downstream cellular components. Yet, little is know about the molecular mechanisms activating the kinase domain. A recent study by Clark *et al*., (2006) strongly suggests that autophosphorylation plays a significant role in target recognition by the TRMP7 kinase domain. Phosphomapping by mass spectrometry has confirmed the massing autophosphorylation of TRPM7 kinase domain, which – in turn – increases the rate of substrate phosphorylation. The phosphomapping has also identified the majority (37 out of 46) of the autophosphor-ylation sites in a Ser/Thr rich region immediately preceding the kinase catalytic domain (Clark *et al*., 2008). Deletion of this region does not affect the intrinsic catalytic activity of the kinase but prevents substrate phosphorylation, confirming the role of this region in substrate recognition (Clark *et al*., 2008). Although this Ser/Thr region is poorly conserved at the amino acid sequence in TRPM6, the kinase domain of this channel appears to require a similar massive autophos-phorylation of its Sr/Thr residues for proper substrate recognition and efficient target phosphorylation (Clark *et al*., 2008).

So far, only annexin I (Dorovkov and Ryazanov, 2004), myosin IIA heavy chain (Clark *et al*., 2008a; Clark *et al*., 2008b), and calpain (Su *et al*., 2006) have been clearly identified as substrates phosphorylated by TRPM7 kinase domain. Although the number of targets is rather restricted, it appears that TRPM7 is playing a double role within cells by regulating Mg^{2+} homeostasis on the one hand, and cellular functions centered on cell adhesion, contractility and (anti)-inflammatory processes on the other hand. This double role of TRPM7 within cells, in particular smooth muscle cells, is emphasized by a recent observation by Touyz and colleagues (Paravicini *et al*., 2009). In this study, the authors report that aortic segments of mice exhibiting low intracellular Mg^{2+} levels present increased medial cross-section and increased TRPM7 expression but decreased levels of annexin-I expression. As annexin-I has a major anti-inflammatory role (Parente and Solito, 2004), the results of this study suggest a potential regulatory role of TRPM7 in regulating vascular structure and integrity, as well as inflammation.

As our understanding of TRPM7 expression and regulation has improved, evidence of a major functional role of the channel in neuronal function and survival under hypoxia or ischemic-reperfusion conditions has increased. Owing to its ability to 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 favours Ca^{2+} fluxes that result in a toxic event for neurons (Aarts *et al*., 2003). In contrast, Mg^{2+} permeation of the channel enhances anti-apoptotic and cell-survival mechanisms, preventing the anoxic death of neurons (Clark *et al*., 2006). The essential role of TRPM7 in detecting extracellular divalent cations is supported by a recent study by Wei *et al*., (2007), which indicates that activation of the channel by low extracellular divalent cations is lethal to the cell. At the same time, Jiang *et al*., (2008) have reported that occlusion of the middle cerebral artery for 1 hour enhances TRPM7 expression in ipsilateral hippocampus, with deleterious consequences for the neurons. The increased expression of TRPM7 and its consequences are largely counteracted by pre-treatment with nerve growth factor via activation of TrkA pathway (Jiang *et al*., 2008). More recently, application of 5-lipoxygenase inhibitors can block TRPM7 current without affecting

protein expression and cell membrane concentration, *de facto* preventing cell death (Chen *et al*., 2010).

The involvement of TRPM7 is not restricted to the sympathetic nervous system but extends to the parasympathetic system as well, in which the channel facilitates the fusion of cholinergic vesicles with the plasma membrane without affecting large dense core vesicle secretion (Brauchi *et al*., 2008).

Despite the data accumulated since its identification as a preferential Mg^{2+} channel, a recent report by Clapham's group has cast some concern about the effective role of TRPM7 in regulating Mg^{2+} homeostasis (Jin *et al*., 2008). In their report, the authors indicate that TRPM7 null mouse present an altered embryonic development, and that tissue specific deletion of the channel in T cell lineage disrupts thymopoiesis, leading to the progressive depletion of thymic medullary cells. Deletion of TRPM7, however, did not affect acute accumulation of Mg^{2+} nor impacted total cellular Mg^{2+} content in T cells. The synthesis of several growth factors, however, was significantly dysregulated, resulting in an altered differentiation of thymic epithelial cells (Jin *et al*., 2008). TRPM7, therefore, appears to be the first TRP channel with a non-redundant and actually essential role in embryogenesis and thymopoiesis. Whether these defects are the result of an altered Ca^{2+} rather than Mg^{2+} homeostasis is presently undefined. It is also unclear how the removal of this protein results in an altered cellular differentiation process.

- *TRPM6*

The unique localization of TRPM6 channels in the colon and the renal distal convolute tubule, two epithelia otherwise highly impermeable to salt reabsorption, highlights the specific role of this channel in controlling intestinal Mg^{2+} absorption and renal Mg^{2+} resorption, and consequently contributing to whole-body Mg^{2+} homeostasis.

The *TRPM6* gene was originally identified as the site of various mutations responsible for Hypomagnesaemia with Secondary Hypocalcaemia (HSH), a rare autosomal recessive disease characterized by Mg^{2+} and Ca^{2+} wasting, whose symptoms could be ameliorated by massive intravenous Mg^{2+} administration followed by oral Mg^{2+} supplementation (Schlingmann *et al*., 2002). Surprisingly, while hypocalcaemia is completely alleviated by this treatment, the patients continue to present serum Mg^{2+} level around 0.5-0.6 mmol/L, i.e. about half the physiological level (Schlingmann *et al*., 2002). Because the primary defect in these patients is at the level of the TRPM6 expressed in the intestine (Schlingmann *et al*., 2002), the excess Mg^{2+} supplementation is rapidly filtered at the glomerular level and increases passive renal absorption via paracellin-1 (see next section). Trans-cellular absorption via renal TRPM6, however, remains depressed and unable to restore physiological serum Mg^{2+} level (Schlingmann *et al*., 2002).

At the functional level, experimental evidence suggests that the channel forms a tetramer within the plasma membrane. As indicated in the previous section, questions remain as to whether the channel forms a homo-tetramer, or a hetero-tetramer with TRPM7, with a varying stoichiometry. Irrespective of the possibilities, several TRPM6 mutations have been identified (Walder *et al*., 2002). The majority of these mutations result in the expression of a truncated and non-functional channel (Walder *et al*., 2002). The missense mutation $S^{141}L$, on the other hand, occurs at the N-terminus of the channel and prevents its proper assembly as a homo-tetramer, or a hetero-tetramer with TRPM7 (Walder *et al*., 2002). Another missense identified in humans is the $P^{1017}R$ mutation (Walder *et al*., 2002), which appears to occur in a region putatively identified as the pore region of the channel. Yet, this mutation affects negatively and more significantly TRPM7 function when this protein is co-expressed with TRPM6 (Walder *et al*., 2002). More recently, TRPM6 null mice have been developed by Sheffield and his collaborators (Walder *et al*., 2009). The heterozygous $Trpm6^{+/-}$ have for the most part normal electrolyte levels aside for a modest low plasma Mg^{2+} level (~0.67 vs. 0.75, (Walder *et al*., 2009). The majority of the homozygous $Trpm6^{-/-}$ animals die by embryonic day 12.5. Most of the few animals that survive to term present significant neural tube defects, consisting primarily of both exencephaly and spina bifida occulta. Administration of high Mg diet to dams improves offspring survival to weaning (Walder *et al*., 2009).

A peculiarity TRPM6 shares with TRPM7 is the presence of an alpha-kinase domain at the C-terminus. Originally, TRPM6 was known as CHAK2 (channel kinase 2) (Ryazanov, 2002) due to the presence of this kinase domain, which presents a functional homology to eEF2-kinase (Ryazanov, 2002). At variance of other kinases, this domain phosphorylates serine and threonine residues located within an alpha-helix instead of a beta-sheet (Ryazanova *et al*., 2001; Ryazanov, 2002; Middelbeek *et al*., 2010). Owing to their dual function as a channel and a kinase, TRPM6 and TRPM7 are currently referred to as *chanzymes*. As in the case of TRPM7, removal of the kinase domain does not abolish entirely the channel activity but modulates the extent to which the channel is regulated by intra-cellular free Mg^{2+} or MgATP complex (Chubanov *et al*., 2004; Schmitz *et al*., 2005; Chubanov *et al*., 2005; Li *et al*., 2005; Thebault *et al*., 2008). Hence, the targets phosphorylated by the kinase must be located downstream from the protein. At variance of what reported for the kinase domain of TRPM7 (see previous section) no phosphorylation substrate for the TRPM6 kinase has been clearly identified up-to-date, with the exception of TRPM7 itself. Data from the Ryazanov's group clearly indicate that the TRPM6 kinase domain can phosphorylate TRPM7 channel within a heterotetramer structure while the opposite does not occur (Schmitz *et al*., 2005). Due to the limited information available, it is largely undefined as to whether the kinase domain of TRPM7 and TRMP6 phosphorylate similar or different substrates in the tissues in which these chanzymes are specifically expressed.

How exactly the expression and/or activity of TRPM6 channel are modulated *in vivo* is slowly being elucidated. Estrogens (17ß-estradiol) markedly upregulate TRPM6 mRNA in both colon and kidney while having no effect on TRPM7 mRNA (Groenestege *et al*., 2006; Cao *et al*., 2009). In the absence of estrogen, the repressor of estrogen receptor activity (REA) binds to the 6^{th}, 7^{th} and 8^{th} beta-sheets of TRPM6 kinase domain in a phosphorylation-dependent manner and inhibits TRPM6 activity (Cao *et al*., 2009). Short-term estrogen administration dissociates the binding between REA and TRPM6, resulting in an increased channel activity (Cao *et al*., 2009). Dietary Mg^{2+} restriction also upregulates TRPM6 mRNA in both colon and kidney but does not affect TRPM7 mRNA (Groenestege *et al*., 2006; Rondon *et al*., 2008). In contrast, an Mg^{2+} enriched diet upregulates TRPM6 mRNA only in the colon, in keeping with an increased intestinal absorption (Groenestege *et al*., 2006). Mice selected for the their low erythrocyte and plasma Mg^{2+} status exhibit hypomagnesaemia and hypomagnesuria, and increased TRPM6 expression in kidney and intestine when fed a severely Mg^{2+}-deficient diet (Rondon *et al*., 2008). Feeding the mice an Mg^{2+} adequate diet resulted, instead, in hypomagnesaemia and hypermagnesuria, and lower intestinal and renal TRPM6 expression (Rondon *et al*., 2008). These changes in TRPM6 expression and Mg^{2+} level in blood and urine were not observed in mice exhibiting normal or high erythrocyte and plasma Mg^{2+} level (Rondon *et al*., 2008). It is becoming progressively apparent, therefore, that genetic factors control TRPM6 expression and activity, and that dietary Mg^{2+} restriction increases Mg^{2+} resorption, the process correlating well with an increased TRPM6 expression in both intestine and kidney (Groenestege *et al*., 2006; Rondon *et al*., 2008).

As already observed for TRPM7, intracellular ATP specifically decreased TRPM6 current (Chubanov *et al*., 2004; Schmitz *et al*., 2005; Chubanov *et al*., 2005; Li *et al*., 2005; Thebault *et al*., 2008). The inhibitory site resides in the conserved ATP-binding motif GXG(A)XXG within the alpha-kinase domain (Thebault *et al*., 2008). Either the full deletion of the kinase domain or point mutations within the ATP-binding motif ($G^{1955}D$) completely prevents the inhibitory effect of intracellular ATP. The effect of ATP, however, is independent of alpha-kinase autophosphorylation activity (Thebault *et al*., 2008).

The activity of TRPM6 channels can also be modulated by cellular signalling molecules. Bindels and collaborators (Cao *et al*., 2008) have reported that over-expression of RACK1 (receptor for activated protein kinase C) results in a direct binding of this protein to the alpha-kinase domain of TRPM6, and possibly TRPM7 due to the high homology (>84%) between the two kinase domains. The TRPM6 site binding RACK1 is restricted to the region between amino acids 1857 and 1885 (6^{th}, 7^{th} and 8^{th} b sheets). Interestingly, these are the same sheets involved in REA regulation (Cao *et al*., 2009). The interplay between REA and RACK1 in modulating the

channel activity, however, still remains undefined. Accessibility analysis of the RACK1 binding site suggests that 18 of the 28 amino acids of this site are localized at the surface of the TRPM6 alpha-kinase domain (Cao *et al*., 2008). As a result of this interaction, the channel activity of TRPM6 and TRPM7 are inhibited. As it could be anticipated, co-expression of RACK1with a alpha-kinase deleted TRPM6 mutant fails to suppress channel activity. The inhibitory effect of RACK1 fully depends on the autophosphorylation of threonine 1851 (T^{1851}) within the kinase domain. This residue is localized at the end of the 4th alpha-helix adjacent to the RACK1 binding site. Mutation of T^{1851} to alanine ($T^{1851}A$) or to aspartate ($T^{1851}D$) significantly decreases TRPM6 autophosphorylation while leaving unaltered RACK1 binding. The inhibitory effect of RACK1 on the channel activity, however, is completely abolished only in the case of $T^{1851}A$ mutation while it persists in the case of $T^{1851}D$ mutation (Cao *et al*., 2008). Interestingly, $T^{1851}D$ autophosphorylation strongly depends on Mg^{2+} concentration, steadily increasing for concentrations between 0.1 to 1mM. In contrast, $T^{1851}A$ mutant is less sensitive to intracellular Mg^{2+} concentrations as compared to the wild type (IC_{50} ~0.7 vs. 0.5mM, respectively). Under conditions in which protein kinase C is activated (e.g. pre-incubation with PMA), the inhibitory effect of RACK1 on TRPM6 channel activity is completely prevented (Cao *et al*., 2008). The inhibition, however, can be restored by pre-treatment with the PKC inhibitor chelerythrine (Cao *et al*., 2008), suggesting a competing effect of PKC for RACK1.

A recent study of Groenestege *et al*., (2007) has evidenced the role of EGF as an autocrine/ paracrine magnesiotropic hormone. By engaging its receptor in the basolateral domain of the distal convolute tubule, EGF is able to activate TRPM6 at the apical domain of the cell and induce cellular Mg^{2+} accumulation. Point mutation in the pro-EGF ($P^{1070}L$) retains EGF secretion to the apical membrane and disrupts this cascade of events, ultimately resulting in the Mg^{2+} wasting typical of isolated recessive renal hypo-magnesemia (IRH). An alteration of the axis EGF/TRPM6/Mg^{2+} reabsorption/renal Mg^{2+} wasting is also observed in cancer patients undergoing treatment with antibodies anti-EGFR (Cunningham *et al*., 2004; Dimke *et al*., 2010), as the antibody antagonizes the stimulation of TRPM6 activity via EGF. The modality by which EGF modulates TRPM6 activity and/or expression appears to involve ERK1/2 signalling. A report by Ikari *et al*., (2008) indicates that the stimulation of NRK-52E cells by EGF results in an increased phosphorylation of ERK1/2 and an increased expression of TRPM6 in a time-dependent manner (Ikari *et al*., 2008) via modulation by adaptin protein-1 (AP-1) (Ikari *et al*., 2010). The process is prevented by the use of antagonists for integrin a_vb3 or for MEK1/MEK2 activity, or by the use of siRNA for TRPM6 (Ikari *et al*., 2008). How exactly EGF, integrin, and ERK1/2 interact to enhance TRPM6 expression needs further elucidation. It is in fact unclear whether the activation of this signalling axis is connected to the release of RACK1-mediated inhibition of TRPM6 activity mentioned previously (Cao *et al*., 2008). The modality by which apically accumulated Mg^{2+} is transported across the cell to be delivered to basolateral domain of the cell to be extruded into the blood stream also needs further elucidation. One hypothesis is that parvalbumin and calbindin-D_{28k}, two proteins abundantly present within cells of the distal convolute tubule of the nephron, can operate the transcellular transport of Mg^{2+} accumulated at the apical domain, or at least accelerate the rate of delivery at the basolateral domain. However, as mentioned previously, parvalbumin null mice do not show detectable defects in Mg^{2+} excretion or homeostasis (Belge *et al*., 2007), *de facto* questioning whether parvalbumin does play a role in the process or other proteins can compensate for its absence in the null model.

Claudins

Paracellin 1 (claudin 16) was the first Mg^{2+} transporting protein to be identified in mammals (Simon *et al*., 1999). The identification was rendered possible by the genetic analysis of patients affected by Familial Hypomagnesaemia with Hypercalciuria and Nephrocalcinosis (FHHNC), a disease characterized by massive renal Mg^{2+} and Ca^{2+} wasting that leads rapidly and irreversibly to renal failure (Simon *et al*., 1999). At variance of what is described for patients with TRPM6 mutations, the symptoms and the progressive renal deterioration in FHHNC patients are not ameliorated by Mg^{2+} supplementation (Simon *et al*., 1999). The gene responsible for this disease was identified by

Lifton and collaborators in 1999 and named *Paracellin-1* (*PCLN-1*) (Simon *et al*., 1999). More than 20 mutations affecting paracellin-1 trafficking or permeability have been identified up-to-date (Kausalya *et al*., 2006). *PCLN-1* encodes for paracellin-1 (PCLN-1), also termed claudin-16. This protein is a member of the claudin family ((Lal-Nag and Morin, 2009), which comprehends a group of tight junction proteins that present 4 transmembrane spans coordinated by 2 extracellular loops, and both C- and N-termini on the cytoplasm side.

Claudin-16 mediates paracellular Ca^{2+} and Mg^{2+} fluxes throughout the nephron. Yet, discrepancy exists about the modality by which these fluxes are generated. Data obtained in LLC-PK1 (a porcine renal cell line) suggest that claudin-16 mediates paracellular Na^{+}-permeation which, in turn, generates a positive potential within the lumen that acts as the driving force for Mg^{2+} and Ca^{2+} resorption (Hou *et al*., 2005). In contrast, data in MDCK cells indicate a decrease in Na^{+}-permeability and an increase in Mg^{2+} permeability (Ikari *et al*., 2006). 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. It is evident, however, that *PCLN-1* expression is modulated based upon the magnesium concentration present in the extracellular medium (Efrati *et al*., 2005).

At the functional level, claudin-16 has to be delivered correctly to the tight junction where it interacts with the scaffolding protein ZO-1 (Muller *et al*., 2003). The association and dissociation of claudin-16 and ZO-1 appear to be regulated via PKA-mediated phosphorylation of Ser^{217} in claudin-16 (Muller *et al*., 2003). The dephosphorylation of this residue, as it occurs upon activation of the Calcium Sensing Receptor (CaSR) (Khan and Conigrave, 2010) results in the dissociation of claudin-16 from ZO-1 and its accumulation in the lysosomal compartment (Ikari *et al*., 2006). Mutations of Ser 217 can therefore accelerate claudin-16 turnover and modulate its function. Mutation of Threo233 (T233R) also impairs the interaction between claudin-16 and ZO-1, and favours the accumulation of claudin-166 into lysosomes (Muller *et al*., 2003; Ikari *et al*., 2006). More recently, evidence has emerged indicating the involvement of another claudin isoform, claudin-19, in mediating Mg^{2+} and Ca^{2+} resorption (Hou *et al*., 2009) by forming a head-to-head cation-selective complex with claudin-16 at the level of the tight junction. While the channel function of claudin-16 may not depend on its association with claudin-19, claudin-19 plays an indispensable role in recruiting claudin-16 to form a co-polymer at the level of the tight junction and in switching the channel from anion to cation selective (Hou *et al*., 2009). The heteromeric association between claudin-16 and claudin-19 is dramatically 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 $G^{123}R$), which abolish the physiological synergism between the two proteins and result in the development of FHHNC.

MagT1

This protein was identified by Goytain and Quamme (2005a) in human epithelial cells that upregulate the encoding gene following exposure to low-Mg^{2+} concentrations in the culture medium. The protein encoded by this gene has an estimated molecular weight of 38 KDa and 5 transmembrane domains. The mature MagT1 protein, however, would contain 4 transmembrane spans owing to the cleavage of the first transmembrane segment located near the C-terminus. At variance of SLC41 (Section 3.3.1) and Mrs2 (discussed in the next Section), MagT1 does not exhibit any significant degree of homology to prokaryotic Mg^{2+} transporters, but has some similarities with the oligosaccharide transferase complex OST3/OST6 that regulates protein glycosylation in the endoplasmic reticulum in yeast (Shibatani *et al*., 2005). The murine orthologue of MagT1 is highly expressed in liver, heart, kidney and colon, with detectable levels in lung, brain and spleen (Goytain and Quamme, 2005a). 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 (Goytain and Quamme, 2005a). At variance of the other transporters described in this section, MagT1 appears to possess high specificity for Mg^{2+} (K_m = 0.23mM). The Mg^{2+}-elicited currents are not inhibited by Ca^{2+} but can be inhibited by Ni^{2+}, Zn^{2+} and Mn^{2+}, although the required concentrations (>0.2mM) far exceed the physiological concentrations of these cations in extracellular

fluids. Interestingly, nitrendipine at ~10mM can inhibit the Mg^{2+} current whereas the more common nifedipine does not, even at much higher concentrations (Goytain and Quamme, 2005a). Virtually no information is available about N33, a second member of the MagT family. Although able to transport Mg^{2+}, this protein does not show the same high specificity presented by MagT1 for the cation, and can also mediate the transport of Fe^{2+}, Mn^{2+} and Cu^{2+} (Goytain and Quamme, 2005a).

Based upon these observations, MagT1 appears to possess channel-like characteristics and a high selectivity for Mg^{2+}. The latter evidence strongly suggests that this transporter can play an essential role in regulating Mg^{2+} homeostasis in mammalian cells. Support to this hypothesis is provided by the report of Zhou and Clapham (2009) that knock-out of MagT1, and its human homolog TUSC3, in HEK-293 cells results in a major reduction of cellular Mg^{2+} content. These authors also provide evidence that either protein can complement the yeast Mg^{2+} transporter ALR1 (Zhou and Clapham, 2009). Interestingly, the mRNA levels of MagT1 but not those of TUSC3 increase markedly following exposure of expressing cells to low extracellular Mg^{2+} concentrations for 1 day and 2 days. Incubation of the cells in high extracellular Mg^{2+} concentration has no effect on the expression of either protein (Zhou and Clapham, 2009).

Mrs2

This protein was identified during a screening aimed at isolating nuclear genes suppressing RNA splicing defects in yeast mitochondrial introns (Wiesenberger *et al*., 1992). The three main characteristics observed in yeasts deficient in Mrs2 are: 1) a splicing phenotype, 2) a significant reduction in cytochromes, and 3) a deficit in mitochondria respiration to the point that the yeasts become unable to grow on non-fermentable substrates.

Structurally, Mrs2 shows short regions of homology to the bacterial transporter CorA (Bui *et al*., 1999), and shares a similar membrane topology with 2 transmembrane domains. Mutants lacking Mrs2 can be rescued by CorA fused to the mitochondrial N-terminus leader sequence of Mrs2, which guarantees proper insertion in the mitochondrial membrane. These mutants also present a decrease in mitochondrial Mg^{2+} content, which strongly supports a key role of Mrs2 in regulating mitochondrial Mg^{2+} homeostasis. Studies carried out with the fluorescent indicator Mag-Fura indicate a marked decrease in mitochondrial matrix Mg^{2+} level in yeast lacking Mrs2 protein whereas the over-expression of the protein results in a rapid and marked increase in matrix free Mg^{2+} (Kolisek *et al*., 2003). Mrs2 apparently functions as a channel, and its function is modulated by mitochondrial Dy as well as by inhibitors of F0-F1-ATPase or ANT, which substantially decrease Mg^{2+} influx. Highly conserved motifs in the middle region of the protein, corresponding to the coiled-coil portion of the channel, appear to be essential to form functional channels, or to gate the channel. More recently, Schweyen and his collaborators (Piskacek *et al*., 2009) have confirmed in HEK293 cells some of the mitochondrial modifications observed in yeasts. These authors, in fact, have reported that HEK 293 cells deprived of Mrs2 (Piskacek *et al*., 2009) lack complex I expression in mitochondria and present reduced level of mitochondrial Mg^{2+}. Furthermore, the cells show a change in configuration as well as an increased incidence in apoptosis, which within 2 weeks results in a complete loss of cell viability (Piskacek *et al*., 2009). It still remains to be elucidated whether the decrease in mitochondrial Mg^{2+} simply depends on the absence of Mrs2, or is related to some extent to the absence of complex I, which affects mitochondrial Dy and consequently Mg^{2+} retention within the organelle (Akerman, 1981).

Mammalian cells express a single orthologue of Mrs2, which can rescue Mg^{2+} deficient yeast strain (Zsurka *et al*., 2001). Hence, it appears that the mammalian/human Mrs2 homologue functions in a manner similar to the yeast homologue in mediating Mg^{2+} entry in mitochondria. Under conditions in which Mrs2p is absent, the operation of an alternative but much slower mitochondrial Mg^{2+} entry mechanism is observed. Although this pathway restores Mg^{2+} homeostasis only partially, it does rescue the phenotype of Mrs2 deficient yeast, ensuring their survival. No information is presently available about the identity, abundance, and regulation of this alternative transporter in mitochondria. Taken together, the data on Mrs2 suggest that Mg^{2+} is dynamically regulated within the

mitochondrion, in which it plays a significant role in modulating mitochondrial dehydrogenases and oxygen consumption (Panov and Scarpa, 1996a; Panov and Scarpa, 1996b).

MMgTs

This gene family comprehends two proteins termed MMgT1 and MMgT2 (for membrane Mg^{2+} transporter 1 and 2) by Goytain and Quamme, who identified them by microarray analysis screening (Goytain and Quamme, 2008). The chromosomal location of these proteins in the mouse is XA5 for MMgT1 and 11B2 for MMgT2. In the rat, the respective locations are Xq36 for MMgT1 and 10q23 for MMgT2. Human MMgT1 is instead located on Xq26.3 (Goytain and Quamme, 2008). Immunohistochemistry assessment indicates that MMgT1 and MMgT2 are essentially located in the Golgi complex and post-Golgi vesicles, where they may contribute to the regulation of Mg^{2+} dependent enzymes involved in protein assembly and glycosylation (Goytain and Quamme, 2008). This localization, however, does not exclude that these proteins may play a role in modulating Mg^{2+} homeostasis at sites downstream the Golgi network. Widely distributed in tissues, these proteins are formed by 131 (MMgT1) and 123 (MMgT2) amino acids assembled into two predicted transmembrane domains. This suggests that these proteins can form homo-oligomeric and possibly hetero-oligomeric channels to favour Mg^{2+} permeation. MMgT-mediated Mg^{2+} uptake is saturable with a K_m ~1.5mM for MMgT1 and ~0.6mM for MMgT2, and these values do not vary significantly with voltage. However, MMgT1 and MMgT2 are not specific for Mg^{2+} but they can transport other cations as well. Some slight differences in cation permeation exist between the two isoforms. Whereas MMgT1 mediates Sr^{2+}, Fe^{2+}, Co^{2+} and Cu^{2+} transport in addition to Mg^{2+}, MMgT2 favours Sr^{2+}, Co^{2+}, Cu^{2+}, Ba^{2+} and Mn^{2+} transport (Goytain and Quamme, 2008). Electrophysiological experiments indicate that Mg^{2+}-generated current in MMgT1 are inhibited by 0.2mM Mn^{2+} but not by Gd^{3+} or Ni^{2+}. Consistent with what reported for other Mg^{2+} transport mechanisms, 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. Under similar experimental conditions MMgT2 mRNA increases ~1.5 fold in kidney cortex and ~3 fold in MDCT cells (Goytain and Quamme, 2008). We refer the interested audience to reviews by Schmitz *et al*., (2007), Bindels' group (Alexander *et al*., 2008), and Quamme (2010) for a more in-depth elucidation of the specifics of TRPM6/7 channels and other Mg^{2+} entry mechanisms summarily described in this section.

Exchangers

While Mg^{2+} entry appears to be mediated by channels or channels-like mechanisms, Mg^{2+} extrusion is mediated by two mechanisms operating as exchangers. Based upon the electrochemical requirements favouring Mg^{2+} extrusion, these mechanisms are referred to as Na^+-dependent and Na^+-independent Mg^{2+} exchanger, respectively (Table 1). Because neither of these two mechanisms has been cloned, information about their operation, abundance and tissue specificity remains mostly circumstantial or indirect based upon experimental conditions or pharmacological inhibition.

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

The first evidence of the operation of a Mg^{2+} transport mechanism in mammalian cells was provided by Gunther *et al*., (1984). In this and a subsequent publication (Gunther and Vormann, 1985), these authors detailed the presence and operation of an amiloride-inhibited, Na^+-dependent, Mg^{2+} extrusion mechanism in chicken red blood cells. This initial observation has been subsequently confirmed by other groups in mammalian red blood cells (Feray and Garay, 1986; Flatman and Smith, 1990; Xu and Willis, 1994) including human erythrocytes (Ludi and Schatzmannm 1987; Vormann *et al*., 1984; Raftos *et al*., 1999), and in a variety of mammalian cell types (see Romani and Scarpa, 2000 for a list). In addition, observation from Vormann and Gunther (Vormann and Gunther, 1987; Gunther and Vormann, 1992a), Wolf and collaborators (Wolf *et al*., 1996; Wolf *et al*., 1997), and our laboratory (Romani and Scarpa, 1990a; Romani and Scarpa 1990b; Romani *et al*., 1993a; Romani *et al*., 1993b; Fagan and Romani, 2000; Fagan and Romani, 2001; Cefaratti and Ruse, 2007; Cefaratti and Romani, 2007) has provided compelling evidence that this Na^+-dependent, amiloride-inhibited Mg^{2+} extrusion mechanisms is specifically coupled to cAMP-production within the cells. From the experimental stand-point it is irrelevant whether cellular cAMP increases via

stimulation of β-adrenergic, glucagon, or PGE2 receptors, or via administration of forskolin or cell-permeant cyclic-AMP analogs. All these conditions, in fact, result in the activation of the Na^+-dependent Mg^{2+} extrusion mechanism via phosphorylation. Conversely, pre-treatment of cells with inhibitors of adenylyl cyclase (e.g. Rp-cAMP) completely blocks Mg^{2+} mobilization irrespective of the modality utilized to enhance cAMP level (Wolf *et al*., 1997).

Because the Mg^{2+} extrusion mediated by this exchanger strictly depends on the presence of a physiological concentration of Na^+ in the extracellular milieu (Romani *et al*., 1993a; Fagan and Romani, 2000), it is generally accepted that the Na^+-dependent Mg^{2+} extrusion mechanisms is a Na^+/Mg^{2+} exchanger. As this Mg^{2+} extrusion mechanism has not been functionally cloned, we lack detailed information about its membrane abundance, structure, proximity to other cellular transporters with whom it may possibly interact, and stoichiometry. Early reports by Gunther and Vormann (1985) suggest the operation of electroneutral bases ($2Na^+_{in}$:1 Mg^{2+}_{out}) at least in chicken or turkey erythrocytes. This notion has not been confirmed in mammalian erythrocytes including human red blood cells, in which the exchanger appears to operate electrogenically on a $1Na^+_{in}$:1 Mg^{2+}_{out} ratio (Ludi and Schatzmann, 1987; Flatman, 1990; Xu and Willis, 1994). The discrepancy between these reports is not apparent although it may depend on the experimental model (i.e. cell isolation vs cultured cells), composition of incubation medium, or modality of cellular Mg^{2+} loading. Irrespective of the stoichiometry of exchange and the experimental model, however, all the obtained results consistently indicate a K_m for Na^+ between 15 to 20 mM (Gunther, 1996; Tashiro and Konishi, 1997; Cefaratti *et al*., 1998). Pharmacological inhibition has done little to enhance our understanding of the modality of operation of the putative Na^+/Mg^{2+} exchanger. Amiloride, imipramine and quinidine represent the three most widely utilized inhibitors of Na^+-dependent Mg^{2+} extrusion (Gunther and Vormann, 1984; Feray and Garay, 1988; Gunther and Vormann, 1992a). However, because of their limited specificity, it is unclear as to whether they inhibit the Na^+/Mg^{2+}-exchanger directly, or indirectly by operating on other transport mechanisms including Na^+ and K^+ channel, ultimately altering the cell membrane potential and the driving force for Mg^{2+} transport across the plasma membrane.

Despite intense research, the Na^+/Mg^{2+} exchanger is not cloned as yet. Work by Schweigel, Martens and colleagues (Schweigel *et al*., 2000) in mammalian rumen support the operation of a Na^+/Mg^{2+} exchanger with kinetic parameters and characteristics similar to those described by other groups in a various mammalian cell types. Furthermore, by using a hybridoma screening procedure, this group has generated monoclonal antibodies against the Na^+/Mg^{2+} exchanger present in porcine red blood cells. Incubation of sheep rumen epithelial cells in the presence of these antibodies has resulted in a significant inhibition of Mg^{2+} extrusion *via* this exchanger (Schweigel *et al*., 2000). Western blot analysis utilizing these antibodies has evidenced a protein band of ~70 KDa mr, which could tentatively correspond to the Na^+/Mg^{2+} exchanger (Schweigel *et al*., 2000). This is the first time that information about the molecular size of the elusive Na^+/Mg^{2+} exchanger is obtained, and the utilization of these antibodies could represent an ideal tool to identify and recognize this transporter in mammalian tissue.

Na^+-independent

Under conditions in which no extracellular Na^+ is available to exchange for intracellular Mg^{2+}, an alternative Na^+-independent Mg^{2+} extrusion mechanism becomes evident. The specificity of this transporter, however, is far from being characterized. Different cations, including Ca^{2+} or Mn^{2+}, as well as anions (e.g. HCO_3^-, Cl^-, or choline) (Gunther, 1993; Ebel *et al*., 2002) have been reported to be utilized by this mechanism to extrude Mg^{2+} from the cell. Hence, it remains unclear whether we are in the presence of distinct transport mechanisms, or in the presence of a transporter that can operate as an antiporter for cations or a synporter for cations and anions based upon the experimental conditions. Also, it is unclear whether the Na^+-independent pathway is activated by hormonal stimulation. Results obtained in liver cells (Keenan *et al*., 1996; Fagan and Romani, 2000; Fagan and Romani, 2001) indicate that the stimulation by mix adrenergic agonists (e.g. epinephrine) elicit a Mg^{2+} extrusion that is equivalent to the sum of the amounts of Mg^{2+} mobilized by the separate stimulation of a_1- and b-adrenergic receptors. More specifically, the

selective stimulation of a_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 (Fagan and Romani, 2000; Fagan and Romani, 2001). As phenylephrine stimulation appears to operate via Ca^{2+}-CaM to induce Mg^{2+} extrusion, it is undefined whether this signalling pathway represents an alternative modality of activation of the Na^+/Mg^{2+} exchanger, or it activates instead a different Mg^{2+} extrusion mechanism that can be reconciled at least in part with the aforementioned Na^+-independent mechanism. Adding to the uncertainty, Ebel and collaborators (Ebel *et al*., 2002) have suggested that in red blood cells and hepatocytes the Na^+-independent Mg^{2+} extrusion occurs via the choline transporter, which can be inhibited rather specifically by cinchona alkaloids.

One controversial issue is whether the Na^+-dependent and Na^+-independent mechanisms operate as ATPases or require ATP for their operation. Reports by Gunther and collaborators (Gunther *et al*., 1990; Ebel *et al*., 2004) indicate a certain dependence of Na^+-dependent Mg^{2+} extrusion on the presence of a physiological concentration of cellular ATP, the absence or decrease in cellular ATP content resulting in a reduced Mg^{2+} efflux from the cell (Gunther *et al*., 1990; Ebel *et al*., 2004). In the particular case of red blood cells, ATP and 2,3 bisphosphoglycerate both contribute to Mg^{2+} homeostasis and transport (Gunther *et al*., 1995). A regulatory effect of ATP on Mg^{2+} extrusion is not observed in purified liver plasma membrane vesicles (Cefaratti *et al*., 1998). It is true that no Mg^{2+} extrusion is observed in alkaline phosphatase-treated basolateral liver plasma membrane vesicles in the absence of ATP (Cefaratti and Romani, 2007), but this observation can reasonably be explained by the requirement of ATP to phosphorylate and activate the Na^+/Mg^{2+} exchanger in the presence of PKA catalytic subunit (Cefaratti and Romani, 2007).

Mg^{2+}/H^+

This exchange mechanism, originally identified in *A. thaliana* and termed AtMHX, appears to be present in all plants (Shaul *et al*., 1999). This transporter presents 11 putative transmembrane domains, is exclusively localized in the vacuolar membrane of the plant, and electrogenically exchanges protons with Mg^{2+} or Zn^{2+}. Interestingly, the ectopic overexpression of the transporter in tobacco plants sensitizes the plant to grow in the presence of elevated concentrations of Mg^{2+} (or Zn^{2+}) (Shaul *et al*., 1999). Presently, no corresponding gene and transporter have been identified in mammalian cells, although evidence for a direct or indirect exchange of Mg^{2+} for H^+ under certain conditions has been provided by Gunther (Gunther and Vormann, 1990a). An enhanced extrusion of cellular Mg^{2+} has been observed in cells incubated in an acidic extracellular environment, in which an inwardly oriented H^+ gradient is imposed, provided that extracellular Na^+ is present (Gunther and Vormann, 1990a; Dalal and Romani, 2010). Amiloride derivates that inhibit with high affinity the Na^+/H^+ exchanger are ineffective at blocking Mg^{2+} extrusion under these experimental conditions (Gunzel and Schlue, 1996), thus excluding that Mg^{2+} extrusion depends on the operation in reverse of the Na^+/H^+ exchanger in parallel with the forward operation of the Na^+/Mg^{2+} exchanger.

Carriers

This section groups several novel Mg^{2+} transport mechanisms of murine or human origin identified as a result of diet restriction (i.e. Mg^{2+}-deficient diet) or medium restriction (i.e. low extracellular Mg^{2+} content). Due to the limited information available and controversies in their *modus operandi*, these transport mechanisms are non-descriptively classified as carriers.

SLC41

This family of Mg^{2+} transport mechanisms includes three members (A1, A2, and A3) that are distantly related to the prokaryotic MgtE channel identified by Maguire (Smith *et al*., 1995). We will discuss predominantly SLC41A1 and A2 isoforms since no study has addressed function and structure of the SLC41A3 isoform.

SLC41A1 was the first member of this family to be identified (Wabakken *et al*., 2003). Based on the hydrophobic profile, this protein of ~56 kDa Mr was predicted to possess 10 transmembrane domains, two of which presented a discrete level of homology with MgtE (Wabakken *et al*., 2003). Northern blot analysis indicates a wide 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 (Wabakken *et al*., 2003). While modest under basal conditions, the expression of this gene is markedly up-regulated in the renal cortex of mice fed low Mg^{2+} diet for several days (Goytain and Quamme, 2005b). Functional expression of mouse SLC41A1 in *X. oocyte* indicates that this protein can transport Mg^{2+} but also Fe^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+} while Ca^{2+} is not transported nor inhibits Mg^{2+} transport (Goytain and Qaumme, 2005b). The original characterization of Mg^{2+} generated current, which would be tentatively consistent with SLC41A1 operating as a channel (Goytain and Qaumme, 2005b) or an electrogenic exchanger similar to the Na/Ca exchanger (Quednau *et al*., 2004), contrasts with a recent report by Kolisek *et al*., (2008). In this study the authors strongly advocate for SLC41A1 operating as a carrier and predominantly favouring Mg^{2+} efflux rather than influx. Following overexpression of SLC41A1 in HEK293 cells 1) no detectable Mg^{2+} currents is observed; 2) incubation of cells in Mg^{2+}-free media results in a significant reduction of total Mg^{2+} content and $[Mg^{2+}]_i$; 3) the amplitude of Mg^{2+} loss depends on the number of SLC41A1 molecules expressed in the membrane and the induction time, and 4) the changes in $[Mg^{2+}]$i are temperature sensitive but insensitive to the Mg^{2+} channel blocker CoHexamine (Kolisek *et al*., 2008). Furthermore, Kolisek and collaborators suggest that SLC41A1 forms high molecular weight complexes within the cell membrane with masses ranging from 1236 KDa to ~360 kDa, in stark contrast to the 56 KDa Mr of the monomer (Kolisek *et al*., 2008). Whether this observation indicates that the SLC41A1 monomer forms large multimeric complexes and/or interacts with auxiliary proteins is presently undefined. The reason for the absence of Mg^{2+}-generated currents in this study as compared to the original observation by Goytain and Quamme (2005b) is not apparent. One possibility is that the murine (Goytain and Qaumme, 2005b) and human orthologs (Kolisek *et al*., 2008) operate differently. Based upon their high degree (>90%) of homology, the mouse and human SLC41A1 are expected to operate in a similar manner. Yet, the possibility that point mutations can dramatically alter SLC41A1 ion specificity and modality of function cannot be completely dismissed. In this respect, it has to be noted that Goytain and Quamme (2005b) did not report a dependency of SLC41A1 operation on Na^+ or other cations or anions following expression in *X. oocytes*, whereas Kolisek and collaborators (Kolisek *et al*., 2008) observed a marked Cl^- conductance following expression in HEK293 cells, which was abolished by DIDS. Whether this reflects the operation of additional transport mechanisms or the presence of structural differences in the cell membrane of HEK293 cells as compared to *X. oocyte* are possibilities that need further investigation.

Two additional SLC41 isoforms were identified in both humans and mice. SLC41A2 also transport Mg^{2+} as well as other divalent cations albeit with a different selectivity and inhibition profile than SLC41A1 (Goytain and Qaumme, 2005c). Aside from 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+} (Goytain and Qaumme, 2005c). Both SLC41A1 and A2 generate Mg^{2+} currents in *X. oocyte*, and the ionic uptake is voltage dependent with an apparent affinity of 0.75 mM and 0.31 mM, respectively (Goytain and Qaumme, 2005b; Goytain and Qaumme, 2005c). Also SLC41A2 is widely expressed in mammalian tissues, but the expression is not affected by low Mg^{2+} diet (Goytain and Qaumme, 2005c). At the structural level, SLC41A2 shares >70% homology with SLC41A1, and is also thought to have 10 trans-membrane domains, although this hypothesis is not supported by a recent study (Sahni *et al*., 2007) that instead suggests a structural arrangement of 2 x five trans-membrane spans linked together by a supplementary span motif. Hydrophobicity analysis indicates that the C- and N- termini are located on different sites of the cell membrane (Sahni *et al*., 2007), a configuration that will be consistent with a total of 11 trans-membrane segments.

ACDP2

The human ACDP gene family was identified by Wang and collaborators (Wang *et al*., 2003a) as a possible candidate of the urofacial syndrome. Mapped to 10q23-10q24 chromosome, this gene family comprises 4 isoforms differentially located in human tissues. ACDP1 is essentially restricted to brain. ACDP2 is more widely expressed, but still retains the highest expression in brain while

being absent in skeletal muscle. Both ACDP3 and ACDP4 are ubiquitous, but have the highest expression in the heart (Wang *et al*., 2003b). The murine distribution of ACDP isoforms is very similar to that observed in humans (Wang *et al*., 2004). Termed ancient conserved domain protein because all isoforms have one domain in common that appears to be phylogenetically conserved from bacteria to man (Wang *et al*., 2003a), these proteins share >50% homology to the CorC transporter, which together with CorB and CorD plays a role in Mg^{2+} efflux in prokaryotes. Over-expression of ACDP2 in *X. oocytes* indicates that this protein can transport a broad range of divalent cations including Mg^{2+}, Co^{2+}, Mn^{2+}, Sr^{2+}, Ba^{2+}, Cu^{2+}, and Fe^{2+} while Zn^{2+} can inhibit its activity (Goytain and Quamme, 2005d). Mg^{2+} transport via ACDP2 is voltage dependent and occurs with a K_m of ~0.5mM. The transport, however, operates independently of Na^+ or Cl^- ions (Goytain and Quamme, 2005d). As in the case of SLC41A1, the *ACDP2* gene becomes over-expressed following a Mg^{2+} deficient diet (Goytain and Qaumme, 2005).

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 and multisystem disorder (Butler, 1990). Located among about 30 genes linked to this disease (Butler, 1990), *NIPA1* has also been implicated in autosomal dominant hereditary spastic paraplegia (HSP). The human and mouse genome contain four members of the NIPA family, termed NIPA1 trough NIPA4, with an overall similarity of ~40%. Homology between human and mice proteins is high at around 98%. Studies conducted by Goytain and Quamme indicate that both NIPA1 and NIPA2 (Goytain *et al*., 2007; Goytain *et al*., 2008a) can operate as Mg^{2+} transporter. Characterized by a sequence of 323 (NIPA1) and 359 amino acids (NIPA2) arranged to form 9 and 8 transmembrane spans, respectively, these two proteins can be distinguished based on their K_m and specificity for Mg^{2+}. While both proteins transport Mg^{2+} in a saturable fashion, NIPA1 has a K_m for Mg^{2+} of ~0.66mM (Goytain *et al*., 2007) as compared to 0.31mM for NIPA2 (Goytain *et al*., 2008a). In addition, NIPA2 is highly specific for Mg^{2+} while NIPA1 can also transport Sr^{2+}, Fe^{2+} or Co^{2+}, albeit to a lesser extent (Goytain *et al*., 2007). NIPA3, instead, transports Sr^{2+}, Ba^{2+}, Fe^{2+} and Cu^{2+} whereas NIPA4 transport Sr^{2+} and Ba^{2+}. Interestingly, point mutations in NIPA1 (i.e. $G^{100}R$ or $T^{45}R$) represent the basis for the insurgence of autosomal dominant HSP (Rainier *et al*., 2003). Both the glycine and threonine residues are conserved among ortholog NIPA1 channels in different species. There are no similar consensus sites in the paralogs NIPA2, NIPA3 and NIPA4, implying that the folding of these proteins might be different. Although NIPA2 appears to be normal in HSP patients, it cannot functionally replace NIPA1 to ameliorate HSP symptoms, nor can NIPA3 or NIPA4 substitute for the defective NIPA1. This is somewhat surprising for NIPA2 as the gene encoding for this protein is part of the 30 gene clusters associated with the Prade-Willi syndrome together with *NIPA1*. Presently, there is no information available as to whether the Prade-Willi syndrome presents alterations in Mg^{2+} homeostasis.

Huntingtin

The use of oligonucleotide microarray analysis to screen for Mg^{2+}-regulated transcripts in epithelial cells indicates Huntingtin-interacting protein 14 (HIP14) and its related protein HIP14-like (HIP14L) as significantly (~ 3fold) upregulated by low-extracellular Mg^{2+} (Goytain *et al*., 2008b). Formed by approximately 532 amino acids organized in 6 transmembrane spans, HIP14 presents a strong sequence similarity to the ankyrin repeat protein Akr1p (Li and Li, 2004), and a 69% homology to HIP14L. In addition, HIP14 possesses a cytoplasmic DHHC cysteine-rich domain. Defined by the Asp-His-His-Cys sequence motif, this domain confers palmitoyl-acyltransferase activity to the protein and gives it the ability to palmitoylate membrane components and modulate their structure. Mg^{2+} accumulation via HIP14 and HIP14L appears to be electrogenic, voltage-dependent, and saturable with K_m of ~0.87 and ~0.74mM, respectively (Goytain *et al*., 2008b). 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)(Yanai *et al*., 2006) has implicated this protein in numerous cellular processes including transcriptional regulation, mitochondrial bioenergetics, structural scaffolding, vesicle trafficking, endocytosis, and dendrite formation (Yanai *et al*., 2006). Its primary location, however, appears to be in the Golgi and post-Golgi vesicles (Yanai *et al*., 2006; Goytain *et al*., 2008b). Hence, it can be hypothesized that the ability of this protein to favour Mg^{2+} accumulation is linked to some extent to the role HIP14 plays in the physiological functioning of the compart-ments in which the protein is located. At the pathological level, the neuropathology of Huntington disease and the occurrence of progressive neurodegenerative disorders, cognitive deficits and choreic movements typical of this disease are linked to the abnormal expansion of glutamine residues from <34 to >37 at the 18^{th} amino acid position (Li and Li, 2004). Presently, the mechanism responsible for the insurgence of these defects is unknown (Li and Li, 2004). Similarly unknown is whether the poly-glutamine expansion alters Mg^{2+} transport, and whether perturbation of Mg^{2+} homeostasis plays any role in the uprising of the neuronal defects typical of Huntington disease.

Mg^{2+} transport in purified plasma membrane vesicles

Because of the lack of functional cloning, several laboratories including ours have resorted to the use of 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: 1) the possibility to provide a well defined ionic extra- and intra-vesicular milieu composition to determine the modality of operation of the various Mg^{2+} transporters; and 2) the ability to investigate the operation of the 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 hepatic subpopulations enriched in basolateral or apical domains, our laboratory has been able to provide a better understanding of the selective location and specificity of the Na^+- dependent and Na^+-independent Mg^{2+} extrusion mechanisms in liver cells and cardiac myocytes.

The Na^+-dependent extrusion mechanism located in the basolateral domain of the hepatocyte is selectively activated by Na^+ (Cefaratti *et al*., 1998; Cefaratti *et al*., 2000), and specifically inhibited by imipramine (Cefaratti *et al*., 2000), but not amiloride and amiloride derivates (Cefaratti *et al*., 2000). Furthermore, the operation of the exchanger is completely inhibited by pre-treatment of basolateral vesicles with alkaline phosphatase, and restored by loading the vesicles with ATP and PKA catalytic subunit (Cefaratti and Ruse, 2007; Cefaratti and Romani, 2007), leaning further support to the notion that this exchange mechanism becomes operative upon phosphoryl-ation by cAMP. The Na^+/Mg^{2+} 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), an indication that Mg^{2+} extrusion does not depend on the trans-membrane gradient for Mg^{2+} but rather on that of Na^+, with a K_m lower than 20mM (Cefaratti *et al*., 1998), in good agreement with kinetic data obtained in isolated hepatocytes (Romani *et al*., 1993b) and other cell types (Tashiro and Konishi, 1997). Experiments based on TPP^+ distribution have confirmed the electro-genicity of this exchange mechanism in plasma membrane vesicles, and suggested a $1Na^+_{in}$ for $1Mg^{2+}_{out}$ exchange ratio under the majority of experimental conditions tested (Cefaratti *et al*., 1998; Cefaratti *et al*., 2000; Cefaratti and Ruse, 2007). Interestingly, removal of intravesicular Cl^- switches the stoichiometric ratio of the exchanger from electrogenic to electroneutral (i.e. $2Na^+_{in}$ for $1Mg^{2+}_{out}$) (Cefaratti and Romani, 2011). Moreover, in the presence of intra-vesicular Cl^- an extrusion of ~35nmol Cl^-/mg protein is observed within 1min from the addition of external Na^+, in concomitance with the extrusion of Mg^{2+} and the accumulation of external Na^+ into the vesicles (Cefaratti and Romani, 2011). Chloride ion extrusion is not inhibited by anion transport inhibitors like DNDS, DIDS, or niflumic acid, nor is it blocked by NKCC1 inhibitors like bumetanide or furosemide (Cefaratti and Romani, 2011), thus excluding that it occurs via one of these mechanisms. The only agent able to block the Cl^- extrusion is imipramine (Cefaratti and Romani, 2011), which specifically

blocks the Na^+/Mg^{2+} exchanger operating in the basolateral domain of the hepatocyte (Cefaratti *et al*., 2000). Hence, it would appear that Cl^- can be extruded either via the Na^+/Mg^{2+} exchanger or via Cl^- channels for partial charge compensation (Cefaratti and Romani, 2011). The possibility of a Cl^- extrusion via the Na^+/Mg^{2+} exchanger has been suggested by (Rasgado-Flores *et al*., 1994) in dialyzed squid axons, and it would also be in good agreement with the observation by Gunther and collab-orators that intracellular Cl- has a stimulatory role on the activity of the Na^+/Mg^{2+} antiport in red blood cells (Ebel and Gunther, 2003).

The basolateral domain of the hepatocyte is not the only site in which the operation of a Mg^{2+} extrusion mechanism has been observed. Experiments carried out in liver plasma membrane vesicles enriched in apical domain indicate the presence of two apparently distinct, unidirectional Mg^{2+} transport mechanisms, which extrude intravesicular Mg^{2+} for extravesicular Na^+ and Ca^{2+}, respectively (Cefaratti *et al*., 2000).

This apical Na^+-dependent Mg^{2+} transporter presents a K_m for Na^+ comparable to the baso-lateral transporter, and selectively uses Na^+ over other monovalent cations in a manner similar to the basolateral exchanger. Like the basolateral antiport, this exchanger transports electro-genically $1Na^+_{in}:1Mg^{2+}_{out}$ (Cefaratti *et al*., 2000). From the pharmacological standpoint the apical and basolateral exchanger can be distinguished based on the specific inhibition of the apical exchanger by amiloride (Cefaratti *et al*., 2000), although it retains a significant level of sensitivity to imipramine inhibition, whereas only imipramine can block the basolateral antiport (Cefaratti *et al*., 2000). The apical exchanger can also be distinguished from the basolateral antiport based on its inability to operate in reverse mode (Cefaratti *et al*., 2000).

The Ca^{2+}-dependent Mg^{2+} extrusion mechanism is specifically located in the apical domain of the hepatocytes, is activated by micromolar Ca^{2+} concentration ($K_m \leq 50\mu M$), and is insensitive to alkaline phosphatase pre-treatment (Cefaratti and Ruse, 2007; Cefaratti and Romani, 2007). The Mg^{2+} extrusion elicited by this antiport occurs on electro-neutral basis (i.e. $1Ca^{2+}_{in}:1Mg^{2+}_{out}$) (Cefaratti and Ruse, 2007). The exchanger, however, is not Ca^{2+} specific, as Mg^{2+} extrusion is observed following the extravesicular addition of micromolar concentrations of other divalent cations ($Ca^{2+}>>Co^{2+}=Mn^{2+}>Sr^{2+}>>Ba^{2+}>Cu^{2+}>>Cd^{2+}$) (Cefaratti *et al*., 2000). Similarly to the apical Na^+/Mg^{2+} antiport, the Ca^{2+}-dependent mechanism is inhibited by amiloride or imipramine (Cefaratti *et al*., 2000). 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+} in purified apical plasma membrane vesicles subpopulations does not appear to significantly enlarge Mg^{2+} extrusion (Romani, personal observation). Second, amiloride inhibits both exchangers to a comparable extent at a similar concentration (Cefaratti *et al*., 2000). Third, alkaline phosphatase treatment does not affect the Mg^{2+} extrusion elicited by either exchanger in apical liver plasma membrane vesicles (Cefaratti and Ruse, 2007; Cefaratti and Romani, 2007). 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 fully clear. Circumstantial evidence, however, might support a possible role of Mg^{2+} in limiting Ca^{2+} sedimentation in the bile with consequent formation of bile stones (Moore, 1990).

The operation of functionally similar Na^+- and Ca^{2+}-dependent Mg^{2+} extrusion mechanisms has also been observed in cardiac sarcolemma vesicles (Cefaratti and Romani, 2007). As in the case of liver plasma membrane vesicles, cardiac sarcolemma vesicles do not require intravesicular ATP for the operation of Mg^{2+} transporters (Cefaratti and Romani, 2007), and pretreatment of the vesicles with alkaline phosphatase specifically inhibits the Na^+-dependent Mg^{2+} extrusion mechanism (Cefaratti and Romani, 2007). For technical reasons, it is presently unknown 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+} accumulation mechanisms has also been observed in plasma membrane vesicles from brush border cells of rabbit ileum (Juttner and Ebel, 1998) and from the duodenum and jejunum of rat (Baillien and Cogneau, 1995). 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 one (Baillien and Cogneau, 1995). This 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 transporter observed in liver plasma membrane, the pathway in ileum vesicles is not reversible and appears to be electroneutral. Yet, it possesses a K_m for Na^+ of 16mM, a value similar to the K_m calculated in liver plasma membranes (Cefaratti *et al*., 1998), in smooth muscle cells from guinea pig tenia caecum (Tashiro and Konishi, 1997), and in chicken erythrocytes (Schatzmann, 1993). Another similarity with the transporter operating in basolateral liver plasma membranes is the lack of inhibition by amiloride analogs. In good agreement with reports from Gunther and collaborators (Ebel and Gunther, 2003), this transporter is modulated by intravesicular anions, especially Cl^- and SCN^-, and markedly stimulated by antagonists of anion transport (e.g., H_2-DIDS) (Juttner and Ebel, 1998).

The main difference between plasma membrane vesicles from duodenum and jejunum (Baillien and Cogneau, 1995) is that a single Mg^{2+} uptake mechanism operates in the duodenum with a K_m of 0.8mM, whereas two transporters operate in the jejunum with K_m values of 0.15 and 2.4mM, respectively. In both these experimental models, Mg^{2+} but not Ca^{2+} accumulation is reduced in the presence of alkaline phosphatase inhibitors (Baillien *et al*., 2005), 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 (Juttner and Ebel, 1998), Mg^{2+} accumulation is stimulated by an intravesicular electronegative potential or an alkaline pH_o (Baillien and Cogneau, 1995). The effect of external pH, however, is lost when $[Mg^{2+}]_o$>1mM (Baillien and Cogneau, 1995). Under the latter condition, Mg^{2+} accumulation is enhanced by the presence of Na^+ or K^+ in the extravesicular space but is inhibited by the presence of divalent cations ($Co^{2+}>Mn^{2+}>Ca^{2+}>Ni^{2+}>Ba^{2+}>Sr^{2+}$) (Baillien and Cogneau, 1995).

Regulation of Mg^{2+} transport and homeostasis

While mammalian cells retains their basal Mg^{2+} content virtually unchanged under resting conditions, compelling evidence supports the ability of different hormones to induce the movement of large amount of Mg^{2+} in either direction across the eukaryotes cell membrane. As a result of these movements, changes in serum, total and to a lesser extent free Mg^{2+} content have been observed. Further, these changes have resulted in detectable variations in Mg^{2+} level within organelles, especially mitochondria, with major repercussions on cellular bioenergetics. A full understanding of the physiological relevance of these changes in cellular Mg^{2+} content is far from complete. Yet, a picture is slowly emerging, which relates changes in total Mg^{2+} content to the utilization of metabolites (e.g. glucose) or to meaningful changes in Mg^{2+} content within discrete portions of the cell or cellular organelles, whereby variations in total Mg^{2+} content translate into changes in concentrations able to modulate the activity of specific enzymes located within these compartments.

Mg^{2+} extrusion

Several classes of hormones induce Mg^{2+} extrusion from various cell types or perfused tissues. For the most part, these hormones are catecholamine or hormones that increase cellular cAMP level by activating different GPCR receptors at the cell membrane level. The extrusion elicited by these agents affects to a varying extent the Mg^{2+} pools present within cytoplasm as well as within cellular compartments. The extrusion across the cell membrane primarily occurs via the Na^+/Mg^{2+} exchanger previously described although a (partial) contribution of the Na^+-independent pathway cannot be excluded. Magnesium extrusion can also be 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 also elicit a glucose output from hepatocytes. Hence, it would appear that at least in this organ Mg^{2+} extrusion is functionally associated with glucose transport and utilization.

Cyclic-AMP Dependent Extrusion

Elliot and Rizack were the first to report an accumulation of Mg^{2+} in adipocytes stimulated by adreno-corticotrophic hormone in 1974, although they did not elucidate the modality of transport (Elliot and Rizack, 1974). The first extensive characterization of an hormonal effect on Mg^{2+} transport was provided by Maguire and colleagues in S49 lymphoma cells and primary lymphocytes stimulated by beta-adrenergic agonist or PGE1 (Bird and Maguire, 1978; Erdos and Maguire, 1980; Erdos and Maguire, 1983; Grubbs *et al*., 1984). Maguire and Erdos (1978) also provided the first observation that stimulation of protein kinase C enhanced Mg^{2+} influx in S49 cells whereas beta-adrenergic stimulation inhibited the process. Observation carried out in S49 cells lacking protein kinase A or adenylyl cyclase, however, indicated that cAMP was not mediating the inhibitory effect of beta-adrenergic agonists (Maguire and Erdos, 1978; Maguire and Erdos, 1980). At variance of what observed in primary lymphocytes (Wolf *et al*., 1997), Mg^{2+} transport in S49 cells appears to be independent of extra-cellular Na^{+} concentration or membrane potential (Grubbs and Maguire, unpublished observation). Further, Mg^{2+} turnover in S49 required more than 40 hours as compared to the much faster Ca^{2+} turn-over, which was accomplished in less than 3 hours (Grubbs *et al*., 1985).

These initial observations were followed by a long series of report supporting the notion that b-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 (Vormann and Gunther, 1987; Romani and Scarpa, 1990a; Romani and Scarpa, 1990b). This effect has been observed in cardiac ventricular myocytes (Vormann and Gunther, 1987; Romani and Scarpa, 1990a; Romani *et al*., 1993a; Howarth *et al*., 1994), liver cells (Romani and Scarpa, 1990b; Gunther *et al*., 1991; Romani *et al*., 1993b; Fagan and Romani, 2000; Fagan and Romani, 2001), red blood cells (Matsuura *et al*., 1993), thymocytes (Gunther and Vormann, 1990b), and Erhlich ascites cells (Wolf *et al*., 1994) among other cells (see Romani and Scarpa, 2000 for a more comprehensive list), as well as in whole anesthetized animals (Gunther and Vormann, 1992; Keenan *et al*., 1995). In all cellular models, Mg^{2+} extrusion is a fast process that reaches the maximum within 8min from the application of the stimulus irrespective of the hormone (catecholamine, isoproterenol, glucagon, PGE1, or arachidonic acid) (Vormann and Gunther, 1987; Gunther and Vormann, 1990a; Romani and Scarpa, 1990a; Romani and Scarpa 1990b; Matsuura *et al*., 1993; Howarth *et al*., 1994; Wolf *et al*., 1994) or agent (i.e. forskolin or cell permeant cyclic AMP analogs) (Vormann and Gunther, 1987; Gunther and Vormann, 1990a; Romani and Scarpa, 1990a; Romani and Scarpa, 1990b; Matsuura *et al*., 1993; Romani *et al*., 1993b; Fagan and Romani 2000; Fagan and Romani, 2001) utilized to increase cellular cAMP level. The key role of cAMP in modulating Mg^{2+} extrusion is further emphasized by the observation that pretreatment with hormones or agents that either decrease cAMP production, such as carbachol (Romani and Scarpa, 1990a; Romani and Scarpa, 1990b; Romani *et al*., 1993b) and insulin (Romani *et al*., 2000), or prevent PKA activation (e.g. Rp-cAMP (Wolf *et al*., 1997)) inhibits cellular Mg^{2+} mobilization. In an open perfusion system, the amount of Mg^{2+} extruded from the organ returns towards baseline level within 8min from the application of the agonist irrespective of its dose or persistence in the perfusate (Romani and Scarpa, 1990a; Romani and Scarpa, 1990b), suggesting a rapid mobilization of Mg^{2+} from a well defined cellular pool that is rapidly depleted of its content. This notion is further supported by the evidence that submaximal doses of agonist sequentially infused within a few minutes from each other elicit Mg^{2+} extrusions of progressively decreasing amplitudes (Gunther and Vormann, 1990a). Under all these conditions, limited changes in cytosolic free $[Mg^{2+}]_i$ are observed (Fatholahi *et al*., 2000; Amano *et al*., 2000), suggesting that Mg^{2+} is rapidly released from its binding and buffering sites, or form cellular organelle(s) and extruded across the cell membrane. Independent of the hormone utilized the cAMP-mediated Mg^{2+} extrusion occurs via the putative Na^{+}/Mg^{2+} exchanger described previously. In fact, either the removal of extracellular Na^{+} (Gunther, 1996) or

the presence of agents like amiloride (Vormann and Gunther, 1987; Gunther, 1996), which inhibits Na^+ transport albeit in a non-specific manner, abolishes to a large extent the Mg^{2+} extrusion. Under either of these conditions, the amplitude of Mg^{2+} extrusion across the cell membrane is hampered and a more sustained rise in cytosolic free $[Mg^{2+}]_i$ is observed (Fatholahi *et al*., 2000; Amano *et al*., 2000), supporting the notion that blocking Na^+ transport prevents Mg^{2+} from being extruded across the cell membrane but not its released from binding/buffering sites and/or cellular pool(s) into the cytoplasm.

Cyclic-AMP Independent Extrusion

In 1989, Jakob and collaborators reported the first observation that phenylephrine can also elicit Mg^{2+} extrusion from liver cells via alpha$_1$-adrenergic stimulation (Jakob *et al*., 1989). Subsequently, our laboratory (Keenan *et al*., 1996; Fagan and Romani, 2000) confirmed this observation and provided the first evidence that the stimulation of α_1- and β-adrenergic receptor are not alternative but rather additive and complementary processes in eliciting Mg^{2+} extrusion from liver cells, especially when the two classes of receptors are stimulated by mix adrenergic agonists such as epinephrine or norepinephrine (Keenan *et al*., 1996; Fagan and Romani, 2000). Pre-treatment with insulin only abolishes β-adrenergic receptor mediated Mg^{2+} extrusion but leaves unaffected the Mg^{2+} mobilization mediated via α_1-adrenergic receptors (Keenan *et al*., 1996). The inhibitory effect of insulin persists even in cells treated with cell-permeant cAMP analogs (Keenan *et al*., 1996). A similar inhibitory effect of insulin on b-adrenergic receptor mediated, cAMP-modulated Mg^{2+} extrusion has been observed in cardiac myocytes (Romani *et al*., 2000). This inhibition has been largely interpreted as the consequence of an inhibitory effect of insulin on the b-adrenergic receptor (Karoor *et al*., 1995) or a positive effect of the hormone on the cytosolic phospho-diesterase degrading cAMP (Smoake *et al*., 1995). A more recent report by Romero and collaborators (Ferreira *et al*., 2004), however, suggests that insulin can also modulate directly the Na^+/Mg^{2+} exchanger, at least in red blood cells.

Fagan and Romani (2000; 2001) further investigated the modality of Mg^{2+} extrusion following α_1-adrenergic receptor stimulation in liver cells. Their observation indicates that phenylephrine-induced Mg^{2+} extrusion strictly depends on the activation of capacitative Ca^{2+} entry (Fagan and Romani, 2001). Inhibition of IP_3-induced Ca^{2+} release from the endoplasmic reticulum, chelation 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 (Fagan and Romani, 2001). The scant information available about possible binding of Mg^{2+} by cellular proteins prevented the authors from ascertaining whether the extruded Mg^{2+} was mobilized from the ER or displaced from cytosolic binding sites following the massive entry of Ca^{2+} across the hepatocyte cell membrane (Fagan and Romani, 2001, and refs therein). Extracellular Na^+ and Ca^{2+} are both required for the phenylephrine-induced Mg^{2+} extrusion to occur (Fagan and Romani, 2001). The absence of extracellular Ca^{2+}, in fact, decreases the amplitude of Mg^{2+} extrusion by ~15% to 20% whereas extracellular Na^+ is responsible for the remaining 80% to 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. It has to be noted, however, that 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+} (Fagan and Romani, 2001), 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 (Fagan and Romani, 2001).

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 of the physiological significance of Mg^{2+} mobilization in mammalian cells. In the case of cardiac myocytes, an increase in extracellular Mg^{2+} level has been associated with a modulatory effect on the open probability of the L-type Ca^{2+}-channels (Wang and Berlin, 2006) and a

temporary decrease in SA node action potential (Howarth *et al*., 1994). In the case of liver cells, instead, Mg^{2+} transport has been associated with a regulatory role on glucose transport and utilization. Under conditions in which hormones like catecholamine (Keenan *et al*., 1996; Fagan and Romani, 2000), glucagon (Fagan and Romani, 2000), or phenylephrine (Keenan *et al*., 1996; Fagan and Romani, 2000) elicit Mg^{2+} extrusion from liver cells, a concomitant release of hepatic glucose, mostly via glycogenolysis, has been observed (Fagan and Romani, 2000). Interestingly, inhibition of Mg^{2+} extrusion by amiloride or imipramine also results in a marked inhibition of hepatic glucose output (Fagan and Romani, 2000). The converse is also true. Inhibition of glucose transport activity by phlorethin results in a qualitatively similar inhibition of Mg^{2+} extrusion from liver cells (Fagan and Romani, 2000). The presence of a close functional 'link' between glucose and Mg^{2+} homeostasis is further emphasized by the observation that overnight starvation results in the complete depletion of hepatic glycogen and glucose as well as in a marked decrease (minus 15%) of total Mg^{2+} content as a consequence of the activation of the pro-glycemic hormones catecholamine and glucagon (Torres *et al*., 2005). Noteworthy, this decrease in hepatic Mg^{2+} content is equivalent to that elicited via *in vitro* stimulation of perfused livers by the same hormones (Torres *et al*., 2005), or that observed in the liver of type-I diabetic animals (Fagan *et al*., 2004), which are markedly decreased in cellular glycogen. The functional link between glucose and Mg^{2+} homeostasis is also observed under conditions in which glucose accumulation is stimulated by insulin or similar hormones in cardiac ventricular myocytes (Romani *et al*., 2000) or pancreatic beta cells (Henquin *et al*., 1983). 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 (Fagan and Romani, 2000; Romani *et al*., 2000).

Although indirect, a clear proof of the glucose/Mg^{2+} relationship is provided by diabetic conditions. Work by Altura's group (Resnick *et al*., 1993) and more recently by Resnick (Resnick, 1993) and Barbagallo (Barbagallo and Dominguez, 2007) indicate that cellular Mg^{2+} content is markedly decreased under type-I and type-II diabetes. Originally observed in red blood cells (Resnick *et al*., 1993; Resnick, 1993; Barbagallo and Dominguez, 2007), the decrease has also been reported in various other tissues including muscles, liver (Fagan *et al*., 2004), and cardiac myocytes (Reed *et al*., 2008). Interestingly, Mg^{2+} extrusion via β-adrenergic signalling remains operative and is actually up-regulated in liver cells from diabetic animals (Fagan *et al*., 2004) while it is markedly inhibited in cardiac myocytes from the same animals (Reed *et al*., 2008). Whether this reflects a differential operation and modulation of β_2-adrenergic receptors in liver cells *vs* β_1-adrenergic receptors in cardiac cells is not completely clear. Both cell models show a marked inhibition of the Mg^{2+} entry mechanism(s), which persists also in liver plasma membrane vesicles (Fagan *et al*., 2004; Cefaratti *et al*., 2004). Addition of glucose or glycogen to plasma membrane vesicles from diabetic animals renormalizes the amplitude of Mg^{2+} extrusion, but is ineffective at restoring Mg^{2+} accumulation in vesicles (Cefaratti *et al*., 2004). The defect in total cellular Mg^{2+} content appears to be strongly associated with the decrease in protein synthesis and ATP production detected in the cells (Reed *et al*., 2008). Supplementation of exogenous insulin restores both these parameters as well as Mg^{2+} homeostasis and extrusion provided that insulin is administered for at least two weeks (reed *et al*., 2008). As indicated previously, it appears that the role of insulin in modulating Mg^{2+} homeostasis is not restricted to controlling glucose homeostasis and accumulation or the release of pro-glycemic hormones like glucagon, but extends to a direct modulation of the Na^+/Mg^{2+} exchanger (Ferreira *et al*., 2004). The latter effect would not only directly increase cellular Mg^{2+} content but it could also reverberate on the insulin receptor itself. Data obtained in animals maintained on low Mg^{2+} diet indicate that a decrease in cellular Mg^{2+} content affects the ability of the insulin receptor to properly phosphorylate the downstream insulin receptor substrate (IRS) and propagate the signalling within muscle cells (Suarez *et al*., 1995). This result might be of relevance in explaining the decrease in glucose accumulation observed in skeletal muscles under diabetic conditions (Suarez *et al*., 1995).

Mg^{2+} homeostasis and ATP

Hormonal stimuli represent the most dynamic modality by which a cell can rapidly extrude 10% to 15% of its total cellular Mg^{2+} content within a few minutes from the application of the agonist. Mg^{2+}, however, can also be extruded following the treatment with various agents that impact cellular ATP content and production. Cyanide (Harman *et al*., 1990; Dalal and Romani, 2010), mitochondrial uncouplers (Akerman, 1981; Kubota *et al*., 2005), fructose (Gaussin *et al*., 1997), ethanol (Tessman and Romani, 1998), or hypoxia (Gasbarrini *et al*., 1992) are just some of the agents whose addition impact cellular Mg^{2+} homeostasis. All these agents, in fact, have in common that they decrease cellular ATP content by either preventing the mitochondrial electron chain from generating ATP at various levels (cyanide or uncouplers), or by acting as an ATP trap (fructose), or by altering the redox state of pyridine nucleotide within the mitochondrion or the cell (ethanol). As ATP represents the major buffering component for Mg^{2+} within the cell (Scarpa and Brinley, 1981; Luthi *et al*., 1999), a decrease in this phosphonucleotide moiety results in an increase in cytosolic free $[Mg^{2+}]i$, and ultimately in a detectable extrusion from the cell (Harman *et al*., 1990; Gasbarrini *et al*., 1992; Gaussin *et al*., 1997; Tessman and Romani, 1998; Dalal and Romani, 2010). The Mg^{2+} extrusion can be observed to a larger extent in erythrocytes, which possess limited cellular buffering capacity and no compartmentation (Hwa *et al*., 1993), but it can also be observed in cells that present additional buffering by proteins or cellular organelles in addition to phosphonucleotides (Harman *et al*., 1990; Gasbarrini *et al*., 1992; Gaussin *et al*., 1997; Tessman and Romani, 1998; Dalal and Romani, 2010). In the case of fructose, the changes in cytosolic Mg^{2+} have been associated with an activation of the glycogen phosphorylase, which ultimately results in glycogenolysis activation and glucose utilization to restore ATP levels (Gaussin *et al*., 1997). In the majority of these experimental conditions, the increase in cytosolic free $[Mg^{2+}]i$ is usually modest, and considerably lower than the increase expected to occur based upon the corresponding decrease in ATP level, which strongly supports the notion that Mg^{2+} is for the most part extruded from the cell. Furthermore, as ATP level decreases as a result of mitochondria poisoning or changes in pyridine nucleotide ratio, it would appear that not phosphorylation but the rise in cytosolic Mg^{2+} albeit modest is sufficient to activate the Mg^{2+} extrusion mechanism and limit the rise in cytosolic free Mg^{2+} concentration to approximately 100-200 mM at the most (Harman *et al*., 1999).

Cellular ATP plays a key role in regulating Mg^{2+} extrusion. Evidence for this role has been provided in the giant squid axon (Di Polo and Beauge', 1988) as well as in mammalian hepatocytes (Gunther and Hollriegl, 1993) or erythrocytes (Gunther *et al*., 1995). In squid axon, the Na^+-dependent Mg^{2+} extrusion requires a physiological level of ATP. As the phospho-nucleotide level decreases, so does the amplitude of extrusion (Gunther and Hollriegl, 1993). In erythrocytes and hepatocytes, instead, ATP appears to regulate the Na^+-independent Mg^{2+} extrusion process (Gunther and Hollriegl, 1993; Gunther *et al*., 1995). The exact role of ATP in regulating the process, however, is unclear as it does not appear that the extrusion process is mediated by 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 (Tessman and Romani, 1998; Fagan *et al*., 2004). Hence, it appears that the role of ATP is predominantly that of a ligand for Mg^{2+} both in the cytoplasm and the mitochondrial matrix (Scarpa and Brinley, 1981; Luthi *et al*., 1999), and that a decrease in ATP results in an increase in free Mg^{2+} and its consequent extrusion from the cell.

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 signalling activating these mechanisms but we lack any structural information about 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 messengers, and their possible cooperation under specific conditions.

Role of Protein Kinase C

Experimental evidence indicates that mammalian cells can accumulate large amounts of Mg^{2+} as a result of hormonal stimulation. Hormones like carbachol, vasopressin, angiotensin-II, or insulin have been indicated as able to either inhibit cAMP-mediated Mg^{2+} extrusion or reverse the extrusion into Mg^{2+} accumulation in various cell types (Romani and Scarpa, 1990a; Romani *et al*., 2000). The list of cells that respond to hormonal stimulation by accumulating Mg^{2+} is rather long [see Romani and Scarpa, 2000 for a list], and involve all kind of cells: cardiac myocytes (Romani and Scarpa, 1990a; Romani *et al*., 2000), smooth muscle cells (Touyz and Schiffrin, 1996), hepatocytes (Romani and Scarpa, 1990b; Romani *et al*., 1992), platelets (Hwang *et al*., 1993), lymphocytes (Grubbs and Maguire, 1986), fibroblasts (Ishijima and Tatibana, 1994), and pancreatic beta cells (Henquin *et al*., 1983), just to name a few. In addition to inhibiting cAMP production, several of the hormones indicated above can activate protein kinase C (PKC) as part of their cellular signalling. Evidence supporting a role of PKC in mediating Mg^{2+} accumulation has been provided by several laboratories. Maguire and collaborators have indicated that administration of phorbol-myristate acetate (PMA), which directly activates PKC, elicits a marked accumulation of Mg^{2+} in S49 lymphoma cells (Erdos and Maguire, 1983). A similar effect of PMA has been reported by Somogyi's group in thymocytes (Csermely *et al*., 1987), and by our laboratory in cardiac myocytes (Romani *et al*., 1992) and hepatocytes (Romani *et al*., 1992). 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 (Romani *et al*., 1992). An inhibitory effect has also been observed following administration of the PKC inhibitors calphostin (Touyz and Schiffrin, 1996) or staurosporine (Gunther and Vormann, 1995). Alteration in PKC distribution and activity and defective accumulation of Mg^{2+} has been observed in arterial smooth muscle cells (Yang *et al*., 2001), hepatocytes of animals exposed to alcohol (Torres *et al*., 2010), and in liver cells of diabetic animals (Tang *et al*., 1993).

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 diacylglycerol (DAG) and IP3. In turn, these two molecules activate protein kinase C and IP3 receptor in the ER, respectively. Activation of the latter receptor results in a marked but transient increase in cytosolic Ca^{2+}, and in a more sustained entry of Ca^{2+} through the capacitative Ca^{2+} entry mechanism. Hence, Ca^{2+} signalling is an integral component of the cellular response elicited by these hormones. Yet, the contribution of this second messenger in mediating Mg^{2+} accumulation is poorly defined. Liver cells loaded with Bapta-AM, which effectively chelates cytosolic Ca^{2+}, are unable to extrude and accumulate Mg^{2+} following stimulation by phenylephrine and PMA, respectively (Romani *et al*., 1993b). The artificial increase in cytosolic Ca^{2+} elicited by thapsigargin administration also prevents Mg^{2+} accumulation (Romani *et al*., 1993b) and actually induces a Mg^{2+} extrusion from the liver cell if applied for more than 3-5 min (Romani *et al*., 1993b; Fagan and Romani, 2001). Because of the different time-scale and amplitude of the changes in cellular Ca^{2+} and Mg^{2+} content (Romani *et al*., 1993b), it is difficult to properly correlate these experimental variations. Cytosolic free Ca^{2+} transiently increases several orders of magnitude while cytosolic free Mg^{2+}, which is already in the millimolar or submillimolar range, increases by ~10-15% (Fatholahi *et al*., 2000) at the most, although in absolute terms this amount is far larger than the overall change in cytosolic Ca^{2+} mass.

An unresolved point of inconsistency in the role of Ca^{2+} and PKC signalling in regulating Mg^{2+} accumulation is provided by the reports that the administration of phenylephrine, which activates PKC signalling in addition to the inositol 1,4,5 trisphosphate/Ca^{2+} pathway, does not elicit Mg^{2+} accumulation but induces a Mg^{2+} extrusion from liver cells (Fagan and Romani, 2001). This point

raises the question as to what modulates the differential response of the cell to the administration of phenylephrine or vasopressin. One possibility could be that different PKC isoforms are activated under one condition and not the other. For example, hepatocytes possess 3 classical and at least 2 novel PKC isoforms (Tang *et al*., 1993). Thus, it is reasonable to envisage that one isoform (or class of isoforms) is involved in mediating Mg^{2+} accumulation whereas another isoform (or class of isoforms) is involved in modulating Mg^{2+} extrusion. Consistent with this hypothesis, recent data from our laboratory suggests that PKCe is essential for Mg^{2+} accumulation to occur (Torres *et al*., 2010). 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 can be detected in liver cells (Torres *et al*., 2010). Interestingly, this PKC isoform has the highest affinity for Mg^{2+} among all PKC isoenzymes, with a K_m of ~1mM (Konno *et al*., 1989), close to the physiological free $[Mg^{2+}]_i$ measured in the cytoplasm of the hepatocyte (Corkey *et al*., 1986; Fatholahi *et al*., 2000) and other mammalian cells as well (Touyz and Schiffrin, 1996). Although the mechanism ultimately responsible for Mg^{2+} accumulation into 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 (Cao *et al*., 2008).

Role of MAPKs

These results mentioned above, however, do not exclude the possibility that additional signalling pathways (e.g. MAPKS) are involved in determining the differing response of the hepatocyte under apparently similar stimulatory conditions. In agreement with previous reports from Altura's group (Yang *et al*., 2000) and Touyz's laboratory (Touyz and Yao, 2003), our laboratory has evidenced that pharmacological inhibition of ERK1/2 and p38 MAPKs in liver cells abolishes PKC mediated Mg^{2+} accumulation (Torres *et al*., 2006). In this respect, it has to be noted that the inhibition of MAPKs hampers Mg^{2+} accumulation in vascular smooth muscle cells, and significantly affect cyclin activity (Touyz and Yao, 2003) and consequently the ability of the cells to progress in the cell cycle (Touyz and Yao, 2003). This effect may occur via changes in nuclear functions directly regulated by Mg^{2+}, as proposed by Rubin (2005) and/or via changes in nuclear functions of ERK2, which depends on Mg^{2+} level to properly dimerize, translocate and activate specific nuclear targets (Waas and Dalby, 2003). The role of ERK1/2 in Mg^{2+} regulation is further emphasized by the recent report that increased phosphorylation of ERK1/2 and increased expression of TRPM6 has been observed upon EGF administration to renal epithelial cells (Ikari *et al*., 2008; Ikari *et al*., 2010). The role of these MAPKs, however, is far from being elucidated as this kinase appears to be involved to some extent in also mediating Mg^{2+} extrusion (Kim *et al*., 2005; Torres *et al*., 2006).

Role of EGF

As mentioned, direct and indirect evidence suggests a key role of EGF in modulating Mg^{2+} accumulation, at least in kidney cells. The administration of EGF controls proper operation of TRPM6 in the apical domain of renal epithelial cells to promote Mg^{2+} accumulation (Ikari *et al*., 2008; Thebault *et al*., 2009; Ikari *et al*., 2010). Point mutations in EGF sequence result in limited function of TRPM6, and limited or no Mg^{2+} accumulation within the cells (van der Wjist *et al*., 2009). The modulation of TRPM6 appears to occur via MAPKs signalling, most likely ERK1/2 coupled to activator protein-1 (AP-1) (Ikari *et al*., 2010). Indirect evidence of EGF regulation is provided by the evidence that antibodies against EGF used in several forms of colon cancer (Cunningham *et al*., 2004; Dimke *et al*., 2010) induce Mg^{2+} wasting and hypomagnesaemia.

Serum Mg^{2+} level and Mg^{2+} sensing mechanism

The experimental evidence that mammalian cells accumulate or extrude Mg^{2+} under a variety of experimental conditions suggests the presence of a *sensor* for the cytosolic Mg^{2+} concentration, whereby the cell would operate accordingly either extruding the excess cation or accumulating it to restore the 'set-point'. Compelling evidence for the presence of such a *sensor* mechanism is provided by the observation that prolonged exposure to 0mM $[Mg^{2+}]_o$ decreases cytosolic *free* Mg^{2+} concentration by approximately 50% in cardiac ventricular

myocytes (Quamme and Rabkin, 1990), MDKC (Quamme and Dai, 1990), or MDCT cells (Dai *et al*., 1997). The new cytosolic Mg^{2+} level is maintained as long as the cells are incubated in the presence of 0 mM $[Mg^{2+}]_o$, but returns to the normal level as soon as $[Mg^{2+}]_o$ is increased (Quamme and Rabkin, 1990; Quamme and Dai, 1990; Dai *et al*., 1997), in a frame of time that is directly proportional to the extracellular Mg^{2+} concentration utilized (Quamme and Rabkin, 1990; Quamme and Dai, 1990; Dai *et al*., 1997). The process is prevented by the presence of the L-type Ca^{2+}-channel inhibitors verapamil or nifedipine, or La^{3+} in the extracellular milieu (Quamme and Rabkin, 1990). The absence of significant changes in cytosolic free Ca^{2+} concentration under these experimental conditions suggests a direct effect of these inhibitory agents on the Mg^{2+} entry mechanism (Quamme and Rabkin, 1990). This observation led the authors to propose the operation of a specific Mg^{2+} channel in these cells, anticipating the identification of TRPM6 (Schlingmann *et al*., 2002) and TRPM7 (Nadler *et al*., 2001) as specific Mg^{2+} channels.

Mg^{2+} accumulation is favoured by ion re-distribution. In fact, renal epithelial cells accumulate Mg^{2+} as a result of phosphate (Dai *et al*., 1997) or potassium (Dai *et al*., 1991) redistribution across the cell membrane. The dependence of Mg^{2+} accumulation on K^+ redistribution across the plasma membrane suggests that Mg^{2+} transport is the result of changes in membrane potential, possibly for charge compensation (Schweigel *et al*., 1999; Tashiro *et al*., 2002; Schweigel and Martens, 2003). In this respect, it is interesting to note that pathological conditions characterized by a marked decrease in tissue Mg^{2+} content (e.g., diabetes) (Fagan *et al*., 2004) are also characterized by an inability to properly transport potassium (Mondon *et al*., 1974; Taylor and Agius, 1988). 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 (Gunther and Vormann, 1995), is a matter for future investigation.

Humans and many mammals present a circulating Mg^{2+} level of ~1.2-1.4 mEq/L (Geigy, 1984; Mudge, 1989). Clinical and experimental evidence indicates that serum Mg^{2+} level decreases in humans and animals in several chronic diseases (Fagan *et al*., 2004). 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 in conscious humans (Joborn *et al*., 1985; Bailly *et al*., 1990) or ovine (Rayssiguier, 1977), or the administration of isoproterenol (Guideri, 1992) or epinephrine in the presence of α_1-adrenoceptor blockade (Rayssiguier, 1977) have provided contrasting results. More recent results, however, would indicate that isoproterenol infusion elicits in a marked dose- and time-dependent increase in circulating Mg^{2+} content (Gunther and Vormann, 1992; Keenan *et al*., 1995). The increase is serum Mg^{2+} is maximal within 20min from the agent administration (Keenan *et al*., 1995), and remains unchanged up to 2 hours even following the removal of the agonist (Keenan *et al*., 1995). This time frame indicates that the increase in serum Mg^{2+} is not dependent on the increase in heart rate and the decrease in mean arterial pressure elicited by the β-adrenergic agonist (Keenan *et al*., 1995). In fact, the infusion of sodium nitroprusside, which mimics the decrease in mean arterial pressure induced by isoproterenol, has no effect on serum Mg^{2+} levels (Keenan *et al*., 1995). The persistence of an elevated serum Mg^{2+} level for up to 2 hours also implies that operation of secondary and not fully elucidated mechanisms activated by the stimulation of β-adrenergic receptor. Consistent with the larger distribution of β_2 versus β_1 adrenergic receptors in the body (Molinoff, 1984; Barnes, 1995), the increase in serum Mg^{2+} occurs *via* the specific activation of β_2-adrenergic receptor, as it is mimicked by the administration of the selective β_2-adrenergic agonist salbutamol, and it is inhibited by the specific β_2-blocker ICI-118551 (Keenan *et al*., 1995). Due to the amplitude of the increase in Mg^{2+} level into the bloodstream, it is reasonable to envisage that the adrenergic agonists are mobilizing Mg^{2+} from more than one tissue (Keenan *et al*., 1995). The involvement of bone has been proposed by Gunther and co-workers (Gunther and Vormann, 1992) based on the observation that the infusion of carbonic anhydrase inhibitor prevents isoproterenol-mediated Mg^{2+} mobilization into the blood of anesthetized rats. Based upon the pre-infusion level of serum Mg^{2+}, the glomerular

filtration rate (1.62 mL/min), and the fractional excretion (17%) (Shafik and Quamme, 1989), changes in renal excretion do not appear to contribute significantly to the increase in serum Mg^{2+} level, at least during the first two hours following adrenergic agonist infusion. It is interesting to note, however, that hormones that increase plasma Mg^{2+} by mobilizing the cation from different organs or tissues usually increase Mg^{2+} resorption in the Henle's loop, *de facto* preventing any net loss of Mg^{2+}.

The inconsistency of changes in serum Mg^{2+} level observed in the literature, however, does not have a clear explanation. Several factors may contribute to this incongruity, including the proportion of b-adrenergic receptor subtypes in different experimental models, the ability of adrenergic agonists to stimulate with various potency different adrenergic receptors subtypes, and the modality, rate and duration of drug infusion. This inconsistency has also hampered our ability to assign a physiological significance to the eventual increase in serum Mg^{2+} level following catecholamine infusion. The observation that serum Mg^{2+} level increases under certain conditions may imply that specific organs or tissues have the ability to *sense* these changes and act accordingly. Presently, no specific Mg^{2+} sensing mechanism has been identified. However, the Ca^{2+} sensing receptor (Brown *et al*., 1993) can senses changes in circulating Mg^{2+} level in a range of concentrations far higher than those of Ca^{2+} (Nemeth and Scarpa, 1987) but consistent with the increase in serum Mg^{2+} level reported in the literature (Gunther and Vormann, 1992; Keenan *et al*., 1995). The observation that in distal convoluted tubule cells (MDCT) of the mouse the Ca^{2+}-sensing receptor can be activated with comparable sensitivity by both extracellular Ca^{2+} and Mg^{2+} (Bapty *et al*., 1998a) suggests interesting hypotheses in terms of whole body physiology. The activation of this sensor mechanism would inhibit glucagon- or vasopressin-mediated entry of Mg^{2+} into the cell (Bapty *et al*., 1998b) and favour the elimination of the cation with the urine. This would then explain the clinical and experimental evidence that hypermagnesemia and hypercalcemia inhibit hormone-stimulated cAMP-mediated resorption of Mg^{2+} and Ca^{2+} along the different segments of the nephron (Quamme and Dirks, 1980). Also, it would represent a distal regulatory mechanism to restore magnesemia to a physiological level following the increase observed in anesthetized animals infused with adrenergic agonists (Gunther and Vormann, 1992; Keenan *et al*., 1995). Whether this sensing mechanism and/or its associated modulatory components are altered under diabetic conditions in which an increased magnesuria is observed it remains to be elucidated.

At variance of an increase in calcemia, which is associated with muscle weakness and arrhythmia, an increase in serum Mg^{2+} level appears to be well tolerated *in vivo*. 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 (Dipette *et al*., 1987). Baboons infused with pharmacological doses of Mg^{2+} sufficient to prevent epinephrine-induced cardiac arrhythmias show a significant attenuation of the epinephrine-induced increase in mean arterial pressure and systemic vascular resistance (Stanbury, 1948). It would appear, therefore, that an elevated level of $[Mg^{2+}]_o$ can regulate catecholamine release from peripheral and adrenal sources (James *et al*., 1988) and modulate cardiac contractility (Howarth *et al*., 1994). Taken together, these observations suggest that an increase in serum Mg^{2+} level following adrenergic stimulation can: 1) act as a feed-back mechanism to modulate catecholamine release and activity; and 2) 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.

Physiological role of intracellular Mg^{2+}

The conclusions presented in the previous sections strongly suggest a key role for Mg^{2+} as an indispensable component for enzymes, phosphometabolites, and channel activity (Grubbs and Maguire, 1987; Romani and Scarpa, 1992). 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 (Otto *et al*., 1974; Garfinkel and Garfinkel, 1988). The adenylate cyclase is another example of an enzyme directly regulated by Mg^{2+} (reviewed in Maguire, 1984). All these processes occur at Mg^{2+} concentrations

between 0.5 to 1mM, which are well within the fluctuations in free $[Mg^{2+}]_i$ measured in the cytoplasm of various cells including hepatocyte (Corkey *et al*., 1986). With the exception of the glycolytic enzymes, studies attempting to evidence 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. At variance from Ca^{2+}, Mg^{2+} concentration in the cytoplasm and extracellular fluids is in the millimolar or submillimolar range. Consequently, an increase or decrease in cytosolic Mg^{2+} level equivalent to the changes observed for Ca^{2+} will go largely undetected by the common 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 the total cellular Mg^{2+} content by 1 to 2mM (or 5-10% of total cell content) result in minor or no changes in cytosolic free $[Mg^{2+}]_i$ (Harman *et al*., 1990; Romani *et al*., 2000). This disconnect can be explained by assuming that the source or destination of transported Mg^{2+} is a cellular compartment or organelle, or a major binding site, or that Mg^{2+} is highly buffered. Hence, regulation of cellular functions by Mg^{2+} should not be necessarily expected to occur in the cytosol but within organelles, and in the plasma, where Mg^{2+} concentration can rapidly increase or decrease more than 20% (Gunther and Vormann, 1992; Keenan *et al*., 1995).

In the following pages we will highlight what is known about regulatory effects of extracellular or intracellular Mg^{2+} on the operation of cation channels in the plasma membrane, as well as on mitochondria respiration and volume following changes in Mg^{2+} concentration within the organelle.

Ca^{2+}- and K^+-channels

The first report of a regulatory effect of intracellular free Mg^{2+} on calcium channels was by White and Hartzell (1988). These authors reported that increasing intracellular free $[Mg^{2+}]_i$ from 0.3 to 3.0mM by internal perfusion had a dual effect in cardiac ventricular myocytes. It had a small effect on basal L-type Ca^{2+}-channels current (I_{Ca}) but decreased by more than 50% the amplitude of I_{Ca} elevated by cAMP-dependent phosphorylation. The effect of Mg^{2+} was not due to changes in cAMP concentration or in the velocity of phosphorylation but appeared to be a direct effect on the phosphorylated channel or on the channel dephosphorylation rate (White and Hartzell, 1988). Similar results were also reported by Agus and Morad (1991) in guinea pig cardiac myocytes. The block induced by Mg^{2+}on the Ca^{2+} current depended on a direct effect on the inactivation state of the channel as the block persisted in the presence and in the absence of cAMP and was not reversed by elevation of extracellular Ca^{2+} concentration or addition of catecholamine (Agus and Morad, 1991). The effect of Mg^{2+} on Ca^{2+}-channels is not restricted to cardiac cells or to an intra-cellular site of action. Bara and Guiet-Bara (2001) have shown that in vascular smooth muscle cells and endothelial cells in human placenta, $MgCl_2$ (and to a lesser extent $MgSO_4$) regulates the influx of Ca^{2+} through voltage-gated Ca^{2+} channels by acting at an extracellular site on the channel, thus modulating the tonus of the vessels. Evidence for a similar block by extracellular Mg^{2+} on T-type Ca^{2+}-channels has been provided by Serrano *et al.*, (2000). The modulatory effect of Mg^{2+} appears to take place at the EF-hand motif of the COOH-terminus of $Ca_v1.2$ channels as recently evidence by Catterall and his group (Brunet *et al*., 2006).

Extracellular Mg^{2+} also modulates the activity of store-operated Ca^{2+} channels. Studies in intact, pressurized rat mesenteric arteries with myogenic tone indicate that 10 mM extracellular Mg^{2+} can mimic nifedipine in preventing or reversing the vasoconstriction elicited by phenylephrine administration, but not that induced by K^+ depolarization (Zhang *et al*., 2002). Therefore, Mg^{2+} abolishes the vasoconstriction attributed to Ca^{2+} entry through store-operated channels, which are activated following phenylephrine administration, contributing to maintain both myogenic tone and α_1-adrenoceptor-induced tonic vasoconstriction. These data may have direct implication to explain some of the modifications that occur under hypertensive conditions, in which a decrease in plasma Mg^{2+} has often been reported.

Intracellular Mg^{2+} also affects he operation of store-operated calcium release-activated Ca^{2+} (CRAC) channels (Prakriya and Lewis, 2002). CRAC channels are highly Ca^{2+}-selective under physiological ionic 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. While an effect of intracellular Mg^{2+} on CRAC channels cannot be completely excluded, a report by Prakriya and Lewis (2002) suggests 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 therefore been termed Mg^{2+}-inhibited cation (MIC) channels, as they present distinctive functional parameters in terms of inhibition, regulation, ion permeation and selectivity (Prakriya and Lewis, 2002).

Potassium channels are also targets for Mg^{2+}. Matsuda (1991) has reported that the presence of Mg^{2+} on the cytoplasmic side of the inwardly rectifying K^{+} channel blocks the outward currents without affecting the inward currents. The Mg^{2+} block is achieved at a half-saturation concentration of 1.7μM. When the Mg^{2+} concentration is elevated to 2-10μM, the outward current fluctuated between two intermediate sublevels in addition to the fully open and closed configuration. However, these concentrations of Mg^{2+} are far from being physiological and it is difficult to envision the occurrence of a similar regulatory effect under normal conditions without invoking Mg^{2+} microcompartmentation. A regulatory role of intracellular Mg^{2+} on K_v channels in vascular smooth muscle cells has been observed by Tammaro *et al.,* (2005). These authors have observed that an increase in intracellular Mg^{2+} in a range of concentrations consistent with its physiological variations can slow down the kinetic of activation of the K_v channel, cause inward rectification at positive membrane potentials, and shift the voltage-dependent inactivation (Tammaro *et al*., 2005). Overall, this represents a novel mechanism for the regulation of this channel in the vasculature. Intracellular Mg^{2+} also modulates large-conductance (BK type) Ca^{2+}-dependent K^{+} channels by either blocking the pore of BK channels in a voltage-dependent manner, or by activating the channels independently of changes in Ca^{2+} and voltage by preferentially 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 (Shi *et al*., 2002).

The inhibitory effect of Mg^{2+} is not restricted to cell membrane channels. Experimental evidence by Bednarczyk *et al*., (2005) indicates that Mg^{2+} in the mitochondrial matrix can modulate gating and conductance of mitochondrial K_{ATP} channels, which play a key role under ischemia/reperfusion conditions.

Mitochondrial dehydrogenases

Mitochondria represent one of the major cellular pools for Mg^{2+}, its concentration being 14 to 16 mM (Gunther, 1986). Evidence from this (Romani *et al*., 1991) and other laboratories (Zhang and Melvin, 1992; Zhang and Melvin, 1996; Kubota *et al*., 2005) suggests that Mg^{2+} can be mobilized from mitochondria under various conditions including hormone-mediated increase in cytosolic cAMP level through a mechanism(s) that has not been fully elucidated but it appears to involve the adenine nucleotide translocase (Romani *et al*., 1991). Several reviews have analyzed in detail how Mg^{2+} homeostasis is regulated in the organelle (Flatman, 1984; Gunther, 1986; Ogoma *et al*., 1992), and we refer to those reviews for further information. This section will focus on some recent evidence about a role of intra- and extra-mitochondrial Mg^{2+} on the activity of specific mitochondrial proteins.

It is commonly accepted that changes in matrix Ca^{2+} can affect the rate of mitochondrial dehydrogenases and therefore the rate of respiration (McCormack *et al*., 1990; Hansford, 1994). Yet, limited evidence supports a similar role for Mg^{2+} although the activity of several mitochondrial dehydrogenases has been observed to increase within minutes from the application of hormonal or metabolic stimuli despite the absence of a detectable increase in mitochondrial Ca^{2+} (Moravec and Bond, 1991; Moravec and Bond, 1992). The role of matrix Mg^{2+} as regulator of dehydrogenases and consequently mitochondrial respiration has been investigated by measuring the activity of several dehydrogenases in

mitochondria under conditions in which matrix Ca^{2+} and/or Mg^{2+} concentration were varied. From those data, it appears that α-ketoglutarate dehydrogenase and pyruvate dehydrogenase are not regulated by changes in mitochondrial Mg^{2+}, whereas Mg^{2+} removal increases several fold the activity of succinate and glutamate dehydrogenases (Panov and Scarpa, 1996a; Panov and Scarpa, 1996b). This evidence would therefore indicate that changes in matrix Mg^{2+} content (in combination with or in alternative to changes in mitochondrial Ca^{2+}) can control mitochondria 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 (Brierley *et al*., 1987), affecting at the same time the amplitude of mitochondria respiration. In addition, data from our laboratory (Romani *et al*., 1991), Zhang and Melvin (1996), and Kubota *et al* (2005) would suggest that mitochondrial Mg^{2+} can be mobilized by catecholamine stimulation via cAMP. Hence, the enhanced mitochondrial respiration elicited by catecholamine could depend, at least in part, on cAMP-mediated modulation of mitochondrial Mg^{2+}, which, in turn, will stimulate directly some dehydrogenases while rendering others more susceptible to the Ca^{2+} concentrations present in the mitochondrial matrix.

The regulatory effect of Mg^{2+} on mitochondrial function is not restricted to the dehydrogenases activity but affects also the anion channel present in the mitochondrial membrane (Beavis and Powers, 2004) as well as the opening of the permeability transition pore (Dolder *et al*., 2003). 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 completely elucidated as yet, experimental evidence suggests that matrix Mg^{2+} and protons inhibit IMAC, maintaining the channel in its closed state (Beavis and Powers, 2004). Kinetic studies by Beavis and collaborators further support a main role of Mg^{2+} in maintaining the channel in a condition that would allow fine modulation by small changes in pH and proton distribution under physiological conditions (Beavis and Powers, 2004).

The permeability transition pore (PTP) is a proteinaceous pore that opens in the inner mitochondrial membrane following a marked decrease in membrane potential and results in the rapid equilibration and redistribution of matrix and extramitochondrial solutes down their concentrations gradient. While it is still debated exactly which proteins participate in the pore formation and how the pore opens, it is well established that an increase in mitochondrial Ca^{2+} content facilitates the opening whereas an increase in mitochondrial Mg^{2+} antagonizes it. This effect can be appreciated well in yeasts, which do not possess a canonical PTP (Bradshaw and Pfeiffer, 2006). According to one model of regulation, creatine kinase can regulate the opening of the permeability transition pore by tightly associating to the mitochondrial membrane and remaining in an active state (Dolder *et al*., 2003). Both these processes are Mg^{2+}-dependent, and Mg^{2+} removal from the extramitochondrial environment results in a decline in creatine kinas activity and in the opening of the permeability transition pore Dolder *et al*., 2003).

Considering the effect of Mg^{2+} on mitochondrial function and channels (see previous section), it appears that Mg^{2+} plays more than one role within this organelle regulating: 1) mitochondrial volume; 2) ion composition; 3) ATP production; and 4) metabolic interaction with the hosting cell.

Reticular G6Pase

The Endoplasmic Reticulum (ER) represents one of the major cellular Mg^{2+} pools, with a total concentration of 14 to 18mM (Romani and Scarpa, 1992). Yet, limited information is available about any major role of luminal Mg^{2+} on reticular functions other than protein synthesis (Rubin, 2005). It is also unknown whether Mg^{2+} is buffered or chelated by endoluminal proteins in the same way that Ca^{2+} is complexed. Furthermore, we do not have any information about the transport mechanism(s) that allow(s) ER (and SR) to attain and maintain such a large luminal Mg^{2+} concentration.

Work by Volpe and collaborators (Volpe *et al*., 1990; Volpe and Vezu', 1993), Gusev and Niggli (2008), and Laver and Honen (2008) suggests that cytosolic and perhaps luminal Mg^{2+} concentration can have a major effect in limiting Ca^{2+} uptake

into the ER/SR and its release from the organelle via IP_3 (Volpe *et al*., 1990) and ryanodine receptor (Laver and Honen, 2008). In the latter case, a direct effect of Mg^{2+} on the receptor has been observed (Gusev and Niggli, 2008; Laver and Honen, 2008). Less clear is whether a similar effect takes also place at the level of the IP_3 receptor. More 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 (Doleh and Romani, 2007). This effect is biphasic, with an optimal stimulatory effect at ~0.5mM $[Mg^{2+}]_i$ and an inhibitory effect at higher concentrations (Doleh and Romani, 2007). The effect of Mg^{2+} appears to take place at the level of the G6Pi transport component of the G6Pase enzymatic complex in that it can be inhibited by EDTA (as Mg^{2+} chelating agent) or taurocholic acid, which permeabilizes the ER membrane and allows G6Pi to bypass the transport mechanism and be delivered directly to the catalytic site of the G6Pase within the ER lumen (Doleh and Romani, 2007). Investigation is in progress to determine whether Mg^{2+} exerts a similar modulatory effect on other ER enzymes.

Cell pH and volume

As mentioned earlier, exposure of the cell to cyanide (Dalal and Romani, 2010), fructose (Gaussin *et al*., 1997), hypoxia (Harman *et al*., 1990; Gasbarrini *et al*., 1992), ethanol (Tessman and Romani, 1998), or choline chloride (Romani *et al*., 1993b) results in a marked cellular acidification associated with a decrease in cellular ATP content and a major Mg^{2+} extrusion. This extrusion is the consequence of a decrease in buffering capacity (ATP loss) as well as binding affinity. Now, a report by Yamaguchi and Ishikawa (2008) highlights a new and important regulatory role of intracellular Mg^{2+} on the operation of the electrogenic Na^+-HCO_3^- co-transporter NBCe1-B in a range of concentrations that are consistent with the variations in Mg^{2+} content measured within the cytoplasm of various cell types (Grubbs and Maguire, 1987). This regulatory effect is exerted by Mg^{2+} and not by MgATP, and requires a functional N-terminus on the NBCe1-B transporter (Yamaguchi and Ishikawa, 2008). It is still unclear whether Mg^{2+} binds the N-terminus of the transporter directly or exerts its effects via an intermediate, Mg^{2+}-modulated regulatory protein (Yamaguchi and Ishikawa, 2008). At a cytosolic $[Mg^{2+}]$ of ~1mM (a physiological Mg^{2+} concentration measured in the cytosol of various cells (Corkey *et al*., 1986; Grubbs and Maguire, 1987), the NBCe1-B current is inhibited by ~50%, and no detectable current can be measured for *free* Mg^{2+} concentrations of 3-5mM.

More or less at the same time, the group of Hirano and collaborators reported that increasing cellular Mg^{2+} content has a stimulatory role on the expression of aquaporin 3 in CaCo-3 cells (Okahira *et al*., 2008). This isoform of aquaporin is highly expressed in the gastro-intestinal tract, in which it absorbs water, glycerol and urea. The effect of Mg^{2+} on aquaporin mRNA expression appears to involve cAMP/PKA/CREB signalling, as well as we MEK1/2 and MSK1 (Okahira *et al*., 2008), suggesting short- and long-term regulation on the activity and expression of this protein. It is presently undefined whether Mg^{2+} exerts a similar regulatory role on other aquaporin isoforms. Moreover, as aquaporin 3 is also expressed in brain, erythrocytes, kidney, and skin, a modulatory role of Mg^{2+} on the protein expression in these tissues may have major relevance for various physiological and/or pathological conditions.

Taken together, these two sets of information suggest that Mg^{2+} can have a major regulatory role on cellular pH, cellular volume, and cellular cation concentration, especially Na^+. Also, as aquaporin 3 mediates glycerol and urea accumulation, possible repercussions on fatty acid metabolism and urea cycle must be taken into proper consideration.

Cell cycle

Cell cycle (Maguire, 1988; Sgambato *et al*., 1999; Touyz et Yao, 2003), cell proliferation (Wolf *et al*., 2009b) and cell differentiation (Covacci *et al*., 1998; Wolf *et al*., 1998; Di Francesco *et al*., 1998) have all been associated with the maintenance of an optimal cellular Mg^{2+} level. Under conditions in which cellular Mg^{2+} accessibility is restricted or reduced, cell proliferation and cell cycle progression are markedly impaired. A decrease in extracellular Mg^{2+} content also affects cell differentiation (Covacci *et al*., 1998; Wolf *et al*., 1998; Di Francesco *et al*., 1998). The mechanisms by which a decrease in cellular Mg^{2+} content affects these cellular processes have been

attributed to defective MAPKs (Touyz and Yao, 2003) and p27 (Sgambato *et al*., 1999) signalling, increased oxidative stress level (Wolf *et al*., 2009b), and decreased MgATP levels (Di Francesco *et al*., 1998; Rubin, 2005). Because cellular MgATP level is at a level optimal for protein synthesis (Rubin, 2005), any alteration in this metabolic parameter will have major repercussion on the proper functioning of the cell. In addition, extracellular Mg^{2+} levels regulate integrins signalling, *de facto* modulating the interaction among cells and between cells and extracellular matrix (Trache *et al*., 2010). Hence, consistent with the long-term regulatory function of Mg^{2+} proposed by Grubbs and Maguire (1987) several years ago, all these observations underlie the important role of Mg^{2+} to guarantee cell cycle progression and retention of proper cell morphology and function, avoiding undesired progression towards cell death or neoplastic destiny (Wolf *et al*., 2009a).

Conclusions

In the last few years, our understanding of cellular and whole body Mg^{2+} homeostasis has significantly advanced. While it still lags behind as compared to the knowledge available for other ions such as Ca^{2+}, H^+, K^+ or Na^+, the identification of putative Mg^{2+} channels and transport mechanisms in the membrane of the cell or mitochondria, as well as an improved understanding of the signalling pathways and conditions regulating Mg^{2+} transport are providing new tools to address essential questions about the physiological role Mg^{2+} plays inside the cell and in the whole body.

References

Aarts M, Iihara K, Wei WL, Xiong ZG, Arundine M, Cerwinski W, MacDonald JF, Tymianski M (2003) A key role for TRPM7 channels in anoxic neuronal death. *Cell* 115:863-77.

Agus ZS, Morad M (1991) Modulation of cardiac ion channels by magnesium. *Annu Rev Physiol* 53:299-307.

Akerman KE (1981) Inhibition and stimulation of respiration-linked Mg^{2+} efflux in rat heart mitochondria. *J Bioenerg Biomembr* 13:133-9.

Alexander RT, Hoenderop JG, Bindels RJ (2008) Molecular determinants of magnesium homeostasis: insight from human disease. *J Am Soc Nephrol* 19:1451-8.

Allouche D, Parello J, Sanejouand YH (1999) Ca^{2+}/Mg^{2+} exchange in parvalbumin and other EF-hand proteins. A theoretical study. *J Mol Biol* 285:857-73.

Amano T, Matsubara T, Watanabe J, Nakayama S, Hotta N (2000) Insulin modulation of intracellular free magnesium in heart: involvement of protein kinase C. *Brit J Pharmacol* 130:731-8.

Baillien M, Cogneau M (1995) Characterization of two mechanisms of ^{28}Mg uptake in rat jejunal brush border membrane vesicles. *Magnesium* 8:331-9.

Baillien M, Wang H, Cogneau M (1995) Uptake of ^{28}Mg by duodenal and jejunal brush border membrane vesicle in the rat. *Magnesium* 8:315-29.

Bailly C, Imbert-Teboul M, Roinel N, Amiel C (1990) Isoproterenol increases Ca, Mg, and NaCl reabsorption in mouse thick ascending limb. *Am J Physiol* 258:F1224-31.

Bapty BW, Dai LJ, Ritchie G, Canaff L, Hendy GN, Quamme GA (1998) Mg^{2+}/Ca^{2+} sensing inhibits hormone-stimulated uptake in mouse distal convolute tubule cells. *Am J Physiol* 275:F353-60.

Bapty BW, Dai LJ, Ritchie G, Jirik F, Canaff L, Hendy GN, Quamme GA (1998) Extracellular Mg^{2+}- and Ca^{2+}-sensing in mouse distal convoluted tubule cells. *Kidney Intern* 53:583-92.

Bara M, Guiet-Bara A (2001) Magnesium regulation of Ca^{2+} channels in smooth muscle and endothelial cells of human allantochorial placental vessels. *Magnes Res* 14:11-8.

Barbagallo M, Dominguez LJ (2007) Magnesium metabolism in type 2 diabetes mellitus, metabolic syndrome and insulin resistance. *Arch Biochem Biophys* 458:40-7.

Barnes PJ (1995) Beta-adrenergic receptors and their regulation. *Am J Respir Crit Care Med* 152:838-60.

Beavis AD, Powers M (2004) Temperature dependence of the mitochondrial inner membrane anion channel: the relationship between temperature and inhibition by magnesium. *J Biol Chem* 279:4045-50.

Bednarczyk P, Dolowy K, Szewczyk A (2005) Matrix Mg^{2+} regulates mitochondrial ATP-dependent potassium channel from heart. *FEBS Lett* 579:1625-30.

Belge H, Gailly P, Schwaller B, Loffing J, Debaix H, Riveira-Munoz E, Beauwens R, Devogelaer JP, Hoenderop JG, Bindels RJ, Devuyst O (2007) Renal expression of parvalbumin is critical for NaCl handling and response to diuretics. *Proc Natl Acad Sci USA* 104:14849-54.

Bessac BF, Fleig A (2007) TRPM7 channel is sensitive to osmotic gradients in human kidney cells. *J Physiol* 582:1073-86.

Bird SJ, Maguire ME (1978) The agonist-specific effect of magnesium ion on binding by β-adrenergic receptors of S49 lymphoma cells: Interactions of GTP and magnesium in magnesium in adenylate cyclase activation. *J Biol Chem* 253:8826-34.

Bogucka K, Wojtczak L (1971) Intramitochondrial distribution of magnesium. *Biochem Biophys Res Commun* 44: 1330-7.

Brauchi S, Krapivinsky G, Krapivinsky L, Clapham DE (2008) TRPM7 facilitates cholinergic vesicle fusion with the plasma membrane. *Proc Natl Acad Sci U S A* 105:8304-8.

Brierley GP, Davis M, Jung DW (1987) Respiration-dependent uptake and extrusion of Mg^{2+} by isolated heart mitochondria. *Arch Biochem Biophys* 253: 322-32.

Brown EM, Gamba G, Riccardi D, Lombardi M, Butters R, Kifor O, Sun A, Hediger MA, Lytton J, Herbert SC (1993) Cloning and characterization of an extracellular Ca^{2+}-sensing from bovine parathyroid. *Nature* 366:575-80.

Brunet S, Scheuer T, Klevit R, Catterall WA (2006) Modulation of $Ca_v1.2$ channels by Mg^{2+} acting at an EF-hand motif in the COOh-terminal domain. *J Gen Physiol* 126:311-23.

Bui DM, Gregan J, Jarosch E, Ragnini A, Schweyen RJ (1999) The bacterial magnesium transporter CorA can functionally substitute for its putative homologue Mrs2p in the Yeast inner mitochondrial membrane. *J Biol Chem* 274:20438-43.

Butler MG (1990) Prader-Willi syndrome: current understanding of cause and diagnosis. *Am J Med Genet* 35:319-32.

Cao G, Thébault S, van der Wijst J, van der Kemp A, Lasonder E, Bindels RJ, Hoenderop JG (2008) RACK1 inhibits TRPM6 activity via phosphorylation of the fused alpha-kinase domain. *Curr Biol* 18:168-76.

Cao G, van der Wijst J, van der Kemp A, van Zeeland F, Bindels RJ, Hoenderop JG (2009) Regulation of the epithelial Mg^{2+} channel TRPM6 by estrogen and the associated repressor protein of estrogen receptor activity (REA). *J Biol Chem* 284:14788-95.

Cefaratti C, McKinnis A, Romani A (2004) Altered Mg^{2+} transport across liver plasma membrane from streptozotocin-treated rats. *Mol Cell Biochem* 262:145-54.

Cefaratti C, Romani A (2007) Functional characterization of two distinct Mg^{2+} extrusion mechanisms in cardiac sarcolemmal vesicles. *Mol Cell Biochem* 303:63-72.

Cefaratti C, Romani A (2011) Modulation of Na^{+}/Mg^{2+} exchanger stoichiometry ratio by Cl^{-} ions in basolateral rat liver plasma membrane vesicles. *Mol Cell Biochem* 351:133-42.

Cefaratti C, Romani A, Scarpa A (1998) Characterization of two Mg^{2+} transporters in sealed plasma membrane vesicles from rat liver. *Am J Physiol* 275:C995-1008.

Cefaratti C, Romani A, Scarpa A (2000) Differential localization and operation of distinct Mg^{2+} transporters in Apical and Basolateral Sides of Rat Liver Plasma Membrane. *J Biol Chem* 275:3772-80.

Cefaratti C, Ruse C (2007) Protein kinase A dependent phosphorylation activates Mg^{2+} efflux in the basolateral region of the liver. *Mol Cell Biochem* 297:209-14.

Chen H-S, Xie J, Zhang Z, Su L-T, Yue L, Runnels LW (2010) Blockade of TRPM7 channel activity and cell death by inhibitors of 5-lipoxygenase. *PLoS One* 5:e11161.

Chubanov V, Gudermann T, Schlingmann KP (2005) Essential role for TRPM6 in epithelial magnesium transport and body magnesium homeostasis. *Pflugers Arch* 451:228-34.

Chubanov V, Waldegger S, Mederos y Schnitzler M, Vitzthum H, Sassen MC, Seyberth HW, Konrad M, Gudermann T (2004) Disruption of TRPM6/TRPM7 complex formation by a mutation in the TRPM6 gene causes hypomagnesemia with secondary hypocalcemia. *Proc Natl Acad Sci U S A* 101:2894-9.

Clark K, Langeslag M, van Leeuwen B, Ran L, Ryazanov AG, Figdor CG, Moolenaar WH, Jalink K, van Leeuwen FN (2006) TRPM7, a novel regulator of actomyosin contractility and cell adhesion. *EMBO J* 25:290-301.

Clark K, Middelbeek J, Lasonder E, Dulyaninova NG, Morrice NA, Ryazanov AG, Bresnick AR, Figdor CG, van Leeuwen FN (2008a) TRPM7 regulates myosin IIA filament stability and protein localization by heavy chain phosphorylation. *J Mol Biol* 378:790-803.

Clark K, Middelbeek J, Morrice NA, Figdor CG, Lasonder E, van Leeuwen FN (2008b) Massive autophosphorylation of the Ser/Thr-rich domain controls protein kinase activity of TRPM6 and TRPM7. *PLoS One* 3:e1876 (1-10).

Corkey BE, Duszynski J, Rich TL, Matchinsky B, Williamson JR (1986) Regulation of free and bound magnesium in rat hepatocytes and isolated mitochondria. *J Biol Chem* 261:2567-74.

Covacci V, Bruzzese N, Sgambato A, Di Francesco A, Russo MA, Wolf FI, Cittadini A (1998) Magnesium restriction induces granulocytic differentiation and expression of p27Kip1 in human leukemic HL-60 cells. *J Cell Biochem* 70:313-22.

Csermely P, Fodor P, Somogyi J (1987) The tumor promoter tetradecanoylphorbol-13-acetate elicits the redistribution of heavy metal in subcellular fractions of rabbit thymocytes as measured by plasma emission spectroscopy. *Carcinogenesis* 8:1663-6.

Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, Verslype C, Chau I, van Cutsem E (2004) Cetuximab monotherpay and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* 351:337-45.

Dai L-J, Friedman PA, Quamme GA (1991) Cellular Mechanism of chlorothiazide and cellular potassium depletion on Mg^{2+} uptake in mouse distal convoluted tubule cells. *Kidney Inter* 51:1008-17.

Dai L-J, Friedman PA, Quamme GA (1997) Phosphate depletion diminishes Mg^{2+} uptake in mouse distal convoluted tubule cells. *Kidney Inter* 51:1710-18.

Dalal P, Romani A (2010) Adenosine triphosphate depletion by cyanide results in a Na^{+}-dependent Mg^{2+} extrusion from liver cells. *Metabolism* 59:1663-71.

Di Francesco A, Desnoyer RW, Covacci V, Wolf FI, Romani A, Cittadini A, Bond M (1998) Changes in magnesium content and subcellular distribution during retinoic acid-induced differentiation of HL60 cells. *Arch Biochem Biophys* 360:149-57.

Di Polo R, Beauge' L (1988) An ATP-dependent Na^{+}/Mg^{2+} countertransport is the only mechanism for Mg^{2+} extrusion in squid axons. *Biochim Biophys Acta* 946:424-8.

Dimke H, van der Wjist J, Alexander TR, Mejier IM, Mulder GM, van Goor H, Tejpar S, Hoenderop JG, Bindels RJ (2010) Effects of the EGFR inhibitor erlotinib on magnesium handling. *J Am Soc Nephrol* 21:1309-16.

Dipette DJ, Simpson K, Guntupalli J (1987) Systemic and regional hemodynamic effect of acute magnesium administration in the normotensive and hypertensive state. *Magnesium* 6:136-9.

Dolder M, Walzel B, Speer O, Schlattner U, Wallimann T (2003) Inhibition of the mitochondrial permeability transition by creatine kinase substrates. *J Biol Chem* 278:17760-6.

Doleh L, Romani A (2007) Biphasic effect of extra-reticular Mg^{2+} on hepatic G6P transport and hydrolysis. *Arch Biochem Biophys* 467:283-90.

Dorovkov MV, Ryazanov AG (2004) Phosphorylation of annexin I by TRPM7 channel-kinase. *J Biol Chem* 279:50643-6.

Ebel H, Gunther T (2003) Stimulation of Na^{+}/Mg^{2+} antiport in rat erythrocytes by intracellular Cl^{-}. *FEBS Lett* 543:103-7.

Ebel H, Hollstein M, Gunther T (2002) Role of the choline exchanger in Na^{+}-independent Mg^{2+} efflux from rat erythrocytes. *Biochim Biophys Acta* 1559:135-44.

Ebel H, Hollstein M, Günther T (2004) Differential effect of imipramine and related compounds on Mg^{2+} efflux from rat erythrocytes. *Biochim Biophys Acta* 1667:132-40.

Efrati E, Arsentiev-Rozenfeld J, Zelikovic I (2005) The human paracellin-1 gene (hPCLN-1): renal epithelial cell specific expression and regulation. *Am J Physiol* 288:F272-83.

Elliot DA, Rizack MA (1974) Epinephrine and adrenocorticotrophic hormone-stimulated magnesium accumulation in adipocytes and their plasma membranes. *J Biol Chem* 249:3985-90.

Erdos JJ, Maguire ME (1980) Independent desensitization of β-adrenergic receptor regulated magnesium transport and cyclic AMP accumulation. *Mol Pharmacol* 18:379-83.

Erdos JJ, Maguire ME (1983) Hormone-sensitive magnesium transport in murine S49 lymphoma cells: Characterization and specificity for magnesium. *J Physiol* 337:351-71.

Fagan TE, Cefaratti C, Romani A (2004) Streptozotocin-induced diabetes impairs Mg^{2+} homeostasis and uptake in rat liver cells. *Am J Physiol* 286:E184-93.

Fagan TE, Romani A (2000) Activation of Na^{+}- and Ca^{2+}-dependent Mg^{2+} extrusion by a_1- and b_1-adrenergic agonists in rat liver cells. *Am J Physiol* 279:G943-50.

Fagan TE, Romani A (2001) a_1-adrenoceptor-induced Mg^{2+} extrusion from rat hepatocytes occurs via Na^{+}-dependent transport mechanism. *Am J Physiol* 280:G1145-56.

Fatholahi M, Lanoue K, Romani A, Scarpa A (2000) Relationship between total and free cellular Mg^{2+} during metabolic stimulation of rat cardiac myocytes and perfused hearts. *Arch Biochem Biophys* 374:395-401.

Feray J-C, Garay R (1986) An Na^{+}-stimulated Mg^{2+} transport system in human red blood cells. *Biochim Biophys Acta* 856: 76-84.

Feray J-C, Garay R (1988) Demonstration of a Na^{+}:Mg^{2+} exchange in human red cells by its sensitivity to tricyclic antidepressant drugs. *Naunyn-Schmied Arch Pharmacol* 338:332-7.

Ferreira A, Rivera A, Romero JR (2004) Na^{+}/Mg^{2+} exchange is functionally coupled to the insulin receptor. *J Cell Physiol* 199:434-40.

Flatman PW (1984) Magnesium transport across cell membrane. *J Membr Biol* 80:1-14.

Flatman PW, Lew VL (1981) The magnesium dependence of sodium-pump-mediated sodium-potassium and sodium-sodium exchange in intact human red cells. *J Physiol* 315:421-46.

Flatman PW, Smith LM (1990) Magnesium transport in ferret red cells. *J Physiol* 431:11-25.

Garfinkel D, Garfinkel L (1988) Magnesium and regulation of carbohydrate metabolism at the molecular level. *Magnesium* 7:249-61.

Gasbarrini A, Borle AB, Farghali H, Bender C, Francavilla A, van Thiel D (1992) Effect of anoxia on intracellular ATP, Na^{+}_{i}, Ca^{2+}_{i}, Mg^{2+}_{i}, and cytotoxicity in rat hepatocytes. *J Biol Chem* 267:6654-63.

Gaussin V, Gailly P, Gillis J-M, Hue L(1997) Fructose-induced increase in intracellular free Mg^{2+} ion concentration in rat hepatocytes: relation with the enzymes of glycogen metabolism. *Biochem J* 326:823-7.

Geigy Scientific Tables. (1984) Ed.: Lentner C., Ciba-Geigy, Basel, Switzerland.

Goytain A, Hines RM, El-Husseini A, Quamme GA (2007) NIPA1(SPG6), the basis for autosomal dominant form of hereditary spastic paraplegia, encodes a functional Mg^{2+} transporter. *J Biol Chem* 282:8060-8.

Goytain A, Hines RM, Quamme GA (2008a) Functional characterization of NIPA2, a selective Mg^{2+} transporter. *Am J Physiol* 295:C944-53.

Goytain A, Hines RM, Quamme GA (2008b) Huntingtin-interacting proteins, HIP14 and HIP14L, mediate dual functions, palmitoyl acyltransferase and Mg^{2+} transport. *J Biol Chem* 283:33365-74.

Goytain A, Quamme GA (2005a) Identification and characterization of a novel mammalian Mg^{2+} transporter with channel-like properties. *BMC Genomics* 6:48.

Goytain A, Quamme GA (2005b) Functional characterization of human SLC41A1, a Mg^{2+} transporter with similarity to prokaryotic MgtE Mg^{2+} transporters. *Physiol Genomics* 21:337-342.

Goytain A, Quamme GA (2005c) Functional characterization of the human solute carrier, SLC41A2. *Biochem Biophys Res Commun* 330:701-5.

Goytain A, Quamme GA (2005d) Functional characterization of ACDP2 (ancient conserved domain protein), a divalent metal transporter. *Physiol Genomics* 22:382-89.

Goytain A, Quamme GA (2008) Identification and characterization of a novel family of membrane magnesium transporters, MMgT1 and MMgT2. *Am J Physiol* 294:C495-502.

Groenestege WM, Hoenderop JG, van den Heuvel L, Knoers N, Bindels RJ (2006) The epithelial Mg^{2+} channel transient receptor potential melastatin 6 is regulated by dietary Mg^{2+} content and estrogens. *J Am Soc Nephrol* 17:1035-43.

Groenestege WM, Thébault S, van der Wijst J, van den Berg D, Janssen R, Tejpar S, van den Heuvel LP, van Cutsem E, Hoenderop JG, Knoers NV, Bindels RJ (2007) Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. *J Clin Invest* 117:2260-27.

Grubbs RD, Collins SD, Maguire ME (1985) Differential compartmentation of magnesium and calcium in murine S49 lymphoma cells. *J Biol Chem* 259:12184-92.

Grubbs RD, Maguire ME (1986) Regulation of magnesium but not calcium by phorbol esters. *J Biol Chem* 261:12550-4.

Grubbs RD, Maguire ME (1987) Magnesium as a regulatory cation: Criteria and evaluation. *Magnesium* 6:113-27.

Grubbs RD, Wetherill CA, Kutschke K, Maguire ME (1984) Magnesium transport in murine S49 lymphoma cells: pharmacology and divalent cation selectivity. *Amer J Physiol* 248:C51-7.

Guideri G (1992) Catecholamine modulation of Mg^{2+} plasma levels in the rat. *Arch Intern Pharmacodyn Therap* 14:122-25.

Gunther T (1986) Functional compartmentation of intracellular magnesium. *Magnesium* 5:53-9.

Günther T (1993) Mechanisms and regulation of Mg^{2+} efflux and Mg^{2+} influx. *Miner Electrolyte Metab* 19:259-65.

Gunther T (1996) Putative mechanism of Mg^{2+}/Mg^{2+} exchange and Na^{+}/Mg^{2+} antiport. *Magnes Bull* 18:2-6.

Günther T, Höllriegl V (1993) Na^{+}- and anion-dependent Mg^{2+} influx in isolated hepatocytes. *Biochim Biophys Acta* 1149:49-54.

Gunther T, Vormann J (1985) Mg^{2+} efflux is accomplished by an amiloride-sensitive Na^{+}/Mg^{2+} antiport. *Biochem Biophys Res Commun* 130:540-45.

Günther T, Vormann J (1990a) Induction of Mn^{2+}/H^{+} antiport in chicken erythrocytes by intracellular Mg^{2+} and Mn^{2+}. *FEBS Lett* 265:55-8.

Gunther T, Vormann J (1990b) Na^{+}-dependent Mg^{2+} efflux from Mg^{2+}-loaded rat thymocytes and HL 60 cells. *Magnes Trace Elem* 9:279-82.

Gunther T, Vormann J (1992a) Activation of Na^{+}/Mg^{2+} antiport in thymocytes by cAMP. *FEBS Lett* 297:132-4.

Gunther T, Vormann J (1992b) Mechanism of beta-agonist-induced hypermagnesemia. *Magnes Bull* 14:122-5.

Gunther T, Vormann J (1995) Reversibility of Na^{+}/Mg^{2+} antiport in rat erythrocytes. *Biochim Biophys Acta* 1234:105-10.

Gunther T, Vormann J, Forster R (1984) Regulation of intracellular magnesium by Mg^{2+} efflux. *Biochem Biophys Res Commun* 119:124-31.

Günther T, Vormann J, Höllriegl V (1990) Characterization of Na^{+}-dependent Mg^{2+} efflux from Mg^{2+}-loaded rat erythrocytes. *Biochim Biophys Acta* 1023:455-61.

Gunther T, Vormann J, Hollriegl V (1991) Noradrenaline-induced Na^{+}-dependent Mg^{2+} efflux from rat liver. *Magnes Bull* 13:122-4.

Günther T, Vormann J, McGuigan JA (1995) Buffering and activity coefficient of intracellular free magnesium concentration in human erythrocytes. *Biochem Mol Biol Int* 37:871-5.

Günzel D, Schlue WR (1996) Sodium-magnesium antiport in Retzius neurones of the leech *Hirudo medicinalis*. *J Physiol* 491:595-608.

Gusev K, Niggli E (2008) Modulation of the local SR Ca^{2+} release by intracellular Mg^{2+} in cardiac myocytes. *J Gen Physiol*. 132:721-30.

Gwanyanya A, Amuzescu B, Zakharov SI, Macianskiene R, Sipido KR, Bolotina VM, Vereecke J, Mubagwa K (2004) Magnesium-inhibited, TRPM6/7-like channel in cardiac myocytes: permeation of divalent cations and pH-mediated regulation. *J Physiol* 559:761-76.

Gwanyanya A, Sipido KR, Vereecke J, Mubagwa K (2006) ATP and PIP2 dependence of the magnesium-inhibited, TRPM7-like cation channel in cardiac myocytes. *Am J Physiol* 291:C627-35.

Hansford RG (1994) Physiological role of mitochondrial Ca^{2+} transport. *J Bioenerg Biomembr* 26:495-508.

Harman AW, Nieminen AL, Lemasters JJ, Herman B (1990) Cytosolic free magnesium, ATP and blebbing during chemical hypoxia in cultured rat hepatocytes. *Biochem Biophys Res Commun* 170:477–83.

Henquin JC, Tamagawa T, Nenquin M, Cogneau M (1983) Glucose modulates Mg^{2+} fluxes in pancreatic islet cells. *Nature* 301:73-4.

Hofer AM, Machen TE (1993) Technique for in situ measurement of calcium in intracellular inositol 1,4,5-trisphosphate-sensitive stores using the fluorescent indicator mag-fura-2. *Proc Natl Acad Sci USA*. 90:2598-602.

Hou J, Paul DL, Goodenough DA (2005). Paracellin-1 and the modulation of ion selectivity of tight junctions. *J Cell Sci* 118:5109-18.

Hou J, Renigunta A, Gomes AS, Hou M, Paul DL, Waldegger S, Goodenough DA (2009) Claudin-16 and claudin-19 interaction is required for their assembly into tight junctions and for renal reabsorption of magnesium. *Proc Natl Acad Sci U S A.* 106:15350-5.

Howarth FC, Waring J, Hustler BI, Singh J (1994) Effects of extracellular magnesium and beta adrenergic stimulation on contractile force and magnesium mobilization in the isolated rat heart. *Magnes Res* 7:187-97.

Hwa J, Romani A, Marfella C, Scarpa A (1993) Mg^{2+} transport in erythrocytes: Regulation by intracellular pH or cAMP. *Biophys J* 63,A307.

Hwang DL, Yen CF, Nadler JL (1993) Insulin increases intracellular magnesium transport in human platelets. *J Clin Endocrinol Metab* 76:549-53.

Ikari A, Matsumoto S, Harada H, Takagi K, Hayashi H, Suzuki Y, Degawa M, Miwa M (2006) Phosphorylation of paracellin-1 at Ser217 by protein kinase A is essential for localization in tight junctions. *J Cell Sci* 119:1781-9.

Ikari A, Okude C, Sawada H, Yamazaki Y, Sugatani J, Miwa M (2008) TRPM6 expression and cell proliferation are up-regulated by phosphorylation of Erk1/2 in renal epithelial cells. *Biochem Biophys Res Commun* 369:1129-33.

Ikari A, Sanada A, Okude C, Sawada H, Yamazaki Y, Sugatani J, Miwa M (2010) Up-regulation of TRPM6 transcriptional activity by AP-1 in renal epithelial cells. *J Cell Physiol* 222:481-7.

Ishijima S, Tatibana M (1994) Rapid mobilization of intracellular Mg^{2+} by bombesin in Swiss 3T3 cells: Mobilization through external Ca^{2+}- and tyrosine kinase-dependent mechanism. *J Biochem* 115:730-7.

Jakob A, Becker J, Schottli G, Fritzsch G (1989) $alpha_1$-adrenergic stimulation causes Mg^{2+} release from perfused rat liver. *FEBS Lett* 246:127-30.

James MFM, Cork RC, Harlen GM, White JF (1988) Interaction of adrenaline and magnesium in the cardiovascular system of the baboon. *Magnesium* 7:37-43.

Jiang H, Tian SL, Zeng Y, Li LL, Shi J (2008) TrkA pathway(s) is involved in regulation of TRPM7 expression in hippocampal neurons subjected to ischemic-reperfusion and oxygen-glucose deprivation. *Brain Res Bull* 76:124-30.

Jiang J, Li M, Yue L (2005) Potentiation of TRPM7 inward current by protons. *J Gen Physiol* 126:137-50.

Jin J, Desai BN, Navarro B, Donovan A, Andrews NC, Clapham DE (2008) Deletion of Trpm7 disrupts embryonic development and thymopoiesis without altering Mg^{2+} homeostasis. *Science* 322:756-60.

Joborn H, Akerstrom G, Ljunghall S (1985) Effects of exogenous catecholamines and exercise on plasma magnesium concentrations. *Clin Endocrinol* 23:219-26.

Jung DW, Apel L, Brierley GP (1990) Matrix free Mg^{2+} changes with metabolic state in isolated heart mitochondria. *Biochemistry* 29:4121-8.

Juttner R, Ebel H (1998) Characterization of Mg^{2+} transport in brush border membrane vesicles of rabbit ileum studied with mag-fura-2. *Biochim Biophys Acta* 1370:51-63.

Karoor V, Baltensperger K, Paul H, Czech MC, Malbon CC (1995) Phosphorylation of tyrosyl residues 350/354 of the beta-adrenergic receptor is obligatory for counterregulatory effects of insulin. *J Biol Chem* 270:25305-8.

Kausalya PJ, Amasheh S, Gunzel D, Wurps H, Muller D, Fromm M, Hunziker W (2006) Disease-associated mutations affect intracellular traffic and paracellular Mg^{2+} transport function of Claudin-16. *J Clin Invest* 116:878-91.

Keenan D, Romani A, Scarpa A (1995) Differential regulation of circulating Mg^{2+} in the rat by b_1- and b_2-adrenergic receptor stimulation. *Circ Res* 77:973-83.

Keenan D, Romani A, Scarpa A (1996) Regulation of Mg^{2+} homeostasis by insulin in perfused rat livers and isolated hepatocytes. *FEBS Lett* 395:241-4.

Kehres DG, Lawyer CH, Maguire ME (1998) The CorA magnesium transporter gene family. *Microb Comp Genomics* 3:151-9.

Khan MA, Conigrave AD (2010) Mechanisms of multimodal sensing by extracellular Ca^{2+}-sensing receptors: a domain-based survey of requirements for binding and signalling. *Br J Pharmacol* 159:1039-50

Kim SJ, Kang HS, Kang MS, Yu X, Park SY, Kim IS, Kim NS, Kim SZ, Kwak YG, Kim JS (2005) alpha$_1$-Agonists-induced Mg^{2+} efflux is related to MAP kinase activation in the heart, *Biochem Biophys Res Commun* 333:1132-8.

Kolisek M, Launay P, Beck A, Sponder G, Serafini N, Brenkus M, Froschauer EM, Martens H, Fleig A, Schweigel M (2008) SLC41A1 is a novel mammalian Mg^{2+} carrier. *J Biol Chem* 283:16235-47.

Kolisek M, Zsurka G, Samaj J, Weghuber J, Schweyen RJ, Schweigel M (2003) Mrs2p is an essential component of the major electrophoretic Mg^{2+} influx system in mitochondria. *EMBO J* 22:1235-44.

Konno Y, Ohno S, Akita Y, Kawasaki H, Suzuki K (1989) Enzymatic properties of a novel phorbol ester receptor ⁄ protein kinase, nPKC. *J Biochem* 106:673–8.

Kubota T, Shindo Y, Tokuno K, Komatsu H, Ogawa H, Kudo S, Kitamura Y, Suzuki K, Oka K (2005) Mitochondria are intracellular magnesium stores: investigation by simultaneous fluorescent imaging in PC12 cells. *Biochim Biophys Acta* 1744:19-28.

Lal-Nag M, Morin PJ (2009) The claudins. *Genome Biol* 10(8):235.

Langeslag M, Clark K, Moolenaar WH, van Leeuwen FN, Jalink K (2007) Activation of TRPM7 channels by phospholipase C-coupled receptor agonists. *J Biol Chem* 282:232-9.

Laver DR, Honen BN (2008) Luminal Mg^{2+}, a key factor controlling RYR2-mediated Ca^{2+} release: cytoplasmic and luminal regulation modeled in a tetrameric channel. *J Gen Physiol* 132:429-46.

Li M, Du J, Jiang J, Ratzan W, Su LT, Runnels LW, Yue L (2007) Molecular determinants of Mg^{2+} and Ca^{2+} permeability and pH sensitivity in TRPM6 and TRPM7. *J Biol Chem* 282:25817-30.

Li M, Jiang J, Yue L (2006) Functional characterization of homo- and heteromeric channel kinases TRPM6 and TRPM7. *J Gen Physiol* 127:525-37.

Li, S-H, Li, X-J (2004) Huntingtin–protein interactions and the pathogenesis of Huntington's disease. *Trends Genet* 20:146-52.

Ludi H, Schatzmann HJ (1987) Some properties of a system for sodium-dependent outward movement of magnesium from metabolizing human red blood cells. *J Physiol* 390:367-82.

Lüthi D, Günzel D, McGuigan JA (1999) Mg-ATP binding: its modification by spermine, the relevance to cytosolic Mg^{2+} buffering, changes in the intracellular ionized Mg2+ concentration and the estimation of Mg^{2+} by 31P-NMR. *Exp Physiol* 84:231-52.

Macianskiene R, Gwanyanya A, Vereecke J, Mubagwa K (2008) Inhibition of the magnesium-sensitive TRPM7-like channel in cardiac myocytes by nonhydrolysable GTP analogs: involvement of phosphoinositide metabolism. *Cell Physiol Biochem* 22:109-18.

Maguire ME (1984) Hormone-sensitive magnesium transport and magnesium regulation of adenylate cyclase. *Trends Pharmacol Sci* 5:73-7.

Maguire ME (1988) Magnesium and cell proliferation. *Ann NY Acad Sci* 551: 215-7.

Maguire ME, Erdos JJ (1978) Magnesium but not calcium accumulation is inhibited by β-adrenergic stimulation in S49 lymphoma cells. *J Biol Chem* 253:6633-6.

Maguire ME, Erdos JJ (1980) Inhibition of magnesium uptake by β-adrenergic agonists and prostaglandin E_1 is not mediated by cyclic AMP. *J Biol Chem* 255:1030-1035.

Matsuda H (1991) Magnesium gating of the inwardly rectifying K^+ channel. *Annu Rev Physiol* 53:289-98.

Matsuura T, Kanayama Y, Inoue T, Takeda T, Morishima I (1993) cAMP-induced changes of intracellular Mg^{2+} levels in human erythrocytes. *Biochim Biophys Acta* 1220:31-6.

McCormack JG, Halestrap A, Denton RM (1990) Role of calcium ions in regulation of mammalian intramitochondrial metabolism. *Physiol Rev* 70:391-425.

Middelbeek J, Clark K, Venselaar HV, Huynen MA, van Leeuwen FN (2010) The alpha-kianse family: an exceptional branch on the protein kinase tree. *Cell Mol Life Sci* 67:875-90.

Molinoff PB (1984) Alpha- and beta-adrenergic receptor subtypes, properties, distribution and regulation. *Drugs* 28:1-14.

Moncrief MB, Maguire ME (1999) Magnesium transport in prokaryotes. *J Biol Inorg Chem* 4:523-7.

Mondon CE, Dolkas CB, Olefsky JM, Reaven GM (1974) Insulin sensitivity of isolated perfused rat liver. *Diabetes* 24: 225-9.

Monteilh-Zoller MK, Hermosura MC, Nadler MJ, Scharenberg AM, Penner R, Fleig A (2003) TRPM7 provides an ion channel mechanism for cellular entry of trace metal ions. *J Gen Physiol* 121:49-60.

Moore EW (1990) Biliary calcium and gallstone formation. *Hepatology* 12:206S-14S.

Moravec CS, Bond M (1991) Calcium is released from the junctional sarcoplasmic reticulum during cardiac muscle contraction. *Am J Physiol* 260:H989-97.

Moravec CS, Bond M (1992) Effect of inotropic stimulation on mitochondrial calcium in cardiac muscle. *J Biol Chem* 267:5310-6.

Mudge GH (1989) Agents affecting volume and composition of body fluids. In: *The Pharmacological Basis of Therapeutics* (Goodman Gilman A, Godman LS, Rall TW, Murad F, eds.) MacMillian, NY.

Muller D, Kausalya PJ, Claverie-Martin F, Meij IC, Eggert P, Garcia-Nieto V, Hunziker W (2003) A novel claudin 16 mutation associated with childhood hypercalciuria abolishes binding to ZO-1 and results in lysosomal mistargeting. *Am J Hum Genet* 73:1293-301.

Nadler MJ, Hermosura MC, Inabe K, Perraud AL, Zhu Q, Stokes AJ, Kurosaki T, Kinet JP, Penner R, Scharenberg AM, Fleig A (2001) LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability. *Nature* 411:590-5.

Nemeth EF, Scarpa A (1987) Rapid mobilization of cellular Ca^{2+} in bovine parathyroid cells by external divalent cations. *J Biol Chem* 262:5188-96.

Ogoma Y, Kobayashi H, Fujii T, Kondo Y, Hachimori A, Shimizu T, Hatano M (1992) Binding study of metal ions to S100 protein: ^{43}Ca, ^{25}Mg, ^{67}Zn and ^{39}K N.M.R.. *Int J Biol Macromol* 14:279-86.

Okahira M, Kubota M, Iguchi K, Usui S, Hirano K (2008) Regulation of aquaporin 3 expression by magnesium ion. *Eur J Pharmacol* 588:26-32.

Oki S, Ikura M, Zhang M (1997) Identification of Mg^{2+} binding sites and role of Mg^{2+} on target recognition by calmodulin. *Biochemistry* 36; 4309-16.

Otto M, Heinrich R, Kuhn B, Jacobasch G (1974) A mathematical model for the influence of fructose 6-phosphate, ATP, potassium, ammonium and magnesium on the phosphofructo-kinase from rat erythrocytes. *Eur J Biochem* 49:169-78.

Panov A, Scarpa A (1996a) Independent modulation of the activity of alpha-ketoglutarate dehydrogenase complex by Ca^{2+} and Mg^{2+}. *Biochemistry* 35:427-32.

Panov A, Scarpa A (1996b) Mg^{2+} control of respiration in isolated rat liver mitochondria. *Biochemistry* 35:12849-56.

Paravicini TM, Yogi A, Mazur A, Touyz RM (2009) Dysregulation of vascular TRPM7 and annexin-1 is associated with endothelial dysfunction in inherited hypomagnesemia. *Hypertension* 53:423-9.

Parente L, Solito E (2004) Annexin 1: more than an anti-phospholipase protein. *Inflamm Res* 53:125-32.

Piskacek M, Zotova L, Zsurka G, Schweyen RJ (2009) Conditional knockdown of hMRS2 results in loss of mitochondrial Mg^{2+} uptake and cell death. *J Cell Mol Med* 13:693-700.

Polimeni PI, Page E (1974) In: *Recent advances in study in cardiac cells and metabolism*. Ed.: Dhalle N S, vol IV, pp. 217-232, University Park Press, Baltimore.

Prakriya M, Lewis RS (2002) Separation and characterization of currents through store-operated CRAC channels and Mg^{2+}-inhibited cation (MIC) channels. *J Gen Physiol* 119:487-507.

Preston RR (1990) A magnesium current in *Paramecium*. *Science* 250:285-8.

Quamme GA (2010) Molecular identification of ancient and modern mammalian magnesium transporters. *Am J Physiol* 298:407-29.

Quamme GA, Dai L-S (1990) Presence of a novel influx pathway for Mg2+ in MDCK cells. *Am J Physiol* 259:C521-5.

Quamme GA, Dirks JH (1980) Effect of intraluminal and contraluminal magnesium on magnesium and calcium transfer in the rat nephron. *Am J Physiol* 238:F187-98.

Quamme GA, Rabkin SW (1990) Cytosolic free magnesium in cardiac myocytes: Identification of a Mg^{2+} influx pathway. *Biochem Biophys Res Commun* 167:1406-12.

Quednau BD, Nicoll DA, Philipson KD (2004) The sodium/calcium exchanger family - SLC8. *Pflugers Arch* 447:543-8.

Raftos JE, Lew VL, Flatman PW (1999) Refinement and evaluation of a model of Mg^{2+} buffering in human red cells. *Eur J Biochem* 263:635-45.

Rainier S, Chai JH, Tokarz D, Nicholls RD, Fink JK (2003) NIPA1 gene mutations cause autosomal dominant hereditary spastic paraplegia (SPG6). *Am J Hum Gene*t 73:967-71.

Rasgado-Flores H, Gonzalez-Serratos H, DeSantiago J (1994) Extracellular Mg^{2+}-dependent Na^{+}, K^{+}, and Cl^{-} efflux in squid giant axons. *Am J Physiol* 266:C1112-7.

Rayssiguier Y (1977) Hypomagnesemia resulting from adrenaline infusion in ewes: its relation to lipolysis. *Horm Metab Res* 9:309-14.

Reed G, Cefaratti C, Berti-Mattera LN, Romani A (2008) Lack of insulin impairs Mg^{2+} homeostasis and transport in cardiac cells of streptozotocin-injected diabetic rats.*J Cell Biochem* 104:1034-53.

Resnick LM (1993) Ionic basis of hypertension, insulin resistance, vascular disease, and related disorders. The mechanism of "syndrome X". *Am J Hypertens* 6:123S-34S.

Resnick LM, Altura BT, Gupta RK, Laragh JH, Alderman MH, Altura BM (1993) Intracellular and extracellular magnesium depletion in type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 36:767-70.

Rogers TA (1961) The exchange of radioactive magnesium in erythrocytes. *J Cell Comp Physiol* 57:119-21.

Rogers TA, Haven FL, Mahan PE (1960) Exchange of magnesium in the rat. *J Natl Cancer Inst* 25:887-8.

Romani A (2007) Regulation of magnesium homeostasis and transport in mammalian cells. *Arch Biochem Biophys* 458:90-102.

Romani A, Dowell E, Scarpa A (1991) Cyclic AMP-induced Mg^{2+} release from rat liver hepatocytes, permeabilized hepatocytes, and isolated mitochondria. *J Biol Chem* 266:24376-84.

Romani A, Marfella C, Scarpa A (1992) Regulation of Mg^{2+} uptake in isolated rat myocytes and hepatocytes by protein kinase C. *FEBS Lett* 296:135-40.

Romani A, Marfella C, Scarpa A (1993a) Regulation of magnesium uptake and release in the heart and in isolated ventricular myocytes. *Circ Res* 72:1139-48.

Romani A, Marfella C, Scarpa A (1993b) Hormonal stimulation of Mg^{2+} uptake in hepatocytes. Regulation by plasma membrane and intracellular organelles. *J Biol Chem* 268:15489-95.

Romani A, Scarpa A (1990a) Hormonal control of Mg^{2+} in the heart. *Nature* 346:841-4.

Romani A, Scarpa A (1990b) Norepinephrine evokes a marked Mg^{2+} efflux from liver cells. *FEBS Lett* 269:37-40.

Romani A, Scarpa A (1992) Regulation of cell magnesium. *Arch Biochem Biophys* 298:1-12.

Romani A, Scarpa A (2000) Regulation of cellular magnesium. *Front Biosci* 5:D720-34.

Romani AM, Maguire ME (2002) Hormonal regulation of Mg^{2+} transport and homeostasis in eukaryotic cells. *Biometals* 15:271-83.

Romani AM, Matthews VD, Scarpa A (2000) Parallel stimulation of glucose and Mg^{2+} accumulation by insulin in rat hearts and cardiac ventricular myocytes. *Circ Res* 86:326-33.

Rondón LJ, Groenestege WM, Rayssiguier Y, Mazur A (2008) Relationship between low magnesium status and TRPM6 expression in the kidney and large intestine. *Am J Physiol* 294:R2001-7.

Rubin H (2005) Central roles of Mg^{2+} and $MgATP^{2-}$ in the regulation of protein synthesis and cell proliferation: significance for neoplastic transformation. *Adv Cancer Res* 93:1-58.

Runnels LW, Yue L, Clapham DE (2001) TRP-PLIK, a bifunctional protein with kinase and ion channel activities, *Science* 291:1043-7.

Runnels LW, Yue L , Clapham DE (2002) The TRPM7 channel is inactivated by PIP(2) hydrolysis. *Nat Cell Biol* 4:329-36.

Rutter GA, Osbaldeston NJ, McCormack JG, Denton RM (1990) Measurement of matrix free Mg^{2+} concentration in rat heart mitochondria by using entrapped fluorescent probes. *Biochem J* 271:627-34.

Ryazanov AG (2002) Elongation factor-2 kinase and its newly discovered relatives. *FEBS Lett* 514:26-9.

Ryazanova LV, Pavur KS, Petrov AN, Dorovkov MV, Ryazanov AG (2001) [Novel type of signalling molecules: protein kinases covalently linked to ion channels]. *Mol Biol* (Mosk) 35:321-32.

Sahni J, Nelson B, Scharenberg AM (2007) SLC41A2 encodes a plasma-membrane Mg^{2+} transporter. *Biochem* J 401:505-13.

Sahni J, Scharenberg AM (2008) TRPM7 ion channels are required for sustained phosphoinositide 3-kinase signalling in lymphocytes. *Cell Metab* 8:84-93.

Scarpa A, Brinley FJ (1981) In situ measurements of free cytosolic magnesium ions. *Fed Proc* 40:2646-52.

Schatzmann HJ (1993) Asymmetry of the magnesium sodium exchange across the human red cell membrane. *Biochim Biophys Acta* 1148:15-8.

Schlingmann KP, Weber S, Peters M, Niemann NL, Vitzthum H, Klingel K, Kratz M, Haddad E, Ristoff E, Dinour D, Syrrou M, Nielsen S, Sassen M, Waldegger S, Seyberth HW, Konrad M (2002) Hypomagnesemia with secondary hypocalcemia is caused by mutations in TRPM6, a new member of the TRPM gene family. *Nat Genet* 31:166–70.

Schmitz C, Deason F, Perraud A-L (2007) Molecular components of vertebrate Mg^{2+} homeostasis regulation. *Magnes Res* 20:6-18.

Schmitz C, Dorovkov MV, Zhao X, Davenport BJ, Ryazanov AG, Perraud A-L (2005) The channel kinases TRPM6 and TRPM7 are functionally nonredundant. *J Biol Chem* 280:37763-71.

Schmitz C, Perraud AL, Johnson CO, Inabe K, Smith MK, Penner R, Kurosaki T, Fleig A, Scharenberg AM (2003) Regulation of vertebrate cellular Mg^{2+} homeostasis by TRPM7. *Cell* 114:191-200.

Schweigel M, Lang I, Martens H (1999) Mg^{2+} transport in sheep rumen epithelium: evidence for an electrodiffusive uptake mechanism. *Am J Physiol* 277:G976-82.

Schweigel M, Martens H (2003) Anion-dependent Mg^{2+} influx and a role for a vacuolar H^{+}-ATPase in sheep rumianl epithelial cells. *Am J Physiol* 285:G45-53.

Schweigel M, Vormann J, Martens H (2000) Mechanisms of Mg^{2+} transport in cultured ruminal epithelial cells. *Am J Physiol* 278:G400-8.

Serrano J, Dashti SR, Perez-Reyes E, Jones SW (2000) Mg^{2+} block unmasks Ca^{2+}/Ba^{2+} selectivity of alpha1G T-type calcium channels. *Biophys J* 79:3052-62.

Sgambato A, Wolf FI, Faraglia B, Cittadini A (1999) Magnesium depletion causes growth inhibition, reduced expression of cyclin D1, and increased expression of P27Kip1 in normal but not in transformed mammary epithelial cells. *J Cell Physiol* 180:245-54.

Shafik IM, Quamme GA (1989) Early adaptation of renal Mg^{2+} reabsorption in response to magnesium restriction. *Am J Phyiol* 257:F974-7.

Shaul O, Hilgemann DW, de-Almeida-Engler J, Van Montagu M, Inz D, Galili G (1999) Cloning and characterization of a novel Mg^{2+}/H^{+} exchanger, *EMBO J* 18:3973-80.

Shi J, Krishnamoorthy G, Wang Y, Hu L, Chaturvedi N, Harilal D, Qin J, Cui J (2002) Mechanism of magnesium activation of calcium-activated potassium channels. *Nature* 418:876-80.

Shibatani T, David LL, McCormack AL, Frueh K, Skach WR (2005) Proteomic analysis of mammalian oligosaccharyltransferase reveals multiple subcomplexes that contain Sec61, TRAP, and two potential new subunits. *Biochemistry* 44:5982-92.

Simon DB, Lu Y, Choate KA, Velazquez H, Al-Sabban E, Praga M, Casari G, Bettinelli A, Colussi G, Rodrigues-Soriano J, McCredie D, Milford D, Sanjad S, Lifton RP (1999) Paracellin-1, a renal tight junction protein required for paracellular Mg^{2+} resorption. *Science* 285:103-6.

Smith RL, Thompson LJ, Maguire ME (1995) Cloning and characterization of MgtE, a putative new class of Mg^{2+} transporter from Bacillus firmus OF4. *J Bacteriol* 177:1233-8.

Smoake JA, Moy G-MM, Fang B, Solomon SS (1995) Calmodulin-dependent cyclic AMP phospho-diesterase in liver plasma membranes: Stimulated by insulin. *Arch Biochem Biophys* 323:223-32.

Somlyo AV, McClellan G, Gonzalez-Serratos H, Somlyo AP (1985) Electron probe X-ray microanalysis of post-tetanic Ca^{2+} and Mg^{2+} movements across the sarcoplasmic reticulum *in situ*. *J Biol Chem* 260:6801-7.

Stanbury JB (1948) The blocking action of magnesium on sympathetic ganglia. *J Pharmacol Exp Ther* 93:52-62.

Su L-T, Agapito M A, Li M, Simpson W, Huttenlocher A, Habas R, Yue L , Runnels LW (2006) Trpm7 regulates cell adhesion by controlling the calcium dependent protease calpain. *J Biol Chem* 281:11260-70.

Suarez A, Pulido N, Casla A, Casanova B, Arrieta FJ, Rovira A (1995) Impaired tyrosine-kinase activity of muscle insulin receptors from hypomagnesaemic rats. *Diabetologia* 38:1262–70.

Takezawa R, Schmitz C, Demeuse P, Scharenberg AM, Penner R, Fleig A (2004) Receptor-mediated regulation of the TRPM7 channel through its endogenous protein kinase domain. *Proc Natl Acad Sci USA* 101:6009-14.

Tammaro P, Smith AL, Crowley BL, Smirnov SV (2005) Modulation of the voltage-dependent K^+ current by intracellular Mg^{2+} in rat aortic smooth muscle cells. *Cardiovasc Res* 65:387-96.

Tang EY, Parker PJ, Beattie J, Houslay MD (1993) Diabetes induces selective alterations in the expression of protein kinase C isoforms in hepatocytes. *FEBS Lett* 326:117–23.

Tashiro M, Konishi M (1997) Na^+ gradient-dependent Mg^{2+} transport in smooth muscle cells of guinea pig tenia cecum. *Biophys J* 73:3371-84.

Tashiro M, Tursun P, Miyazaki T, Watanabe M, Konishi M (2002) Effects of membrane potential on Na^+-dependent Mg^{2+} extrusion from rat ventricular myocytes. *Jpn J Physiol* 52:541-51.

Taylor R., Agius L (1988) The biochemistry of diabetes. *Biochem J* 250:625-40.

Tessman PA, Romani A (1998) Acute effect of EtOH on Mg^{2+} homeostasis in liver cells: Evidence for the activation of an Na^+/Mg^{2+} exchanger. *Am J Physiol* 275:G1106-16.

Thebault S, Alexander RT, Tiel Groenestege WM, Hoenderop JG, Bindels RJ (2009) EGF increases TRPM6 activity and surface expression. *J Am Soc Nephrol* 20:78-85.

Thébault S, Cao G, Venselaar H, Xi Q, Bindels RJ, Hoenderop JG (2008) Role of the alpha-kinase domain in transient receptor potential melastatin 6 channel and regulation by intracellular ATP. *J Biol Chem* 283:19999-20007.

Torres LM, Cefaratti C, Perry B, Romani A (2006) Involvement of ERK1/2 and p38 in Mg^{2+} accumulation in liver cells. *Mol Cell Biochem* 288:191-9.

Torres LM, Konopnika B, Berti-Mattera LN, Liedtke C, Romani A (2010) Defective Translocation of PKCepsilon in EtOH-Induced Inhibition of Mg Accumulation in Rat Hepatocytes. *Alcohol Clin Exp Res* 34:1659-69.

Torres LM, Youngner J, Romani A (2005) Role of glucose in modulating Mg^{2+} homeostasis in liver cells from starved rats. *Am J Physiol* 288:G195-206.

Touyz RM, He Y, Montezano ACI, Yao G, Chubanov V, Gudermann T, Callera GE (2006) Differential regulation of transient receptor potential melastatin 6 and 7 cation channels by ANG II in vascular smooth muscle cells from spontaneously hypertensive rats. *Am J Physiol* 290:R73-8.

Touyz RM, Schiffrin EL (1996) Angiotensin II and vasopressin modulate intracellular free magnesium in vascular smooth muscle cells through Na^+-dependent protein kinase C pathways. *J Biol Chem* 271:24353-8.

Touyz RM, Yao G (2003) Modulation of vascular smooth muscle cell growth by magnesium-role of mitogen-activated protein kinases. *J Cell Physiol* 197:326-35.

Trache A, Trzeciakowski JP, Meininger GA (2010) Mg^{2+} modulates integrin-extracellular matrix interaction in vascular smooth muscle cells studied by atomic force microscopy. *J Mol Recognit* 23:316-21.

van der Wjist J, Hoenderop JG, Bindels RJ (2009) Epithelial Mg^{2+} channel TRPM6: insight into the molecular regulation. *Magnes Res* 22:127-32.

Voets T, Nilliues B, Hoefs S, van der Kemp AW, G Droogmans, Bindels RJ, Hoenderop JG (2004) TRPM6 forms the Mg^{2+} influx channel involved in intestinal and renal Mg^{2+} absorption. *J Biol Chem* 279:19-25.

Volpe P, Alderson-Lang BH, Nickols GA (1990) Regulation of inositol 1,4,5-trisphosphate-induced Ca^{2+} release. I. Effect of Mg^{2+}. *Am J Physiol* 258:C1077-85.

Volpe P, Vezú L (1993) Intracellular magnesium and inositol 1,4,5-trisphosphate receptor: molecular mechanisms of interaction, physiology and pharmacology. *Magnes Res* 6:267-74.

Vormann J, Gunther T (1987) Amiloride sensitive net Mg^{2+} efflux from isolated perfused rat hearts. *Magnesium* 6:220-4.

Vormann J, Magdorf K, Günther T, Wahn U (1994) Increased Na^+/Mg^{2+} antiport in erythrocytes of patients with cystic fibrosis. *Eur J Clin Chem Clin Biochem* 32:833-6.

Waas WF, Dalby KN (2003) Physiological concentrations of divalent magnesium ion activate the Serine/Threonine specific protein kinase ERK2. *Biochemistry* 42:2960-70.

Wabakken T, Rian E, Kveine M, Aasheim H-C (2003) The human solute carrier SLC41A1 belongs to a novel eukaryotic subfamily with homology to prokaryotic MgtE Mg^{2+} transporters. *Biochem Biophys Res Commun* 306:718-24.

Walder RY, Landau D, Meyer P, Shalev H, Tsolia M, Borochowitz Z, Boettger MB, Beck GE, Englehardt RK, Carmi R, Sheffield VC (2002) Mutation of TRPM6 causes familial hypomagnesemia with secondary hypocalcemia. *Nat Genet* 31:171-4.

Walder RY, Yang B, Stokes JB, Kirby PA, Cao X, Shi P, Searby CC, Husted RF, Sheffield VC (2009) Mice defective in Trpm6 show embryonic morality and neural tube defects, *Human Mol Gen* 18:4367-75.

Wang CY, Davoodi-Semiromi A, Shi JD, Yang P, Huang YQ, Agundez JA, Moran JM, Ochoa B, Hawkins-Lee B, She JX (2003) High resolution mapping and mutation analyses of candidate genes in the urofacial syndrome (UPS) critical region. *Am J Med Genet A* 119:9-14.

Wang CY, Shi JD, Yang P, Kumar PG, Li QZ, Run QG, Su YC, Scott HS, Kao KJ, She JX (2003) Molecular cloning and characterization of a novel gene family of four ancient conserved domain proteins (ACDP). *Gene* 306:37-44.

Wang CY, Yang P, Chi JD, Purohit S, Guo D, An H, Gu JG, Ling J, Dong Z, She JX (2004) Molecular cloning and characterization of the mouse Acdp gene family. *BMC Genomics* 5:7.

Wang M, Berlin JR (2006) Channel phosphorylation and modulation of L-type Ca^{2+} currents by cytosolic Mg^{2+} concentration. *Am J Physiol* 291:C83-92.

Wang S, George SE, Davis JP, Johnson JD (1998) Structural determinants of Ca^{2+} exchange and affinity in the C terminal of cardiac troponin C. *Biochemistry* 37:14539-44.

Wei WL, Sun HS, Olah ME, Sun X, Czerwinska E, Czerwinski W, Mori Y, Orser BA, Xiong ZG, Jackson MF, Tymianski M, MacDonald JF (2007) TRPM7 channels in hippocampal neurons detect levels of extracellular divalent cations. *Proc Natl Acad Sci U SA* 104:16323-8.

White RE, Hartzell HC (1988) Effects of intracellular free magnesium on calcium current in isolated cardiac myocytes. *Science* 239:778-80.

Wiesenberger G, Waldherr M, Schweyen RJ (1992) The nuclear gene MRS2 is essential for the excision of group II introns from yeast mitochondrial transcripts in vivo. *J Biol Chem* 267:6963-9.

Wolf FI, Cittadini A (2003) Chemistry and biochemistry of magnesium. *Mol Aspects Med* 24:3-9.

Wolf FI, Cittadini AR, Maier JA (2009a) Magnesium and tumors: ally or foe? *Cancer Treat Rev* 35:378-82.

Wolf FI, Covacci V, Bruzzese N, Di Francesco A, Sachets A, Cord D, Cittadini A (1998) Differentiation of HL-60 promyelocytic leukemia cells is accompanied by a modification of magnesium homeostasis. *J Cell Biochem* 71:441-8.

Wolf FI, Di Francesco A, Cittadini A (1994) Characterization of magnesium efflux from Ehrlich ascites tumor cells. *Arch Biochem Biophys* 308:335-41.

Wolf FI, Di Francesco A, Covacci V, Cittadini A (1997) Regulation of magnesium efflux from rat spleen lymphocytes. *Arch Biochem Biophys* 344:397-403.

Wolf FI, Di Francesco A, Covacci V, Corda D, Cittadini A (1996) Regulation of intracellular magnesium in ascites cells: involvement of different regulatory pathways. *Arch Biochem Biophys* 331:194-200.

Wolf FI, Torsello A, Fasanella S Cittadini A (2003) Cell physiology of magnesium. *Mol Asp Med* 24:11-26.

Wolf FI, Trapani V, Simonacci M, Boninsegna A, Mazur A, Maier JA (2009b) Magnesium deficiency affects mammary epithelial cell proliferation: involvement of oxidative stress. *Nutr Cancer* 61:131-6.

Xu W, Willis JS (1994) Sodium transport through the amiloride-sensitive Na-Mg pathway of hamster red cells. *J Membr Biol* 141:277-87.

Yamaguchi S, Ishikawa T (2008) The electgrogenic Na^+-HCO_3^- cotransporter NBCe1-B is regulated by cellular Mg^{2+}. *Biochem Biophys Res Commun* 376:100-4.

YamaguchiH, Matsushita M, Nairn A, Kuriyan J (2001) Crystal structure of the atypical protein kinase domain of a TRP channel with phosphotransferase activity. *Mol Cell* 7:1047-57.

Yanai A, Huang K, Kang R, Singaraja RR, Arstikaitis P, Gan L, Orban PC, Mullard A, Cowan CM, Raymond LA, Drisdel RC, Green WN, Ravikumar B, Rubinsztein DC, El-Husseini A, Hayden MR (2006) Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. *Nat. Neurosci* 9:824-31.

Yang Z, Wang J, Altura BT, Altura BM (2000) Extracellular magnesium deficiency induces contraction of arterial muscle: role of PI3-kinases and MAPK signalling pathways. *Pflugers Arch* 439:240-7.

Yang ZW, Wang J, Zheng T, Altura BT, Altura BM (2001) Ethanol-induced contractions in cerebral arteries: role of tyrosine and mitogen-activated protein kinases. *Stroke* 32:249-57.

Zhang GH, Melvin JE (1992) Secretagogue-induced mobilization of an intracellular Mg^{2+} pool in rat sublingual mucous acini. *J Biol Chem* 267:20721-7.

Zhang GH, Melvin JE (1996) Na^{+}-dependent release of Mg^{2+} from an intracellular pool in rat sublingual mucous acini. *J Biol Chem 271*:29067-72.

Zhang J, Wier G, Blaustein MP (2002) Mg^{2+} blocks myogenic tone but not K^{+}-induced constriction: Role for SOCs in small arteries. *Am J Physiol* 283:H2692-705.

Zhou H, Clapham DE (2009) Mammalian MagT1 and TUSC3 are required for cellular magnesium uptake and vertebrate embryonic development. *Proc Natl Acad Sci U S A* 106:15750-5.

Zsurka G, Gregan J, Schweyen RJ (2001) The human mitochondrial Mrs2p protein functionally substitutes for its yeast homologue, a candidate magnesium transporter. *Genomics* 72:158-68.