Cell signaling, beyond cytosolic calcium in eukaryotes

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ABSTRACT

Calcium functions as a secondary messenger within the cytosols of eukaryotes. This serves as a reference point to evaluate three related questions:

1. Calcium, as well as cyclic AMP, also functions as a paracrine messenger; how specific and extensive is this use?
2. Calcium binding proteins and calcium extrusion mechanisms have been identified in prokaryotes; does it function as a messenger?
3. The concentrations of other divalent cations, especially zinc and magnesium, are well regulated and perturbations have specific physiological impacts; are these divalents involved in information transfer? Exploration of these three interrelated questions indicates the importance of more sensitive techniques and of a refined concept of information transfer and transduction.

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1. Introduction

The role of calcium functioning as a secondary messenger within the cytosols of eukaryotes is well established and several homolog families of calcium modulated proteins have been characterized. The importance of cell signaling in general, and of calcium in particular, suggests that Nature may have put calcium to broader use and employed other divalents as well. This review addresses three interrelated questions:

1. Does calcium function as an extracellular messenger in metazoa?
2. Does calcium function as a secondary messenger in prokaryotes?
3. Are other divalent cations including, but not restricted to, Zn^{2+} and Mg^{2+} also involved in information transduction?

We first summarize cell signaling by calcium in eukaryotes as well as the involved calcium modulated proteins. We next address the function of calcium as an extracellular, or paracrine messenger. Given these precedents we ask whether calcium serves similar functions in bacteria. Finally, we consider whether other divalents are involved in information transduction.

2. Calcium as a secondary messenger in eukaryotes

2.1. Calcium as a cytosolic messenger

Douglas in 1963 [1] reviewed the research of many groups, including his own, and argued that "Calcium acts as a crucial link in the process of stimulation-secretion coupling..." Rasmussen [2] summarized "Cell communication", "The basic elements of this system are two interrelated messengers, 3′,5′-AMP and Ca^{2+}. Activation of excitation of the cell leads to an increase in both." We now understand that the information contained in a pulse of calcium functioning as a secondary messenger is transduced via its binding to calcium modulated proteins in the cytosol.

The second and related breakthrough was the gradual realization that the concentration of the free Ca^{2+} ion, within the cytosols of all organisms, including prokaryotes, is very low, 1.0–5.0×10^{-8} M [3,4]; while $[Ca^{2+}]$ in extracellular fluids of most metazoa is 1.0–5.0×10^{-3} M, Table 1, [5]. The pumps that extrude calcium from the cytosol and the channels that admit it are highly selective; they are, however, like any calcium binding protein, subject to competition by other divalents [6].

Calcium signals in the cell are generated by influx through voltage- and ligand-gated Ca^{2+} ion channels, release from internal stores, and sequestration by calcium pumps and exchangers [6,7]. Changes in $[Ca^{2+}]_{cyt}$, in response to agonist stimulation or cell depolarization, can be visualized, using calcium specific dyes, engineered green fluorescent protein [8,9], or aequorin as highly localized pools of calcium in sub-cellular structures or as calcium waves

Table 1
Concentrations of metals, total and ionized, in the cytosol and extracellular milieu

Cytosol			Extracellular millieu	
	Total	Ionic	Total	Ionic
Na		19.4 mM [135]		146 mM [136]
K		150 mM		3.0–3.5 mM [137]
Mg	2.3 mM [138]	0.95 mM [139]		1.0–1.5 mM [137]
Ca	1.5–1.75 mM [140]	50–100 nM [141]	1–40 mM [26]	1.0–1.5 mM [137]
Mn	10–100 μM [142]			
Zn	200 μM [77]	pM [78,79]		

that spread throughout the cell including the nucleus [10,11]. Corresponding dyes should be engineered for the non-calcium divalents.

From a toolkit of basic components – pumps, channels, and buffers – eukaryotic cells can tailor a broad range of calcium signals, ranging from localized calcium spikes that regulate fast responses to slower global calcium transients or waves [12]. The same toolkit that coordinates cell division and interactions during embryogenesis [13] is also responsible for the rise in $[Ca^{2+}]_{cyt}$ and depletion of the endoplasmic pool of calcium that precedes apoptosis [14].

Agonist-stimulated Ca^{2+} signaling events involve (1) the release of Ca^{2+} from internal storage compartments into the cytoplasm via intracellular release channels e.g., the inositol 1,4,5-triphosphate (IP_3) receptor; (2) the activation of Ca^{2+} entry through store-operated channels, such as the recently identified Ca^{2+} release-activated pathways [15]; and (3) the extrusion of Ca^{2+} into the extracellular space by plasma membrane Ca^{2+} ATPases or other export mechanisms, e.g. Na^{+}/Ca^{2+} exchangers.

2.2. Calcium modulated proteins

Each homolog family of calcium binding proteins has its own saga; each subfamily its own story; and each member or mutant its personal idiosyncrasies. Nonetheless, there are many characteristics common to most calcium binding proteins (CaBP). As a reference point we consider a hypothetical, generic CaBP that has the following characteristics, which we will assume for every CaBP, unless there is convincing experimental evidence to the contrary.

Our generic protein has several, interacting or non-interacting calcium binding sites. The affinities of most CaBP sites for the Ca^{2+} ion are ~10^4 greater than for the Mg^{2+} ion. This surely reflects not the energies of the divalent cation, oxygen bonds, per se, but natural selection for a protein conformation(s) whose change(s) releases more energy when binding calcium than when binding magnesium. Our generic CaBP binds magnesium at only the nominal calcium binding sites. If magnesium, or any other cation, binds non-cooperatively to only calcium binding sites, then its affinity can be determined by competition experiments.

Calmodulin, an exemplar of EF-hand proteins, is found in the cytosol of (nearly) all eukaryotic cells and transduces the information in a pulse of Ca^{2+} ions into the activation of scores of target proteins, including calmodulin-dependent protein kinases, calcineurin, phosphodiesterase, adenylyl cyclase, and many other enzymes [16–18]. It is calcium modulated in that its interaction with messenger calcium alters its interaction with other molecules in a physiologically meaningful way (Fig. 1). Calmodulin can interact with target proteins in both its apo- and its calci-forms. For example, apo-calmodulin activates the ryanodine receptor, and calci-calmodulin inhibits calcium release from sarcoplasmic reticulum [19].

2.3. Criteria for Information transduction

Sutherland suggested criteria essential to a secondary messenger system [20]. By analogy, one might consider four criteria for
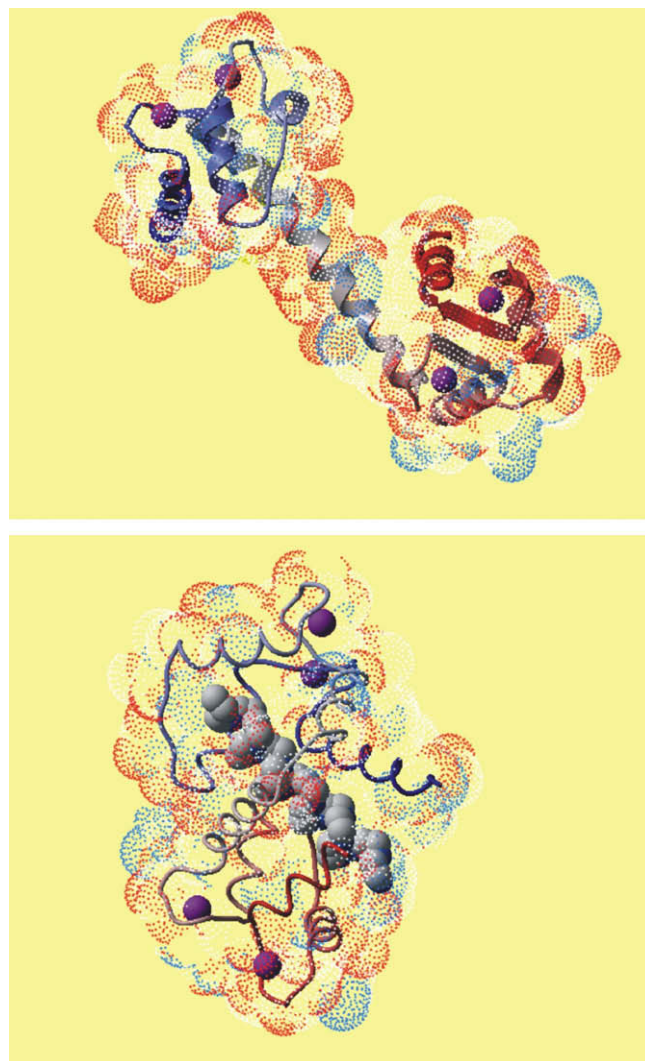
Fig. 1. Calmodulin and information transduction. Panel a: Tetra-calci-calmodulin: CaM consists of two pairs of EF-hands, each consisting of α-helix E, a Me^{2+} binding loop, and α-helix F. The van der Waals surface of CaM is stipled-red, electronegative; blue electropositive. The four Ca^{2+} ions are indicted by purple spheres. Panel b: CaM complexed with a target peptide ARRKWQKTGHAVRAIGRLSS from myosin light chain kinase (represented as space filling, PDB file 1cdl): CaM, usually in the tetra-calci form, interacts with scores of targets; CaM interacts with a few in the apo-(magnesi-)form. Lobe 1,2 is linked to lobe 3,4 by a flexible tether. The two lobes enfold an α-helix of the target; however, the relations of the two lobes to one another and to the helix vary with the target, perhaps part of the reason that CaM can recognize so many different targets. The information contained in a pulse of Ca^{2+} ions is transduced by CaM to altered functions of the targets (figure drawn with ICM Browser program).

establishing the role, if any, of divalents in information transduction:

1. The divalent should bind to a target molecule in vitro under physiological conditions.

2. This interaction of divalent and target should occur in vivo.

3. The concentration of the divalent should vary in a purposeful way in vivo.

4. A physiological response should be associated with the divalent, target interaction.

We consider only divalents naturally present in living organisms and do not discuss heavy metals such as lead, mercury, and chromium, whose detoxifications merit a separate review.

As will be discussed these criteria are met for extracellular calcium as a paracrine messenger. Although prokaryotes have specific calcium binding proteins and regulate cytosolic concentrations of

calcium and other divalents, there is still not convincing evidence of messenger function. Several of these four criteria are satisfied for zinc and magnesium. The question is whether all four have been met for a single system, i.e. whether the circle has been closed.

## 3. Calcium as an extracellular messenger in eukaryotes

Temporal and spatial separation of calcium entry and efflux across the plasma membrane can give rise to physiologically significant changes in extracellular $[Ca^{2+}]$ [21], especially in neurons and polarized epithelial cells. The best known of the cell surface receptors for calcium is the extracellular calcium sensing receptor (CaSR) [22]. CaSR is a member of family C of the G protein coupled receptor superfamily, which also includes three taste receptors (T1–T3), the GABAB receptors, eight metabotropic glutamate receptors (mGluR1–mGluR8), and six orphan receptors, including GPRC6A [23,24]. It interacts with Gαi protein to inhibit adenylyl cyclase and activate ERK, with Gαq protein to stimulate phospholipases C and A2, and with Gβγ protein to stimulate phosphatidylinositol 3-kinase.

CaSR is expressed in osteoblasts, kidney, and gut – the three major organs involved in calcium homeostasis [22,25]. There is a single gene encoding CaSR in mammals; variations in sequences of cDNA arise from splice variants at exonic junction I/II [26,27]. Mutations in the CaSR cause both inactivation, as seen in hypocalciuric hypercalcemia, and hyperactivation, as in autosomal dominant hypocalcemia, e.g. E640K in HEK-293 cells [27,28]. Intracellular signal pathways to which the CaR couples via its associated G proteins include phospholipase C, protein kinase B, and mitogen-activated protein kinases [29,30].

Although the crystal structure of CaSR is not known, it is probably similar to that of its homolog, the metabotropic glutamate receptor (mGluR) (Fig. 2). The extracellular region of mGluR consists of a pair of ligand binding (LB) domains, the first comprising residues 33–206 and the second, 207–344. Glutamate binds in a cleft, the so called Venus flytrap, between LB1 and LB2 and causes a shift of 70° in the relative orientation of LB1 to LB2; calcium probably binds in the homologous site in CaSR. The remainder of the ligand binding region to residue 522, the cysteine rich region (residues 523–592), the seven transmembrane helices, 593–640, and the intracellular region to the C-terminus at residue 1199 were not included in the construct that was crystallized. A pair of LB1 and LB2's are related by an approximate two fold axis and are cross linked by a disulfide bond between Cys's 140 [31].

The CaSR responds to several calcium mimics, especially extracellular spermine, as seen in regulation of fluid secretion in rat colonic crypt cells [32]. One assumes that the binding of two glutamates to the mGluR dimer (or of two Ca^{2+} ions to CaSR) induces a change in conformation that is transmitted through the transmembrane region altering the interaction of the intracellular region with its cognate G-protein(s). Half maximal activation occurs at 3.5 mM $[Ca^{2+}]$, the Hill coefficient is ~ 3 [25,26]. These observations imply that the CaSR is in the apo state under normal conditions, $[Ca^{2+}]_{ext} \sim 1.3$ mM, and that the active form consists of two or more dimers and/or that other poly-cations contribute to its activation.

The binding of extracellular Ca^{2+} ions by the CaSRs results in transient increases in $[Ca^{2+}]_{cyt}$ by release of calcium from thapsigargin sensitive intracellular stores and through increased calcium influx though voltage insensitive channels in the cell membrane [26]. In chief cells of the parathyroid a slight increase in $[Ca^{2+}]_{ext}$ causes decreased synthesis and release of parathyroid hormone, as well as decreased cell proliferation [27]. Human embryonic kidney-293 cells, stably transfected with the CaSR gene, increase their
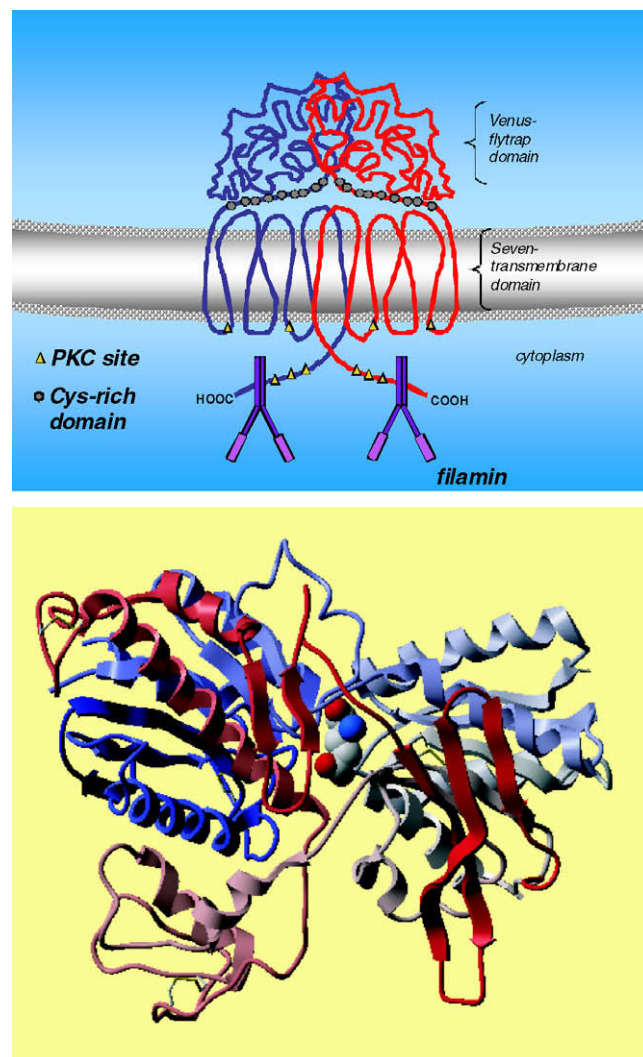
Fig. 2. Model of the structure of the calcium sensing receptor. The calcium sensing receptor is homologous to the metabotropic glutamate receptor. The extracellular region of mGluR consists of a pair of ligand binding domains – the first, residues 33–206 and the second, 207–344 (lower panel) [143], PDB file 1isr. Glutamate (space filling molecule in the lower panel; oxygen, red; nitrogen, blue; carbon, white) binds in a cleft, so called Venus flytrap, between LB1 and LB2 and causes a shift of 70° in the relative orientation of LB1–LB2; calcium probably binds in the homologous site in CaSR. The remainder of the ligand binding region to residue 522, the cysteine rich region 523–592, the seven helices of the transmembrane region 593–640, and the intracellular region to the C-terminus at 1199 were not included in the construct that was crystallized. A pair of LB1 and LB2's are related by an approximate two fold axis and are cross linked by a disulfide bond at Cys's140; the two monomers are red and blue in the upper panel. One assumes that the binding of two glutamates to the mGluR dimer (or of two Ca^{2+} ions to CaSR) induces a change in conformation that is transmitted through the transmembrane region and alters the interaction of the intracellular region with its cognate G-protein(s) (with permission from Hofer and Brown [25]).

synthesis and secretion of parathyroid hormone related protein in response to increased $[Ca^{2+}]_{ext}$ [33]. In Madin-Darby canine kidney cells, CaSR couples to Gα12/13 to regulate phospholipase D [34]. CaSR enables osteoblasts to sense high $[Ca^{2+}]_{ext}$, resulting from osteoclast mediated dissolution of bone, and to lay down new hydroxyapatite, as demonstrated by introducing a mutated CaSR into cultured rat calvaria cells [35]. The expression of pituitary tumor transforming gene, which is over expressed in many cancers, is up regulated in H-500 Leydig tumor cells by small increases in $[Ca^{2+}]_{ext}$; this expression presumably is mediated by CaSR because the dominant negative mutation in CaSR, R185Q, attenuates this effect [30].

CaSR appears to participate in coordinating interactions among several different homeostatic control systems, including those for water, magnesium, sodium, extracellular volume, and/or blood pressure. In clusters of cultured HEK-293 cells, various extracellular stimuli give rise to oscillations in $[Ca^{2+}]_{cyt}$ that correlate with oscillations in $[Ca^{2+}]_{ext}$; it is possible that CaSR is involved in generating and in decoding calcium oscillations [36].

There appear to be sensors of $[Ca^{2+}]_{ext}$ other than CaSR; these include gap-junction hemichannels, the acid sensing ion channels, ASIC1a and ASIC1b, and the 36pS cation channel found in hippocampal neurons [25]. "Uncoupling of gap-junctions with octanol or heptanol significantly inhibited intercellular calcium wave propagation." In enteric glia from neonatal guinea pig; intracellular phospholipase C and inositol triphosphate, as well as extracellular ATP are involved [37]. A component of the L-type calcium channel of sensory hair cell synapses is encoded by the cav1.3a gene of zebra fish [38]. External sodium can pass inward through T-type low voltage calcium channels, CaV3.1 and CaV3.2 [39]. Intercellular calcium waves in astrocytes are strengthened by reduction of $[Ca^{2+}]_{ext}$ and/or of $[Mg^{2+}]_{ext}$, acting apparently by release of extracellular ATP [40]. The influx of calcium associated with synaptic transmission causes reduction of $[Ca^{2+}]_{ext}$ from ~2.0 to ~1.5 mM; this in turn regulates several forms of synaptic plasticity [41]. "These results suggest that cells can take advantage of the obligate cycling of Ca^{2+} between intracellular and extracellular compartments during agonist stimulation, using Ca^{2+} as an extracellular 'third' messenger to direct different subsets of tissue interaction." [42].

A CaSR, not homologous to that from animals [43], has been cloned from Arabidopsis. It is responsible for the stomatal closure response of guard cells; this is triggered by elevated calcium in extracellular fluid [42,44]. The uptake of calcium by *Saccharomyces cerevisiae* increases as $[Ca^{2+}]_{ext}$ decreases, suggesting the presence of an extracellular calcium sensor [45].

Many other cell surface proteins are susceptible to physiological fluctuations in external $[Ca^{2+}]$ [42]. These include gap-junction hemichannels [46], which can open in response to a modest (~200 μM) decrease in $[Ca^{2+}]_{ext}$, and the receptor Notch, which may sense $[Ca^{2+}]_{ext}$ to drive the establishment of right-left symmetry during embryogenesis [47].

These results indicate that controlled variations in $[Ca^{2+}]_{ext}$ affect many cytosolic processes; that is, calcium functions as a paracrine messenger.

4. Calcium as a secondary messenger in prokaryotes?

Calcium is involved in bacterial motility, chemotaxis, transport, cell division, sporulation, and heterocyst formation [48–52]. For example, in *Escherichia* coli repellents cause an increase in intracellular calcium concentration and provoke tumbling of the bacteria; whereas, in the presence of attractants, the calcium concentration is reduced and bacteria move smoothly [53].

Calcium homeostasis has been demonstrated in some bacteria, including *E. coli* and *Bacillus* subtilis [54–56] and is inferred to exist in most or all prokaryotes [57,58]. The concentration of free Ca^{2+} ion in *E. coli* is maintained at 90 ± 10 nM, irrespective of the calcium concentration in the extracellular medium [54]. In starved cells $[Ca^{2+}]_{cyt}$ varies from 0.2 to 0.7 μM when $[Ca^{2+}]_{ext}$ varies from 10 μM to 10 mM. Several bacteria have primary ATP-dependent Ca^{2+} pumps, as well as secondary calcium antiporters that use proton or sodium electrochemical gradients to remove calcium from the cell [49,59].

Several CaBPs (calerythrin, calsymin, etc.) have been isolated from prokaryotes; however, functions have yet to be assigned. Calerythrin contains four EF-hand domains, of which three bind calcium and one is nonbinding. It was suggested that this protein might function in calcium buffer or transport rather than having a transducing function [60]. Several additional EF-hand proteins have been identified in prokaryotes [52]. Other CaBPs in prokaryotes include extracellular polysaccharide degrading enzymes from Clostridium, Ruminococcus and Bacteroides, and periplasmic D-galactose-binding proteins, which are involved in active transport of galactose and glucose, as well as chemotaxis [52].

The components for calcium signaling have been identified; however, a single, integrated system involving physiological changes in $[Ca^{2+}]_{cyt}$ has yet to be characterized.

5. Divalents in information transduction

5.1. Overview of biometals

Several metals – Na, K, Mg, Ca, Mn, Fe, Co, Ni, Cu, Zn, Mo, V, and W – are essential for life; many of their ligands have been characterized. There are mechanisms for detecting and regulating their extracellular and intracellular concentrations; there are channels and/or transporters for their uptake or extrusion; and there are pathologies associated with perturbations of their homeostasis. However, the existence of targets, transporters, detectors, and pathologies does not, per se, establish a role in information transduction beyond homeostasis. Most ligands for these metals are proteins; however, other important ligands include phosphorylated compounds for magnesium and heme groups for iron. The specificities and mechanisms of most transporters and detectors have yet to be established. Some divalents may enter or leave the cell via calcium channels.

Most of the biologically significant metals are located in the fourth row of the periodic table, except sodium and magnesium, which are in the third row as well as molybdenum and tungsten which are in the fifth and sixth rows, respectively. These biologically significant metals are divided into two groups: non-transition elements (Na, K, Mg, Ca, and Zn) and transition elements (Mn, Fe, Co, Ni, Cu, Mo, V, and W). Non-transition elements are characterized by constancy of their oxidation state and formation of ions with completely filled electron shells. In contrast, transition elements are characterized by formation of ions with incompletely filled electron shells and variable oxidation state. The transition elements that are significant for biological processes have incompletely filled d-orbitals. They form colored complexes and paramagnetic compounds whose physical properties facilitate their studies.

The alkali elements, sodium and potassium, have one *s*-electron in the outer shell beyond that of the rare gas atom; the alkali earths, magnesium and calcium, have two outer shell electrons. All four have very weak tendencies to form covalent bonds. The completely filled octets of the K^+, Na^+, Mg^{2+} and Ca^{2+} ions have no preferences for direction of formation of ionic bonds and can be modeled as spheres.

The Ca^{2+} ion, of radius 0.99 Å, is usually coordinated in proteins by seven oxygen atoms in an approximate pentagonal bipyramid. There are one to five carboxylate groups in this primary coordination sphere. One of the carboxylates, more often from Glu than Asp, is a bidentate ligand coordinating through both oxygen atoms. In most cases, there is little correlation between the number, beyond two, of carboxylates in the primary coordination sphere and the affinity of that site for calcium [61]; this implies that specific changes in conformation throughout the protein accompany binding of the divalent.

The 2+ charge of magnesium is contained within a smaller volume, 0.65 Å ionic radius; it is more electronegative than is calcium. Magnesium is almost always coordinated by six oxygen atoms arranged octahedrally. The bidentate carboxylate that contributes

two oxygens to the hepta-coordination of the Ca^{2+} ion adjusts its side chain to present only one oxygen to the Mg^{2+} ion [62,63]. There is a slight contraction of the coordination sphere of a calcium binding protein to adapt to the smaller radius of magnesium. The affinities of magnesium for many small, oxygen-containing ligands are greater than those of calcium, e.g. alanine: $pK_d(Ca)$ 1.24, $pK_d(Mg)$ 1.96; ATP: $pK_d(Ca)$ 3.97, $pK_d(Mg)$ 4.22.

All metal cations are hydrated in solution. The rate at which they bind to a protein is often determined by the rate of dissociation of the first water, or hydroxyl, from the primary coordination spheres. Because the six oxygens of hexa-aquo-magnesium are in optimal van der Waals contact and leave slowly, $k_{on}(Mg)$ for proteins is relatively low. In contrast, the waters of hepta-aquo-calcium have more lateral mobility; thus $k_{on}(Ca)$ is much faster accounting for most of the difference in affinity of CaBPs for calcium versus magnesium. In some processes, such as muscle contraction, one must consider not only the relative binding affinities but also the relative kinetics of calcium verses other divalents [64].

The Na^+ ion has a radius of 1.00 Å, similar to that of calcium, but only half the charge density. It is either seven- or eight-coordinate with oxygen. Although it competes with calcium, its affinity for these sites is usually lower than that of magnesium and it often binds to multiple sites on the protein. K^+ has ionic radius 1.3 Å and has few specific interactions with CaBPs or with other divalent binding proteins. The interactions of the "hard" cations Ca^{2+}, Mg^{2+}, Na^+, and K^+ with their electronegative ligands are essentially purely ionic; there is very little orbital overlap.

Biologically significant "soft" cations, primarily Zn^{2+} but also Mn^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, V^{2+}, and Mo^{2+}, bind to specific sites on numerous proteins. These interactions have partial covalent character and involve the d-orbitals of the divalents and the p orbitals of nitrogen, sulfur, and oxygen ligands from the protein. The formation of a coordination bond in complexes of transition metals can be considered as a transfer of a lone electron pair from the coordinated group or ligand to the metal ion. Whereas zinc prefers tetrahedral binding sites in proteins, manganese is typically found in square pyramidal or trigonal bipyramidal binding sites, with coordination number = 5, or octahedral protein-binding sites with coordination number = 6. All of these geometries are subject to distortion, and the range of metal, ligand bond lengths is greater than observed for the hard cations. A notable similarity between the d_{10} Zn^{2+} ion and the high-spin d_5 Mn^{2+} ion is that neither is subject to the complications of ligand-field stabilization effects. Manganese is capable of redox behavior under biological conditions; zinc is not [63]. Cu^{2+} is characterized by coordination numbers 4 (square planar), 5 (trigonal bipyramid or square pyramid) or 6 (octahedron). The complexes of Fe^{2+} and Fe^{3+} have predominantly octahedral structure. These empirical generalizations regarding coordination geometry and ligands are usually valid; however, one cannot predict the selectivities or affinities for these divalents from the structures of the proteins [66].

Zinc is not a transition element since it has no empty d-orbitals. Zn^{2+} ions are very different from the other non-transition metal ions. While the radius of Zn^{2+} ion is close to the radius of Mg^{2+} ion (0.65 Å), its ionization potentials are higher than are those of calcium and magnesium. These high values of zinc ionization potentials are reflected in its stronger tendency to form covalent bonds. It does not participate in redox reactions but rather functions as a Lewis acid to accept a pair of electrons. Zn^{2+} ions do not bind to the calcium binding sites in proteins; however, Mn^{2+} ions bind to both calcium and zinc sites. For example, α-lactalbumin possesses a single strong calcium binding site and several separate zinc binding sites and Mn^{2+} ions bind to both [67]. The simplest model is that these cations do not (normally) compete for calcium binding sites and that, when they bind to other sites on CaBPs, they do not affect calcium binding. There are, however, a few examples in which binding of another cation affects calcium binding, e.g. the zinc binding sites of recoverin [66] and of α-lactalbumin [67]. In practice, however, it is difficult to distinguish between direct competition for a calcium binding site and binding at an alternative site with an allosteric effect, negative or positive, on calcium binding.

Several metal cations – especially cadmium, lanthanum, and the fourteen lanthanides – are used as mimics of calcium and other divalents since they have valuable spectral properties. Their relative affinities vary with specific binding site; there is no evidence of their functioning in vivo.

Na^+ and K^+ ions are essential in maintaining transmembrane potentials and specifically in transmitting nervous impulses. However, they do not function as secondary messengers in the sense of binding to specific targets and thereby transmitting information.

The seven transition elements (Mn, Fe, Co, Ni, Cu, Mo, V, and W) all have (at least) two oxidation states available to biological systems. Their homeostasis involves, in addition to the sensing and transport required for Na, K, Mg, Ca, and Zn, mechanisms to sense and to maintain the appropriate intracellular, and for metazoa extracellular, redox potentials. Although these homeostatic mechanisms are complex and perturbations can have profound effects on physiology, there is little evidence that they function as messengers. Kretsinger [68] suggested that "Cells initially evolved pumps to extrude Ca^{2+} in order to use (Mg^{2+}) HPO_4^{2-} as their basic energy currency. $Ca_3(PO_4)_2$ is insoluble. Having first developed a four decade Ca^{2+} gradient, eukaryotic cells evolved the use of this Ca^{2+} gradient as an information potential." Though a great oversimplification, this concept still seems valid.

We focus our attention on zinc and magnesium – ligands (in vitro and in vivo), variations in concentration, and functions. We then ask which, if any, systems might meet Sutherland's four criteria – bind to target in vitro; target interaction in vivo; $[Zn^{2+}]$ or $[Mg^{2+}]$ vary in vivo; and physiological response from $[Me^{2+}]$, target interaction.

5.1.1. Zinc

Four types of zinc sites are apparent from examination of the zinc binding proteins: structural, catalytic, co-catalytic, and protein interface [65,66,69]. Structural sites for zinc have four protein ligands and no bound water molecule. The zinc finger proteins, many of which function as transcription factors, are the best known and most widely distributed zinc binding proteins. There are at least 14 different classes of zinc fingers; three classes are most common [29,70–74]. The Zn^{2+} ion is tetrahedrally coordinated by two Cys and two His residues in the C_2H_2 domains. There are many other zinc finger domains – RING, FYVE, LIM and so on [72,74]. Several S100's have zinc binding sites distinct from those that bind calcium; the binding of zinc affects the affinities of these proteins for calcium [75].

Zinc serves as a co-factor for more than 300 various enzymes, including representatives of all the six main classes of enzymes. This broad distribution contrasts with that of iron, where 80% of a total of about 3 g in a human is in the heme group alone. In catalytic sites, zinc generally forms complexes with water and any three nitrogen, oxygen, or sulfur donors, with His most often chosen. Water is always a ligand in such sites. For example, the ABC transporter, ZnuA, coordinates the Zn^{2+} ion with three His's [76] (Fig. 3). Co-catalytic sites contain two or three metals in close proximity, with two of the metals bridged by the side chain of a single amino acid residue – such as Asp, Glu, or His – and sometimes a water molecule. Asp and His are the preferred amino acids for these sites; no Cys ligands have been observed in co-catalytic sites. The interface between two subunits, or two domains, may involve zinc ligands from both subunits.

The total zinc concentration in a eukaryotic cell is about 200 μM; most of it is bound tightly to proteins [76]. The concentration free Zn^{2+} ion is in the picomolar range [78,79]. Thirty to 40% of the cellular zinc is localized in the nucleus, 50% in the cytosol and cytosolic organelles and the remainder is associated with membranes [80].

Zinc homeostasis in eukaryotic cells is controlled at the levels of uptake, intracellular sequestration in zinc storing vesicles ('zincosomes'), distribution between nucleus and cytoplasm, and elimination from the cell [81]. The cellular homeostasis of zinc is partially controlled by metallothionein, which has been shown to play a role in the regulation of cell proliferation [82]. It is over expressed in proliferating tissues, e.g. in regenerating rat liver, in developing rat liver and in various tumors. Metallothionein is normally found in the cytoplasm and not in the nucleus. It is accumulated transiently in the nuclear fraction of fetal and newborn rat livers, followed by redistribution to the cytoplasm two to three weeks post partum [83,84]. In cultured human colonic cancer cells (HT-29) cellular metallothionein levels oscillate with the cell cycle and reach a maximum in successive G1-phases and at the G1–S transitions.

Addition of 0.1 mM zinc to the extracellular medium triggers massive release of calcium from thapsigargin sensitive intracellular stores in the human colonocytic cell line HT29 with no detectable entry of zinc into the cells [85]. They suggested that this implies the existence of a zinc-specific extracellular sensor, with an apparent K_d, 0.08 mM, that communicates by an unknown mechanism and subsequently causes generation of IP$_3$ in the cytosol. The postulated extracellular zinc sensor might be a G coupled protein analogous to, but distinct from, the CaSR.

Two families of transporters, the Zrt-, Irt-like Protein (ZIP) and cation-diffusion facilitator, have been found to play a number of roles in zinc transport in eukaryotic organisms. Many transporters of the ZIP family are involved in cellular zinc uptake, and at least one member, the Zrt3 transporter of S. cerevisiae, transports stored zinc out of an intracellular compartment during adaptation to zinc deficiency [86]. In contrast, CDF family members mediate zinc efflux out of cells or facilitate zinc transport into intracellular compartments for detoxification and/or storage. The activities of many of these transporters are regulated through transcriptional and post-transcriptional mechanisms to maintain zinc homeostasis at both the cellular and organismal levels [87].

Since zinc ions play essential roles in many cell processes, organisms must maintain adequate intracellular zinc concentrations. Cells have evolved efficient uptake systems to allow accumulation of zinc even when it is scarce. These uptake systems use integral membrane transport proteins to move zinc across the plasma membrane [87]. Some of the cytosolic zinc must be transported into intracellular organelles to serve as a co-factor for various zinc dependent enzymes and processes. Zinc can also be stored in intracellular compartments when supplies are high and used later. Zinc transporters are required to facilitate this transport in and out of organelles.

E. coli concentrates zinc, as well as iron, to about 0.1 mM total, several orders of magnitude higher than the concentration in a typical growth medium. Zinc homeostasis in bacteria is achieved by both uptake and export systems, which are separately regulated. Several ABC transporters have been identified. Zinc ABC transporters are regulated by Zur repressors, which belong to the Fur protein family of iron regulators. Three types of zinc-export systems that protect cells from toxic concentrations of zinc have been identified: RND multi-drug efflux transporters, P-type ATPases, and cation-diffusion facilitators [88].

Excess zinc can be toxic to cells [89]. The mechanism(s) of zinc toxicity is not known in detail but it may bind to inappropriate intracellular ligands or compete with other metal ions for enzyme active sites, transporter proteins. A possible detoxification mechanism involves zinc's binding to metallothionein proteins. Zinc transporters can also take part in detoxification by facilitating intracellular sequestration within organelles, or efflux of zinc across the plasma membrane.

Frederickson and Bush [90] identified three distinct classes of zinc signals which they called: Zn^{2+}-SYN, Zn^{2+}-TRANS, and Zn^{2+}-INT. The first of these signals, 'synaptic zinc' (zinc SYN) is a conventional, transmitter like, synaptic signal, between presynaptic bouton and postsynaptic spine of dendrite. The Zn^{2+} ions for this signal are stored in presynaptic vesicles at low millimolar concentrations. Upon the arrival of an action potential at the bouton, the calcium and impulse frequency dependent exocytosis of these zinc filled vesicles produces a rapid transients of ionic Zn^{2+} in the extracellular fluid surrounding the boutons. These transients, or puffs, have rise times of a few milliseconds, and can reach apparent concentrations of 10–30 μM in the extracellular fluid [91,92]. Zinc puffs comparable to those seen during synaptic release can also be seen during exocytosis of a similar zinc rich secretory granule, the insulin containing beta pancreatic cell, and could presumably be seen during exocytosis of mast cell granules, salivary cell granules, and that of the many other cell types that have zinc filled secretory granules.

Approximately 10% of the total zinc in the brain is in synaptic vesicles [90,93]. It is sequestered in the presynaptic vesicles of a specialized type of neurons called 'zinc-containing' neurons, reviewed in [90]. In glutamatergic neurones, zinc is secreted with glutamate during neuronal activity, and is believed to act as a modulator of synaptic transmission [94,95]. After being released into the cleft it is recycled into the presynaptic terminus. Zinc also passes into postsynaptic neurons during synaptic events, perhaps functioning as a transmembrane neural signal.

The two pore, potassium channel, TASK-3, is inhibited by both zinc and copper in the extracellular medium; TREK-1, in contrast, is inhibited by zinc and activated by copper. These activities may reflect one of the functions of zinc and copper in the exocytotic vesicles of glutamatergic neurons [96].
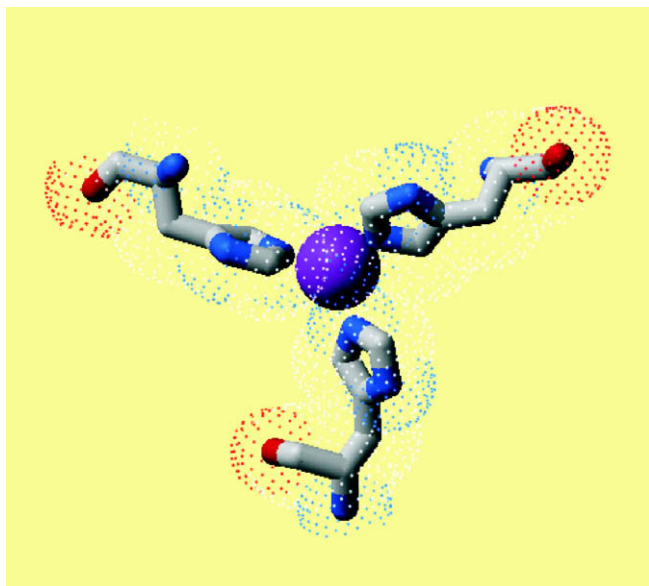
Fig. 3. Example of zinc coordination. A Zn^{2+} ion (dark) is coordinated by ε-nitrogens (dark) of His's 83, 179, and 243 in the extracellular domain of the ABC transport protein ZnuA (PDB file 1pq4) [76]; carbons are light. In this structure a water may occupy a fourth coordination site; however, it was not visible in the electron density. Note that in this structure the 2+ charge of the divalent is not balanced in the first coordinate sphere (Figure drawn with ICM Browser program).

The second zinc signal that has been observed and characterized in the brain occurs roughly simultaneously with the conventional zinc SYN signal. This signal is analogous to the transmembrane calcium signals, and consists of a transmembrane flux of Zn^{2+} ions from the extracellular milieu, through gated, zinc permeable channels into the somata [91] or dendrites of postsynaptic neurons. Because the presynaptic release of zinc is the only known source of appreciable amounts of extracellular zinc, this zinc TRANS signal is both a transynaptic and a transcellular signal. The temporal and spatial characteristics of these zinc TRANS sparks in neuronal cytosol have not been established, but the rise times can be within a few tens of milliseconds of the initial stimulation of the zinc containing presynaptic fiber system [91].

The third zinc signal is analogous to the intracellular calcium signal. In the case of calcium, the sarcoplasmic or smooth endoplasmic reticulum are established storage sites for intracellular release of the ion. The pumps and channels that mediate the calcium movement into and out of those depots are well characterized. In the case of zinc on the other hand, there is (as yet) no candidate organelle for the storage pools for intracellular release. The neuronal vesicle has no detectable zinc until it moves out of the Golgi apparatus and into the orthograde axoplasmic flow down axons, and no other zinc filled organelle is found in healthy neurons.

Zinc can also affect cellular signal recognition, second messenger metabolism, as well as protein kinase and protein phosphatase activities, reviewed in [82]. Zinc has been shown to affect the transduction pathways of the second messengers, cAMP and cGMP, by modulating phosphodiesterase activities. There have been several reports of both activating and inhibiting effects of zinc on different phosphodiesterase subfamilies [97–99]. It was found, that not only zinc modulates cGMP signaling, but that cGMP also modulates the uptake of zinc [82].

It can specifically modify the metabolism of cGMP and modulate the activities of protein kinase C. A regulatory function of zinc for protein kinase C is inferred from the observation that nanomolar concentrations of zinc can activate the enzyme and cause a translocation to the plasma membrane, a central event in the activation of protein kinase C [100,101]. Zinc seems to affect mitogenic signaling pathways evoked by growth factors [102]. Zinc interferes with different aspects of calcium regulation. In some cell types, elevation of extracellular zinc evoked intracellular Ca^{2+} mobilization [103].

Zinc is essential for cell proliferation and differentiation, especially for the regulation of DNA synthesis and mitosis. Zinc was shown to be a structural element in enzymes involved in DNA synthesis, transcription, aminoacyl tRNA synthesis and ribosomal function, reviewed in [82]. Furthermore, zinc is present in the zinc finger structures of transcription factors that control the activity of genes responding to growth factors [104]. It can stimulate or inhibit the activities of transcription factors, including MTF-1, which controls the transcription of the genes for metallothionein and the zinc transporter, ZnT-1 [105]. Metallothionein has been shown to play a role in the regulation of cell proliferation. The over expression of metallothionein in developing tissues and at the transition from fetal to newborn rat development suggests a role for metallothionein in differentiation, too.

Store operated calcium channels in the plasma membranes of salivary cells are inhibited by extracellular zinc binding at a site distinct from the channel [106]. In airway epithelial cells exposure to extracellular zinc causes an increase in $[Ca^{2+}]_{cyt}$ by its interaction with the P2X receptor channel [107]. Exposure of cerebro cortical cultures to peroxynitrite ($ONOO^-$) evokes the release of intracellular zinc, which in turn causes production of O_2^- and more $ONOO^-$; all leading to neuronal cell death [108]. "Zn^{2+}, applied at either the extracellular or intracellular side of the membrane is a potent, reversible activator of [SUR2A/Kir6.2] KATP channels."

[109]. However, neither the mechanism nor physiological relevance is known.

Zinc seems to play a regulatory role in the immune system by activating or inhibiting several signaling pathways that interact with the signal transduction of pathogen sensing receptors [110]. These sense pathogen-derived molecular structures and, upon activation, lead to secretion of pro-inflammatory cytokines. The interaction of zinc with protein tyrosine phosphatases and protein kinase C, and a direct modulation of lipopolysaccharide binding to its receptor (TLR-4) all result in enhanced cytokine production. At the same time, a complex interaction between zinc, NO and cyclic nucleotide signaling, inhibition of interleukin-1 receptor associated kinase-1, and inhibition of kappa B kinase all counteract the production of pro-inflammatory cytokines. By acting on all these signaling molecules, the zinc status of monocytes can have a direct effect on inflammation.

Zinc is a strong candidate for an apocrine messenger and appears to be involved in neurotransmission. Zinc ions can also be regarded as possible intracellular messengers [82]. As with calcium, $[Zn^{2+}]_{cyt}$ is strictly controlled and excess zinc is sequestered to cytoplasmic vesicles. The stimulus induced increases in $[Ca^{2+}]_{cyt}$ are short-lived transients of a few seconds; in contrast, changes in $[Zn^{2+}]_{cyt}$ are much slower and longer lasting than those of calcium. Zinc is viewed as an enduring component of proteins and an ion that is sometimes temporarily 'free'; whereas, calcium is typically viewed as a 'free' extracellular ion, that is generally bound in the intracellular milieu, (i.e., between signaling duties) in calcium-sequestering proteins that serve to 'send' calcium signals [90].

5.1.2. Magnesium

Magnesium is the preferred counter ion for phosphorylated compounds including adenosine triphosphate and other nucleic acids. As noted, this need for phosphate necessitated the extrusion of calcium [68]. It activates many enzymes that act on nucleic acids, such as restriction nucleases, ligases, and topoisomerases, and is essential for the fidelity of DNA replication. Both Mg^{2+} and Ca^{2+} ions increase the melting temperature of biological membranes by binding to the carboxylated or phosphorylated head groups of lipids [111].

Cromie et al. [112] reported an example of an RNA serving as sensor for cytoplasmic Mg^{2+}. The expression of the Mg^{2+} transporter, MgtA, of Salmonella typhimurium is controlled by its 5′untranslated region (5′UTR). Interestingly, the 5′UTR of the mgtA gene can adopt different stem-loop structures depending on the Mg^{2+} levels; this determines whether transcription reads through into the mgtA coding region or stops within the 5′UTR. The initiation of mgtA transcription responds to $[Mg^{2+}]_{ext}$; while its elongation into the coding region responds to $[Mg^{2+}]_{cyt}$ providing an example in which the same ligand is sensed in different cellular compartments to regulate disparate steps in gene transcription.

Magnesium binds to various EF-hands with about 10^{-4} the affinity of calcium [113] (Fig. 4). A change in $[Mg^{2+}]_{cyt}$ can affect the kinetics and apparent affinity of proteins for calcium [113]. Unlike Ca^{2+} and Zn^{2+} ion binding sites, only a few specific binding sequence motifs for the Mg^{2+} ion have been identified. These include –NADFDGD– observed in RNA polymerases, DNA Pol I, and HIV reverse transcriptase, and –YXDD– or –LXDD– found in reverse transcriptase and in telomerase [65].

Mg^{2+} ions compete for most of the calcium binding sites. As a rule, while the Ca^{2+}, and Mg^{2+} sites bind calcium very tightly ($K_d \sim 10^{-9}$–10^{-8} M), they bind magnesium 10^4–10^5 less strongly. Variation in $[Mg^{2+}]_{ext}$ can transitorily affect $[Ca^{2+}]_{cyt}$. Such variations have been correlated with cardiovascular abnormalities; however, no specific mechanism has been identified. Increase in $[Mg^{2+}]_{ext}$ from 0.3 to 3.0 mM inhibits both influx of calcium and

its release from intracellular stores in cultured smooth muscle cells; however, it remains to be established whether this reflects a physiological response [114].

Magnesium is the fourth most abundant cation in the human body, after Ca^{2+}, K^+, and Na^+, and the second most abundant cation, after K^+, in the cytosol, Table 1. The free Mg^{2+} ion is present in both cytosol and extra cytosolic spaces at 0.5–1.0 mM and represents ~5% of total cell magnesium. Homeostasis of $[Mg^{2+}]_{ext}$ is tightly regulated and depends on the balance between intestinal absorption and renal excretion. "No specific [extracellular] magnesium sensing mechanism has been identified." "Within the cell, total Mg^{2+} content is distributed almost homogeneously among nucleus, mitochondria and endo-(sarco)-plasmic reticulum." [115].

Of the total magnesium in the body approximately 52% is found in bone, 46% in skeletal muscle and 2% in extracellular fluids [116]. Serum magnesium levels, which amount to less than 1% of total magnesium, are controlled by the gastrointestinal tract and the kidneys. About 30–50% of dietary magnesium is absorbed in jejunum and ileum. Magnesium is essentially an intracellular ion: approximately 98% of non-skeletal magnesium in humans is located within the cell. It is the second most abundant intracellular divalent cation, exceeded only by potassium. The intra and extra cellular concentration gradient of Mg^{2+} in most cell types is small, as is the diffusion force for magnesium entry into cells. In excitable cells, $[Mg^{2+}]_{cyt}$ is several hundred times lower than expected if Mg^{2+} ions were at electrochemical equilibrium. In squid giant axons and barnacle muscle cells a $2Na^+$, $2K^+$, $2Cl^-$: $1Mg^{2+}$ exchanger is supposedly responsible for transporting Mg^{2+} ions across the plasmalemma and for maintaining $[Mg^{2+}]_{cyt}$ at steady-state conditions [117].

Genetic analyses of inborn errors of magnesium metabolism have revealed several new proteins as well as several previously known molecules that are involved in renal epithelial magnesium transport, e.g. claudin and paracellin-1. They are involved in creating charge-selective channels through the tight-junction barrier in the thick ascending limb of the distal convoluted tubule [118].

Intracellular magnesium concentration is precisely regulated within a narrow range in spite of a wide variation in external magnesium concentration. This implies the existence of a specialized Mg^{2+} transport system. Based on the structural and sequence similarities among individual transient receptor potential proteins, three subfamilies of ion channels are distinguished – the canonical TRPC; the vanilloid-like TRPV; and the melastatin-like TRPM. Most members of the TRPC and TRPV subfamilies have been characterized as calcium permeable channels. The TRPM6 channel displays strong outward rectification and has a five fold higher affinity for magnesium than for calcium [119]. TRPM6 is localized along the apical membrane of the renal distal convoluted tubule and the brush-border membrane of the small intestine, epithelia particularly associated with active (re)absorption of magnesium. In kidney, parvalbumin and calbindin-D28K are co-expressed with TRPM6 and might buffer intracellular magnesium levels [120] in addition to their generally accepted role in buffering intracellular calcium. TRPM7 facilitates magnesium entry into the cell and may be a regulator of magnesium homeostasis [121]. In contrast, other putative magnesium transporters apparently operate in the opposite direction [122].

In *Salmonella enterica* three transporters – the P-type ATPases, CorA, MgtA, and MgtB – mediate magnesium uptake [123]. Their expressions are induced in low magnesium. The magnesium level is regulated by the PhoP/PhoQ system and by CorA, whose transcription is regulated either by the levels of $[Mg^{2+}]_{cyt}$ or by the PhoP/PhoQ system [123,124]. The CorA transport system is expressed constituitively and is the major magnesium transporter in Eubacteria and Archaea. It has three transmembrane domains, a uniquely large periplasmic domain, and no significant sequence similarity to other known proteins [125]. In contrast "...potential homologs of the prokaryotic MgtE Mg^{2+} transporter are widespread in eukaryotes..." [126].

Within cells, magnesium plays a vital role in numerous regulatory processes. Magnesium activates many enzymes that act on nucleic acids, such as restriction nucleases, ligases, and topoisomerases, and is essential for the fidelity of DNA replication. When bound to ATP in the active site of an enzyme, not all of the normal six coordination positions for ligands are filled by interaction with either the protein or ATP. One or more waters remain coordinated with magnesium. The function of magnesium binding to the phosphoryl moieties of ATP in many cases appears to be activation of the phosphate ester toward hydrolysis [127,128]. Both Mg^{2+} and Ca^{2+} ions increase the melting temperature of biological membranes by binding to the carboxylated or phosphorylated head groups of lipids [111].

$[Mg^{2+}]_{cyt}$ can change in response to physiological stimuli; deoxygenation of human erythrocytes increases $[Mg^{2+}]_{cyt}$ due to the increased binding of free ATP and 2,3-diphosphoglycerate by
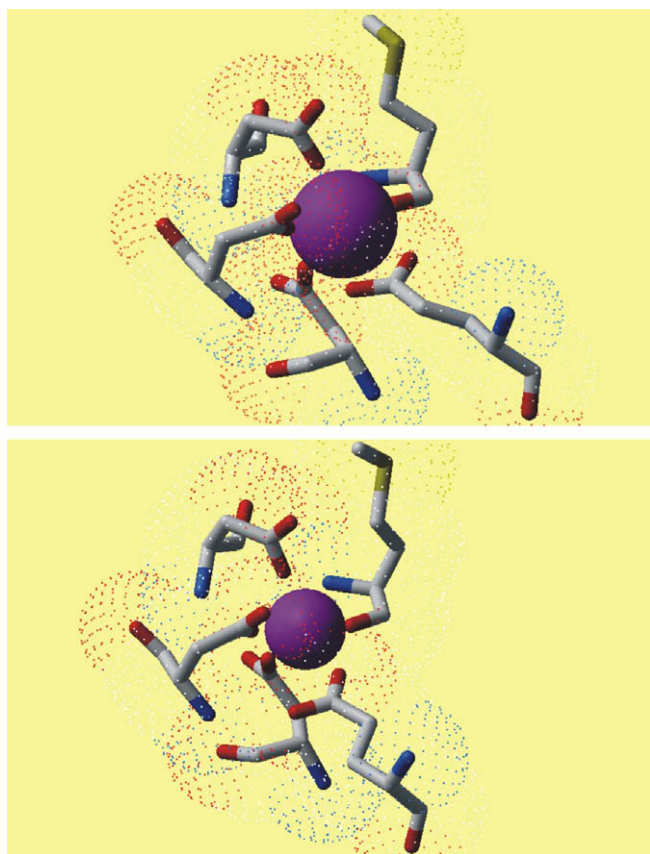
Fig. 4. Comparison of the coordinations of the Mg^{2+} and the Ca^{2+} ions. The crystal structures of dicalci- and dimagnesi-parvalbumin, from pike, were viewed in the same orientation. One sees the slight shift in the second (the EF) of the two Me^{2+} ion binding loops. The divalent ligands of the loop can be assigned to the vertices of an octahedron – X* Y* Z* –Y* –X* –Z. The X, Y, Z and –X ligands of a canonical EF-loop are side chains of Asp, Asn, or Ser. The –Y ligand is the carbonyl oxygen of the main chain; oxygens are dark and carbons are light. The –Z ligand is the carboxylate of Glu; when coordinating the Ca^{2+} ion it presents both oxygen atoms, making calcium seven coordinate. The two oxygens of Glu (α-carbon near the viewer, side chain pointing toward the divalent cation) are in the equatorial plane of a pentagonal bipyramid. The larger sphere (upper panel) represents the Ca^{2+} ion. In the resting or unstimulated state the EF-loops are (partially) occupied by the Mg^{2+} ion. It is six coordinate; the carboxylate of Glu (-Z) rotates slightly to present only one oxygen (lower panel). The pair of EF-hands is stabilized by many hydrophobic contacts between the two domains. Most crystal structures of EF-hand proteins are of the calci-forms because the apo- or magnesi-form probably are more flexible and less readily crystallized. A water at vertex –X provides the seventh ligand for the Ca^{2+} ion and the sixth for the Mg^{2+} ion (figure drawn with ICM Browser program).

deoxyhemoglobin [129]. There are several reports on the inhibitory effects of $[Mg^{2+}]_{cyt}$ of K^+-channels. For example, $[Mg^{2+}]_{cyt}$ dose- and voltage-dependently blocks the outward K^+ current through the channel and induces a dose-dependent increase in the channel activation in guinea pig atrial cells [130]. Magnesium can modulate Ca^{2+} release from sarcoplasmic reticulum of canine cardiac cells [131]. In rat pancreatic acinar cells elevated magnesium concentration significantly reduces the secretory responses and Ca^{2+} mobilization [132]. Hormonally-controlled systems could affect the function of ion channels and second messenger systems in cells. In murine S49 lymphoma cells a highly selective Mg^{2+} uptake mechanism is under hormonal control [133]. A rise in intracellular magnesium leads to activation of several Mg^{2+}-dependent enzymes. These changes result in phosphorylation of proteins and production or activation of various transcription factors required for cell activation. These results show that Mg^{2+} might function as a modulator of the mobilization of Ca^{2+}, which in turn mediates enzyme secretion.

At the same time, it seems unlikely that magnesium acts as a trigger for functioning of enzyme systems as does calcium. However, many experimental facts suggest that Mg^{2+} does play a role in cellular homeostasis, reviewed in [134].

6. Conclusions

The calcium sensing receptor certainly transduces the information of changes in $[Ca^{2+}]_{ext}$ to physiological responses in the cytosol via a system of G proteins. Several of these responses involve more than calcium homeostasis; calcium does seem to function as an extracellular messenger [42].

There is inferential evidence of calcium's involvement in signaling in bacteria; however, Sutherland's four criteria have yet to be met for a single system.

Many targets and functions of non-calcium biometals have been studied. Their channels and transporters are well characterized. The concentrations of these biometals are tightly regulated. Perturbations in their concentrations correlate with (patho) physiological responses, many of which might involve modulation of calcium signaling. However, their concentrations, cytosolic or extracellular, have yet to be demonstrated to vary in a physiologically meaningful way.

Zinc may well function as a paracrine modulator or messenger in the nervous system. Meaningful changes in concentrations of $[Mg^{2+}]$ might modulate the effects of calcium functioning as a secondary messenger.

7. Abbreviations

3′,5′-AMP	3′,5′-cyclic-adenosine monophosphate
ABC	ATP-binding cassette transporter
ASIC	acid sensing ion channels
CaBP	calcium binding protein
CaSR	calcium sensing receptor
CaV	T-type low voltage calcium channels
CDF	cation diffusion facilitator
ERK	extracellular signal-regulated kinase
LB	ligand binding
mGluR	metabotropic glutamate receptor
P2X	ATP-gated cation channel
RND	resistance-nodulation-cell division transporter
TASK	TWIK (tandem pore domain weak inward rectifying K^+ channel)-related acid-sensitive K^+ channel
TREK	TWIK-related K^+ channel
TRPC	transient receptor potential channel
ZIP	Zrt-, and Irt-like protein

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