Why Calcium? How Calcium Became the Best Communicator*

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Calcium, the third most abundant metal in nature, was amply available to cells from the beginning, and was adopted as a regulator at an early evolutionary stage. The basic principles of calcium regulation were already present in prokaryotes and protists (3, 4), but calcium regulation gradually grew to cover nearly all aspects of cell function after the transition to multicellularity. Naturally, agents that carry messages to intracellular targets must be maintained within cells at very low basal levels, to prevent prohibitive energy expenses to modulate their concentration as demanded by the signaling function. The concentration of complex signaling molecules is regulated by biosynthesis and degradation. The means chosen by evolution to regulate calcium within cells was its reversible binding to specifically developed molecules. Here, calcium had a decisive advantage over the other metals present in the primordial ambient, chiefly over magnesium, which was far more abundant in the seawater where life had begun (5).

Chemical Properties of Calcium

The advantage of calcium was its peculiar coordination chemistry. The interaction of metals, including calcium, with coordinating ligands is determined by a number of properties: 1) the valency (i.e. the charge of the metal); 2) the ionic radius; 3) the so called polarizability, which defines the aptitude of the metal electron cloud to be distorted by external electrical forces; 4) the hydration energy (i.e. the ease with which the water molecules are stripped off the metal); and 5) the radius of the hydrated metal ion, which determines the charge density. Taken together, these properties explain why calcium is readily accepted by sites of irregular geometry, such as those offered by the complex molecules (proteins) developed by evolution (6, 7), and why these sites would not, for instance, accommodate magnesium (Fig. 1). The coordination flexibility of calcium (the coordination number is usually 6–8, but up to 12 is possible) and its variable bond length and angle are at sharp variance with those of magnesium, which, because of its smaller size (0.65 Å as compared with 0.99 Å) and much lower polarizability, requires a fixed octahedral geometry with six coordinating ligands and minimal bond length variability (Fig. 1). In sites offered by proteins, calcium ligation usually occurs via carboxylates (monodentate) or neutral oxygen (oxygen is the preferred ligand). In these sites, calcium is generally bound by seven oxygen atoms in a conformation that is best represented by a pentagonal bipyramid.

The evolutionary choice of calcium as a carrier of signals thus exploited the ease of decreasing its concentration within cells, which in most cytosols oscillates in the low-to-mid nm range. Here, a point of general importance becomes obvious. The lowering of the cellular calcium concentration, in addition to being required for the efficient signaling function, was an evolution-
ary necessity, dictated by the early choice of phosphate as the energy currency of life. Calcium phosphate salts, unlike those of magnesium, have poor solubility, and would have made phosphate-based bioenergetics impossible in the presence of mM calcium concentrations within cells.

Calcium in Tissues and within Cells

In animal fluids and tissues, the concentration of calcium varies between 2.1 and 2.6 mM (6), subdivided in three forms: ionized, complexed to organic compounds, and bound to small molecular weight inorganic molecules. The equilibrium among these three forms in the fluids and in the extracellular spaces may vary somewhat, but the proportion of ionized calcium (Ca^{2+}) is generally not distant from that of complexed calcium. Total calcium concentration is also mM inside cells; however, in the cytosol of most cells, the concentration of free calcium is about 10,000-fold lower. Also, within cells, calcium is complexed by inorganic compounds and low molecular weight organic molecules. However, they normally bind calcium with low affinity, and cannot lower its free concentration to the mM range, which is needed for Ca^{2+} to efficiently perform its signaling function. The achievement of the mM concentration demands the ligation of calcium by specific proteins that contain sites with the necessary affinity and specificity for Ca^{2+} (see below). They belong to two broad classes. The first class consists of proteins that are soluble in the cytoplasm, sequestered inside cellular organelles, or organized in insoluble non-membranous structures such as the cytoskeleton. They buffer Ca^{2+} to the mM range without modifying its total content in the cells. Also, a number of these proteins, in addition to buffering Ca^{2+}, perform an additional task: they process its information. This is a very important concept, which deserves an extended comment. Ca^{2+} can deliver the information to targets directly, as enzymes may contain sites that bind it. As these sites bind Ca^{2+}, the activity of the enzyme increases or (more rarely) decreases (Ca^{2+} is not an active site metal; it is the allosteric metal par excellence). However, in the majority of cases, the Ca^{2+} message is not transmitted to targets directly. Prior to this, it must be decoded by Ca^{2+} “sensor” proteins that bind it and conserve its information in the form of a conformational change that is then transmitted to the targets to which they bind. There are also cases that could be considered as intermediate, i.e. some target enzymes incorporate the sequence of a Ca^{2+} sensor protein within their structure. The second class of proteins that control cell Ca^{2+} is intrinsic to membranes, and transports Ca^{2+} in or out of cells, or between the cytosol and the lumen of the organelles. When the traffic of Ca^{2+} occurs across the plasma membrane, the ion obviously modifies its total cellular content.
Regulating Calcium Concentration

Calcium Binding and Sensor Proteins

The EF-hand

In 1973, Kretsinger and Nockolds (8) published the crystal structure of parvalbumin, a Ca\(^{2+}\)-binding protein that contained the structural features that later became known as the EF-hand motif (9). In this helix-loop-helix motif, a sequential arrangement of about 30 amino acids ligates Ca\(^{2+}\) with high affinity in a loop flanked by two helical segments. Parvalbumin contains three EF-hands, with a total of six α helices, A through F. The spatial orientation of the fifth (E) and sixth (F) α helix, which enclose the Ca\(^{2+}\)-coordinating loop, resembles a hand, as shown in Fig. 2, top (10). The EF-hand domain was later shown to be highly conserved in cellular Ca\(^{2+}\)-binding proteins, which contain a variable number of EF-hand motifs. The family is steadily increasing, and presently includes more than 800 proteins, with more than 100 three-dimensional structures solved. However, a precise function has not been assigned to most of them (11).

Evolution of the EF-hand—Ca\(^{2+}\)-binding proteins were already developed in prokaryotic organisms (12, 13). The analyses of prokaryotic genomes have shown the presence of proteins with EF-hands and other Ca\(^{2+}\)-binding motifs (13–15). The EF-hand protein family is likely to have arisen from a founder protein with a single EF-hand from which proteins with multiple EF-hands were then derived by gene duplication (16). Important EF-hand proteins are Ca\(^{2+}\) sensors such as calmodulin, troponin C, recoverin, S-100, and STIM\(^{2}\)

\(^{2}\)The abbreviations used are: STIM, stromal interaction molecule; CaM, calmodulin; ER, endoplasmic reticulum; SR, sarcoplasmic reticulum; SOCE, store-operated calcium entry; RyR, ryanodine receptor; PMCA, plasma membrane Ca\(^{2+}\) ATPase; NCX, Na/Ca exchanger; MCU, mitochondrial Ca\(^{2+}\) uniporter; InsP\(_3\), inositol trisphosphate.

FIGURE 2. Top, the EF-hand-binding motif, proposed by Kretsinger et al. (10) from the crystal structure of parvalbumin, can be represented by the forefinger (helix E) and the thumb (helix F), enclosing the Ca\(^{2+}\)-binding loop, represented by the bent middle finger. Adapted from Ref. 10. Bottom left, C2b motif of synaptotagmin I (Protein Data Bank (PDB) file 1TJX). Bottom right, full-length annexin A1 (PDB file 1MCX), with repeats 1–4 in red, yellow, purple, and green, respectively. The calcium ions are depicted as orange spheres, and the residues involved in its coordination are shown as sticks. Adapted from Ref. 7.
(stromal interaction molecule). They all bind Ca\(^{2+}\) and process its signal. Other EF-hand proteins, *e.g.* parvalbumin, calbindin and calretinin, are instead pure Ca\(^{2+}\)-buffering proteins that maintain the appropriate Ca\(^{2+}\) homeostasis. Thus, for instance, the concentration of parvalbumin in mammalian muscles correlates well with their relaxation speed (17). EF-hands can even serve as internal regulators of the activity of proteins. This is for instance the case of the ryanodine receptor (see below) in which EF-hands mediate the gating of its Ca\(^{2+}\) channel (18). Most of these proteins contain an even number of EF-hands (between 2 and 12), usually as tandem copies related by a two-fold symmetry axis. A different EF-hand family has also been described that contains an uneven number of EF-hands, usually five (hence the name penta-EF or PEF family (19)). Recently solved structures, *e.g.* that of the protease calpain (20), have indicated that the functional motifs of these proteins occur in pairs, either by homodimerization or by heterodimerization, whereas the non-functional fifth EF-hand acts as a dimerization domain.

**Calmodulin**—Calmodulin (CaM) is the most conserved EF-hand protein. Together with histones, actin, or ubiquitin, it is one of the most conserved proteins known to date (21). It is ubiquitously expressed in all eukaryotic organisms, the sequences in all vertebrates being 100% identical. It transmits the Ca\(^{2+}\) message to a large number of cellular functions. In humans it is encoded by three non-allelic genes; even if they encode an identical protein, the coding sequences differ substantially (22). The CaM homologue troponin C differs instead in sequence depending on whether it is of skeletal or cardiac muscle origin (23).

The structure of CaM has been solved for both the Ca\(^{2+}\)-bound and the Ca\(^{2+}\)-free form (for a recent review, see Ref. 24). The structure shows a dumbbell shape, with two terminal lobes connected by a long flexible helix. The EF-hands are located in pairs in the lobes. The binding of Ca\(^{2+}\) induces a conformational change that exposes hydrophobic patches on the surface of the protein, conferring to it the ability to interact with targets. Structures of Ca\(^{2+}\)-loaded CaM complexed to binding peptides from various targets have been solved by NMR and by x-ray crystallography (24). The binding to target domains induces a large conformational change of CaM, which tends to a variable extent from the extended dumbbell shape into a more globular structure in which its two halves wrap around the target domain (24).

**STIM**—STIM1 (stromal interaction molecule-1) is a protein of the endoplasmic reticulum (ER) membrane that detects the Ca\(^{2+}\) level of the lumen. As it decreases, a conformational change of STIM (25) promotes its interaction with, and the gating of, the channel-forming plasma membrane Orai protein (26) at specialized junctions between the ER and the plasma membrane (the SOCE channel mechanism (27); see below). Remarkably, STIM senses Ca\(^{2+}\) in the very high concentration of the ER lumen, *i.e.* with an affinity that is 3 orders of magnitude lower than that of CaM and other EF-hand proteins. The EF-hand pair of STIM is thus unusual, as it is composed of a canonical and of a non-canonical EF-hand, in which amino acids critical to Ca\(^{2+}\) binding are replaced (24). The SOCE system was developed early in evolution, as homologs of the STIM and Orai proteins have been found in the genome of unicellular organisms (28).

**S100 Proteins**—S100 proteins are a family of EF-hand-containing proteins that are soluble in 100% ammonium sulfate, hence their name (29). To date 21 S100 proteins have been identified in humans (30). They are small, and usually contain two EF-hands. The C-terminal one is canonical, whereas the N-terminal one is a pseudo-EF-hand, in which a 14-residue loop coordinates Ca\(^{2+}\) with low affinity (31). These proteins also bind other divalent metal ions such as zinc and copper, and usually occur as homodimers. They regulate numerous cellular targets, *e.g.* transcription, the cell cycle, cell growth, motility, differentiation. Peculiarly, some of them also interact with targets, *e.g.* RAGE (receptor for advanced glycosylation end products), outside the cell. It is not clear whether they are actively secreted or passively released by them. Dysregulation in the expression of a number of S100 proteins leads to different disease phenotypes, including cancer (30).

**Other Calcium-binding Motifs in Proteins**

The **C2-domain**—C2-domains of proteins interact with phospholipids of different membranes, mostly in a Ca\(^{2+}\)-dependent manner. These domains, first identified in protein kinase C, have now been identified in more than 100 proteins. They consist of 120–130 amino acids, organized as a β-sandwich of two four-stranded β-sheets connected by three loops. Ca\(^{2+}\) is coordinated at the loops that connect β-sheets 2–3 and 6–7 at the edge of the β-sandwich by carbonyl and mono- and bidentate aspartate side chain oxygens, and a water oxygen (Fig. 2, bottom left). Most of the proteins that contain C2-domains are involved in either signal transduction pathways or membrane traffic (32). Among the best studied C2 proteins is synaptotagmin, a transmembrane protein that contains two such domains. It acts in synaptic vesicles as the Ca\(^{2+}\) sensor in the process of exocytosis and a fast, synchronous neurotransmitter release (33, 34). Recently, a different C2-domain-containing protein, Doc2, was found in which isoforms α and β function as Ca\(^{2+}\) sensors for the slow phase of asynchronous neurotransmitter release (34). At variance with the Ca\(^{2+}\) binding to EF-hand sites, which causes a conformational change, Ca\(^{2+}\) binding to C2-domains only leads to the structural stabilization of the protein.

**The Annexin Ca\(^{2+}\)-binding Fold**—Annexins are a broad family (more than 200 members are now known) of Ca\(^{2+}\)-dependent phospholipid-binding proteins that bind to membranes in a Ca\(^{2+}\)-dependent manner (35). They act inside cells, but some (A1, A2, A5) are found outside cells. They are involved in numerous cell processes, among them the inhibition of phospholipases, endo- and exocytosis, Ca\(^{2+}\) channel formation, and anchoring of proteins to the plasma membrane. Their name reflects the role in bringing together different cellular structures. Outside cells, they have a role in blood coagulation and fibrinolysis. All annexins consist of a divergent N-terminal head domain and a conserved C-terminal core domain (35). The core domain nearly always contains four repeat units consisting of five α helices A–E, which are separated by intervening sequences of variable length, and in which helices A, B, D, and E form a coiled-coil curved disk with loops connecting helices
A-B and D-E on its convex side. The loops bind Ca\(^{2+}\), whereas the N-terminal domain is on the concave side of the disk (Fig. 2, bottom right). Annexins contain three types of Ca\(^{2+}\)-binding sites, formed by the AB, DE, and AB’ loops. Up to 12 Ca\(^{2+}\)-binding sites may exist along the membrane-binding surface of annexins. A number of annexinopathies have been described, ranging from diabetes, to different forms of heart failure, to leukemia (36). Annexin A2 complexed to protein S100A11 can be recruited by a system for the repair of the damage to the plasma membrane caused by cancer cells induced by the stress of navigating through dense extracellular matrix (37).

**Membrane-intrinsic Calcium-transporting Proteins**

The buffering of cell Ca\(^{2+}\) to the nM level is the task of proteins that transport Ca\(^{2+}\) across membranes. The channels allow the downhill passage of Ca\(^{2+}\) across the plasma membrane and the membranes of organelles. ATPases transport Ca\(^{2+}\) with high affinity, and exchangers transport it with lower affinity in exchange for another ion (normally Na\(^{+}\)). Mitochondria take up Ca\(^{2+}\) with an electrophoretic uniporter and release it with a variant of the Na\(^{+}\)/Ca\(^{2+}\) exchanger. The properties of all these systems have been extensively covered in numerous reviews (e.g. see Refs. 6, 7, and 38). This review will mention striking new developments, but will consider in more detail points of special interest to Ca\(^{2+}\) signaling in general. The Ca\(^{2+}\) channels of the plasma membrane are regulated by different mechanisms: 1) the interaction with ligands (e.g. glutamate in neurons); 2) the emptying of the cellular Ca\(^{2+}\) stores (the SOCE channels mentioned above (27), in which the STIM proteins sense the Ca\(^{2+}\) depletion in the ER to reach and gate the plasma membrane channel-forming Orai protein); 3) the changes in the membrane electrical potential (the voltage-gated Ca\(_\text{v}\) channels), which exist in several subfamilies; and 4) the interaction with environmental signals, e.g. temperature, pH, and odorants (the five families of transient receptor potential (TRP) channels, which are variously permeable to Ca\(^{2+}\), and which have also been located in intracellular membranes). The plasma membrane Ca\(^{2+}\) channels are selectively distributed in tissues, as their gating properties and responses to agonists/antagonists respond to the distinct needs of cells. For instance, the SOCE channels, although also present in excitable cells such as the skeletal muscle, are the major Ca\(^{2+}\) influx system in non-excitable cells. A very recent striking finding has been the solution of the three-dimensional structure of the voltage-gated Ca\(^{2+}\) channel of skeletal muscle cells (39), which is a complex of a pore-forming subunit \(\alpha\) and of auxiliary regulatory subunits \(\alpha_2\delta\beta\gamma\) (Fig. 3A).

The membranes of ER/SR (sarcoplasmic reticulum) contain the most important intracellular Ca\(^{2+}\) channels. Ca\(^{2+}\) channels have also been described, or hypothesized, in other cellular organelles, but they are less well characterized. The ER channel is gated by the second messenger InsP\(_3\) (40), and the channel in the SR is gated by Ca\(^{2+}\) itself (Ca\(^{2+}\), however, plays a role in the gating of the InsP\(_3\) channel as well). The SR channel, routinely called the ryanodine receptor (RyR) as it is inhibited by the alkaloid ryanodine, is critical in the control of cytosolic Ca\(^{2+}\) during the excitation-contraction cycle of muscles. It is a gigantic tetrameric molecule of four monomers of more than 5000 residues, which is regulated by numerous mechanisms. Recent cryo-EM work has led to the solution of the three-dimensional structure of the RyR (18, 41, 42) (Fig. 3B) and the InsP\(_3\) receptor (InsP\(_3\)R) (40). These spectacular achievements have greatly advanced the molecular understanding of the movement of Ca\(^{2+}\) across membranes. Ca\(^{2+}\) is removed from the cytosol to the external space or to the lumen of organelles by different systems. Ca\(^{2+}\) ATPases (Ca\(^{2+}\) pumps) are located in the plasma membrane (PMCA pump), in the membrane of the ER/SR (sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) pump), and in the Golgi network (secretory pathway Ca\(^{2+}\) ATPase (SPCA) pump). The SERCA pump is the most powerful system for the clearing of Ca\(^{2+}\) from the cytosol of all eukaryotic cells. It coexists in animal tissues with the PMCA pump, which is expressed only in very low amounts. The concept has thus recently emerged (43, 44) that in most tissues the main role of the PMCA is not the global control of cytosolic Ca\(^{2+}\), but the control of the Ca\(^{2+}\) signaling in selected sub-plasma membrane domains where important Ca\(^{2+}\)-regulated enzymes also reside. The PMCA pump is a target of CaM. In the presence of Ca\(^{2+}\), CaM becomes bound to the pump, making it active. As result, Ca\(^{2+}\) in the environment decreases, promoting the detachment of CaM. Thus, the activation of the pump by CaM must necessarily have oscillatory character (44).

The other plasma membrane Ca\(^{2+}\)-ejecting system is the Na/Ca exchanger (Ncx). It is particularly active in excitable cells and complements the action of the ATPases, as it has high transport capacity, but low Ca\(^{2+}\) affinity. It thus cannot lower Ca\(^{2+}\) to the nM level of resting cells. It operates electrogenically, exchanging three Na\(^{+}\) ions for one Ca\(^{2+}\) ion, and thus responds to both the transmembrane potential and the concentrations of Ca\(^{2+}\) and Na\(^{+}\) inside and outside cells. Thus, it can also operate in the reverse mode, bringing Ca\(^{2+}\) into the cell.

The mitochondrial Ca\(^{2+}\)-handling system and its role in the handling of cytosolic Ca\(^{2+}\) have had a peculiar history. The poor Ca\(^{2+}\) affinity of mitochondria (apparent \(K_m\) values in excess of 5–10 \(\mu\)M) had been measured soon after the discovery of the process (45, 46) and had ruled them out as efficient controllers of cytosolic Ca\(^{2+}\). Because early work had discovered that three tricarboxylic acid (TCA) cycle matrix dehydrogenases were exquisitely sensitive to Ca\(^{2+}\) (47), for a long time it was tacitly accepted that the role of mitochondria was to control their own Ca\(^{2+}\), not Ca\(^{2+}\) in the cytosol. Even if work in rats injected with radioactive Ca\(^{2+}\) had shown that the energy-linked uptake process did somehow occur *in vivo* (48), mitochondria were brought back as important controllers of cytosolic Ca\(^{2+}\) only in the 1990s, by the discovery that they sense micropools of high Ca\(^{2+}\) concentration created in their vicinity by the InsP\(_3\)-mediated opening of the Ca\(^{2+}\) store of adjacent ER (49, 50). Very recent discoveries have shown that Ca\(^{2+}\) penetrates into mitochondria through a channel (51) and have identified the components of both the uptake and the release legs of the Ca\(^{2+}\)-transporting system. The uptake leg has special complexity, as it consists of the transmembrane uniporter channel (MCU) (52, 53), aided by a number of accessory membrane extrinsic proteins (mitochondrial calcium uptake proteins (MICUs)) (54, 55) (Fig. 4). The release leg, which had originally been discovered as a Na\(^+\)-promoted path that, unlike the MINIREVIEW: How Calcium Became the Best Communicator
plasma membrane NCX, also accepted Li$^+$ in exchange for Ca$^{2+}$ (56), is a variant of the plasma membrane NCX (NCMX), which, indeed, also accepts Li as a Ca$^{2+}$ exchange partner (57). (Some cell types contain a mitochondrial Ca$^{2+}$/H$^+$ mitochondrial exchanger (58).) The multitude of Ca$^{2+}$-transporting systems, coupled to the vast array of buffering and sensor proteins, underlines the vital importance of the precise control of the Ca$^{2+}$ signal, which is reflected in the large list of enzymes and other cell processes that are controlled by Ca$^{2+}$ (Table 1).

**Ambivalence and Other Distinctive Properties of the Ca$^{2+}$ Signal**

The canonical way of transmitting messages to cells involves the interaction of “first messengers” with plasma membrane receptors. The interaction activates the production of diffusible “second messengers” that convey the information to cellular targets. Ca$^{2+}$ is one of these diffusible second messengers. However (see above), Ca$^{2+}$ can also penetrate directly into cells to carry signals to cellular targets, bypassing the interaction of first messengers with plasma membrane receptors. However, it can also be a real first messenger, as in a growing number of cells in which it interacts with a canonical plasma membrane ryanodine receptor (RyR1) in complex with the modulator immunophilin FKBP12. The nine cytosolic domains of the receptor are shown in different colors; FKBP12 is in red. Atomic coordinates in the Protein Data Bank: accession code 3J8H (from Ref. 42).

Figure 3. A, cryo-EM structure of the rabbit voltage-gated skeletal muscle Ca$^{2+}$ channel Ca$_{1.1}$ complex. Left, overall EM density map; right, structure. The pore-forming subunit a1, and the auxiliary subunits a2dm b and g, are shown in different colors. Atomic coordinates are in the PDB (accession code 3JBR) (from Ref. 39). VWA, von Willebrand factor type A domain; CTD, C-terminal domain. B, overall cryo-EM structure and domain organization of the rabbit skeletal muscle ryanodine receptor (RyR1) in complex with the modulator immunophilin FKBP12. The nine cytosolic domains of the receptor are shown in different colors; FKBP12 is in red. Atomic coordinates in the Protein Data Bank: accession code 3J8H (from Ref. 42).
controlled way. For instance, Ca\textsuperscript{2+} demands, but they must occur in a spatially and temporally controlled way. For instance, Ca\textsuperscript{2+} can be delivered to targets in the form of high concentration oscillatory transients spatially confined to specific cellular (micro) domains. These deviations are normal means to deliver the Ca\textsuperscript{2+} signal. The ambivalence of the signal is instead defined as the abnormal, uncontrolled deviation of Ca\textsuperscript{2+} from its normal homeostatic level, including, albeit less frequently, in the direction of its persistent unplanned decrease. Cell life became evolutionarily organized on a given, tightly controlled homeostasis level of Ca\textsuperscript{2+}. However, having decided to use Ca\textsuperscript{2+} as a determinant for function, cells have at the same time accepted to live in a permanent state of controlled risk. Given the unlimited availability of Ca\textsuperscript{2+} in the environment, and the enormous inwardly directed Ca\textsuperscript{2+} pressure, the risk of large increases of Ca\textsuperscript{2+} entry by the damage to the plasma membrane is a distinct possibility; the unwanted persistent activation of proteases, phospholipases, and nucleases would then terminate cell life. For a while, cells can delay the onset of the catastrophe by storing excess Ca\textsuperscript{2+} within the mitochondria, which take it up together with phosphate and store it in the matrix as osmotically inactive hydroxyapatite deposits. However, this defense only provides cells with the time necessary to survive brief Ca\textsuperscript{2+} storms. If the membrane toxicant does not rapidly disappear, doom unavoidably follows. Importantly, this toxic Ca\textsuperscript{2+} death is completely different from the death of cells in the process of apoptosis, which instead is but one of the positive ways in which cells use the Ca\textsuperscript{2+} signal. Apoptosis still culminates in the death of cells, but is an essential phenomenon, as it mediates processes such as tissue renewal and organ modeling. It has been calculated that in human adults, about 1.2 kg of cells are actually renewed each day in the process of apoptosis (6). However, the Ca\textsuperscript{2+} signal can also be deranged in subtler ways that concern defects of individual actors in the Ca\textsuperscript{2+}-controlling protein machinery. These defects do not lead to cell death, but induce phenotypes of cell discomfort that can be of very long duration, and very serious. They are most frequently of genetic origin, and have now become an important chapter of the Ca\textsuperscript{2+} signaling topic (60, 61).

**Back to the Title: How (and Why) Did Calcium Become the Best Communicator?**

In summary, the sequence of evolutionary events that have led to the unique signaling role of Ca\textsuperscript{2+} is likely to have been the following. 1) Even in the monacellular stage, it was vital to lower cell Ca\textsuperscript{2+} very significantly if phosphate was to be used as the energetics currency. 2) The chemical properties of Ca\textsuperscript{2+} had uniquely permitted the lowering of its free concentration in cells to levels that would avoid the precipitation of phosphate salts. 3) Once means were developed to constantly maintain Ca\textsuperscript{2+} at very low background levels, cells had acquired an ideal signaling agent. 4) When competition (unicellularity) was replaced by cooperation (multicellularity), Ca\textsuperscript{2+} was the logical choice to begin exchanging signals, as the cellular ambient already had systems to control it. As the complexity of signal transduction increased, additional systems to control Ca\textsuperscript{2+} were then developed.

**References**

1. El Albani, A., Bengtson, S., Canfield, D. E., Bekker, S., Macchiarelli, R., Mazurier, A., Hammarlund, E. U., Boulvais, P., Dupuis, J.-J., Fontaine, C., Fürsich, F. T., Gauthier-Lafaye, F., Janvier, P., Javaux, E., Ossa, F. O., et al. (2010), Large colonial organisms with coordinated growth in oxygenated environments 2.1 Gyr ago. Nature 466, 100–104

**TABLE 1**

A selection of important functions modulated by the calcium signal in eukaryotic cells

| Function Type                       | Examples |
|------------------------------------|----------|
| **Metabolism: generation of fuels**| Glycogenolysis (phosphorylase b kinase) |
|                                    | α-Glycerophosphate dehydrogenase |
|                                    | Pyruvate dehydrogenase phosphate phosphatase |
|                                    | NAD-dependent isocitric dehydrogenase |
|                                    | α-Ketoglutarate dehydrogenase |
|                                    | NADH dehydrogenase (plant mitochondria) |
|                                    | β-Hydroxybutyrate dehydrogenase |
|                                    | Lipases and phospholipases |
| **Membrane-linked functions**      | Excitation-contraction coupling |
|                                    | Excitation-secretion coupling (e.g. neurotransmitter release) |
|                                    | Action potentials |
|                                    | Tight junctions |
|                                    | Cell contact |
|                                    | Calcium-transporting ATPases |
|                                    | Channels in the plasma membrane and organelles |
|                                    | Plasma membrane-vesicles fusion |
| **Hormonal regulation**            | Formation/degradation of cyclic AMP and GMP |
|                                    | Release of several hormones |
| **Contractile and motile systems** | Muscle myofibrils |
|                                    | Cilia and flagella |
|                                    | Microtubules and microfilaments |
|                                    | Cytoplasmic streaming |
|                                    | Pseudopod formation |
| **Miscellaneous functions**        | Fertilization |
|                                    | Bone metabolism |
|                                    | Proteases |
|                                    | Protein kinases |
|                                    | Protein phosphatase (calcineurin) |
|                                    | Production of messengers (e.g. NO) |
|                                    | Gene expression |
|                                    | Neurogenesis and memory |
|                                    | Vision |
|                                    | Apoptosis |

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![A graphic showing a general view of the mitochondrial MCU complex. The channel-forming MCU is a tetrameric 40 KDa protein (a 33-KDa MCUb protein incorporates into the tetrameric MCU channel, reducing its activity). Accessory proteins (MICUs, MCU receptor (MCUR), and essential MCU regulator (EMRE)) variously associated with the mitochondrial inner membrane regulate the gating and the activity of the MCU channel. Adapted from Ref. 54.](Image49x515 to 299x733)
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2. Strother, P. K., Battison, L., Brasier, M. D., and Wellman, C. H. (2011) Earth’s earliest non-marine eukaryotes. *Nature* 473, 505–509

3. Cai, X., Wang, X., Patel, S., and Clapham, D. E. (2015) Insights into the early evolution of animal calcium signaling machinery: a unicellular point of view. *Cell Calcium* 57, 166–173

4. Plattner, H., and Verkrhatsky, A. (2015) The ancient roots of calcium signaling evolutionary tree. *Cell Calcium* 57, 123–132

5. Williams, R. J. P. (1999) Calcium: the developing role of its chemistry in biological evolution. In *Calcium as a Cellular Regulator* (Carafoli, E., and Klee, C., eds), pp. 3–27, Oxford University Press, New York

6. Brini, M., Cali, T., Ottolini, D., and Carafoli, E. (2013) Intracellular calcium homeostasis and signaling. In *Metallomics and the Cell, Metal Ions in Life Sciences* (Banci, L., ed), Vol. 12, pp. 119–168, Springer, Dordrecht

7. Brini, M., Cali, T., Ottolini, D., and Carafoli, E. (2013b) Calcium in health and disease. In *Interrelationships between Metal Ions and Human Disease*. *Metal Ions in Life Sciences* (Sigel A., Sigel, H., and Sigel, R. K. O., eds), Vol. 13, pp. 81–137, Springer, Dordrecht

8. Krebs, J. W. (1986) A model for receptor-regulated calcium entry. *Cell Calcium* 7, 1–12

9. Putney, J. W. (1986) A model for receptor-regulated calcium entry. *Cell Calcium* 7, 1–12

10. Collins, S. R., and Meyer, T. (2011) Evolutionary origins of STIM1 and STIM2 within ancient Ca2+ signaling systems. *Trends Cell Biol.* 21, 202–211

11. Moore, B. W. (1965) A soluble protein characteristic of the nervous system. *Biochim. Biophys. Res. Commun.* 19, 739–744

12. Bresnick, A. R., Weber, D. J., and Zimmer, D. B. (2015) S100 proteins in cancer. *Nat. Rev. Cancer* 15, 96–109

13. Zhou, Y., Yang, W., Kirberger, M., Lee, H. W., Ayalasomayajula, G., and Verkrhatsky, A. (2007) Evolution of calcium homeostasis: from birth of Earth’s earliest non-marine eukaryotes. *Nature* 451, 17055–17062

14. Rigden, D. J., Jedrzejas, M. J., Moroz, O. V., and Galperin, M. Y. (2003) EF-hands? Structural diversity of calcium-binding proteins in bacteria: single-handed EF-hands? *Trends Microbiol.* 11, 295–297

15. Zhou, Y., Wang, W., Kirberger, M., Lee, H. W., AyalaSomayajula, G., and Yang, J. J. (2006) Prediction of EF-hand calcium-binding proteins and analysis of bacterial EF-hand proteins. *Proteins* 65, 643–655

16. Moncrieff, N. D., Krebs, J. W., and Goodman, M. (1990) Evolution of EF-hand calcium modulated proteins. I. Relationships based on amino acid sequences. *J. Mol. Evol.* 30, 522–562

17. Rigden, D. J., Jedrzejas, M. J., Moroz, O. V., and Galperin, M. Y. (2003) Structural diversity of calcium-binding proteins in bacteria: single-handed EF-hand? *Trends Microbiol.* 11, 295–297

18. Zalk, R., Clarke, O. B., de Georges, A., Grassucci, R. A., Reiken, S., Mancia, F., Hendrickson, W. A., Frank, J., and Marks, A. R. (2015) Structure of a mammalian ryanodine receptor. *Nature* 517, 44–49

19. Maki, M., Kitaura, Y., Satoh, H., Ohkouchi, S., and Shibata, H. (2002) Structure, functions and molecular evolution of the penta-EF-hand Ca2+-binding proteins. *Biochim. Biophys. Acta* 1600, 51–60

20. Maki, M., Maemoto, Y., Osako, Y., and Shibata, H. (2012) Evolutionary and physical linkage between calpains and penta-EF-hand Ca2+-binding proteins. *FEBS J.* 279, 1414–1421

21. Copley, R. R., Schultz, J., Ponting, C. P., and Bork, P. (1999) Protein families in multicellular organisms. *Curr. Opin. Struct. Biol.* 9, 408–415

22. Fischer, R., Koller, M., Flura, M., Mathews, S., Streher-Page, M. A., Krebs, J., Penniston, J. T., Carafoli, E., and Streher, E. E. (1988) Multiple divergent mRNAs code for a single human calmodulin. *J. Biol. Chem.* 263, 17055–17062

23. Sia, S. K., Li, M. X., Spyropoulos, L., Gagné, S. M., Liu, W., Putkey, J. A., and Sykes, B. D. (1997) Structure of cardiac muscle troponinC unexpectedly reveals a closed regulatory domain. *J. Biol. Chem.* 272, 18216–18221

24. Marshall, C. B., Nishikawa, T., Osawa, M., Stathopulos, P. B., and Ikura, M. (2015) Calmodulin and STIM proteins: Two major calcium sensors in the cytoplasm and endoplasmic reticulum. *Biochim. Biophys. Res. Commun.* 460, 5–21

25. Sia, S. K., Li, M. X., Spyropoulos, L., Gagné, S. M., Liu, W., Putkey, J. A., and Sykes, B. D. (1997) Structure of cardiac muscle troponinC unexpectedly reveals a closed regulatory domain. *J. Biol. Chem.* 272, 18216–18221

26. Marshall, C. B., Nishikawa, T., Osawa, M., Stathopulos, P. B., and Ikura, M. (2015) Calmodulin and STIM proteins: Two major calcium sensors in the cytoplasm and endoplasmic reticulum. *Biochim. Biophys. Res. Commun.* 460, 5–21

27. Lioi, J., Kim, M. L., Heo, W. D., Jones, I. T., Myers, J. W., Ferrell, J. E., Jr., and Meyer, T. (2005) STIM is a Ca2+-sensor essential for Ca2+-store depletion-triggered Ca2+ influx. *Curr. Biol.* 15, 1235–1241

28. Seske, S., Gwack, Y., Prakriya, M., Sripak, S., Puppel, S. H., Tanasa, B., Hogan, P. G., Lewis, R. S., Daly, M., and Rao, A. (2006) A mutation in Orai causes immune deficiency by abrogating CRAC channel function. *Nature* 441, 179–185

29. Bresnick, A. R., Weber, D. J., and Zimmer, D. B. (2015) S100 proteins in cancer. *Nat. Rev. Cancer* 15, 96–109

30. Chen, J. L., and Ding, Y. H. (2007) C2-domains, structure and function of a universal Ca2+-binding domain. *J. Biol. Chem.* 273, 1579–15882

31. Heizmann, C. W., Berchtold, M. W., and Rowlerson, A. M. (1982) Correlation of parvalbumin concentration with relaxation speed in mammalian muscles. *Proc. Natl. Acad. Sci. U.S.A.* 79, 517, 518

32. Putney, J. W. (1986) A model for receptor-regulated calcium entry. *Cell Calcium* 7, 1–12

33. Collins, S. R., and Meyer, T. (2011) Evolutionary origins of STIM1 and STIM2 within ancient Ca2+ signaling systems. *Trends Cell Biol.* 21, 202–211

34. Moore, B. W. (1965) A soluble protein characteristic of the nervous system. *Biochim. Biophys. Res. Commun.* 19, 739–744
48. Carafoli, E. (1967) In vivo effect of uncoupling agents on the incorporation of calcium and strontium into mitochondria and other subcellular fractions of rat liver. J. Gen. Physiol. 50, 1849–1864

49. Rizzuto, R., Simpson, A. W., Brini, M., and Pozzan, T. (1992) Rapid changes of mitochondrial Ca²⁺ revealed by specifically targeted recombinant aequorin. Nature 358, 325–327

50. Rizzuto, R., Brini, M., Murgia, M., and Pozzan, T. (1993) Microdomains with high Ca²⁺ close to the IP₃-sensitive channels that are sensed by neighbouring mitochondria. Science 262, 744–747

51. Kirichok, Y., Krapivinsky, G., and Clapham, D. E. (2004) The mitochondrial calcium uniporter is a highly selective ion channel. Nature 427, 360–364

52. De Stefani, D., Raffaello, A., Teardo, E., Szabò, I., and Rizzuto, R. (2011) A forty kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature 476, 336–340

53. Baughman, J. M., Perocchi, F., Girgis, H. S., Plovanich, M., Belcher-Timme, C. A., Sancak, Y., Bao, X. R., Strittmatter, L., Goldberger, O., Bogorad, R. L., Kotelsianisky, V., and Mootha, V. K. (2011) Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature 476, 341–345

54. Murgia, M., and Rizzuto, R. (2015) Molecular diversity and pleiotropic role of the mitochondrial calcium uniporter. Cell Calcium 58, 11–17

55. De Stefani, D., Patron, M., and Rizzuto, R. (2015) Structure and function of the mitochondrial calcium uniporter complex. Biochim. Biophys. Acta 1853, 2006–2011

56. Carafoli, E., Tiozzo, R., Lugli, G., Crovetti, F., and Kratzing, C. (1974) The release of calcium from heart mitochondria by sodium. J. Mol. Cell. Cardiol. 6, 361–371

57. Palty, R., Silverman, W. F., Hershfinkel, M., Caporale, T., Sensi, S. L., Parnis, J., Nolte, C., Fishman, D., Shoshan-Barmatz, V., Herrmann, S., Khananshvili, D., and Sekler, I. (2010) NCLX is an essential component of mitochondrial Na⁺/Ca²⁺ exchange. Proc. Natl. Acad. Sci. U.S.A. 107, 436–441

58. Lötscher, H. R., Winterhalter, K. H., Carafoli, E., and Richter, C. (1979) Hydro-peroxides can modulate the redox state of pyridine nucleotides and the calcium balance in rat liver mitochondria. Proc. Natl. Acad. Sci. U.S.A. 76, 4340–4344

59. Brown, E. M., Quin S. M., and Vassilev, P. M. (1999) The plasma membrane calcium sensor. In Calcium as a cellular regulator (Carafoli, E., and Klee, C., eds) pp. 295–310, Oxford University Press, New York

60. Krebs, J., and Michalak, M. (2007), Calcium: A Matter of Life or Death, Elsevier, Amsterdam

61. Brini, M., and Carafoli, E. (2007) Calcium Signaling and Disease, Springer, New York