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Intracellular Ca^{2+} sensing: role in calcium homeostasis and signaling

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Summary

Ca^{2+} is a ubiquitous intracellular messenger that controls diverse cellular functions but can become toxic and cause cell death. Selective control of specific targets depends on spatio-temporal patterning of the calcium signal and decoding it by multiple, tunable and often strategically positioned Ca^{2+} sensing elements. Ca^{2+} is detected by specialized motifs on proteins, which have been biochemically characterized decades ago. However, the field of Ca^{2+} sensing has been reenergized by recent progress in fluorescent technology, genetics and cryo-EM. These approaches exposed local Ca^{2+} sensing mechanisms inside organelles and at the organellar interfaces, revealed how Ca^{2+} binding might work to open some channels, and identified human mutations and disorders linked to a variety of Ca^{2+} sensing proteins. We here, attempt to place these new developments in the context of intracellular calcium homeostasis and signaling.

Keywords

Endoplasmic Reticulum; IP3 receptor; mitochondria; STIM1; MICU1; Miro1

Calcium Homeostasis and Signaling

Intracellular free Ca^{2+} concentration widely varies depending on its location. The cytoplasmic $[Ca^{2+}]$ ($[Ca^{2+}]_c$) under resting conditions is ~10^{-7}M, 10^4 times lower than $[Ca^{2+}]$ in the extracellular millieu (~10^{-3}M). Inside the cell, Ca^{2+} levels in the nuclear matrix ($[Ca^{2+}]_n$) and in the mitochondrial matrix ($[Ca^{2+}]_{mt}$) are similar to that in the cytoplasm. However, other intracellular organelles, known as Ca^{2+} stores, can accumulate Ca^{2+} and maintain a higher $[Ca^{2+}]$ than the cytoplasm (1-5×10^{-4}M). The main internal Ca^{2+} store is the endoplasmic reticulum (ER), and in muscle cells, the sarcoplasmic reticulum.

The low $[Ca^{2+}]_c$ is maintained through the action of the plasma membrane Ca^{2+} transport ATPase (PMCA) and Na^+/Ca^{2+} exchanger (NCX) in a resting cell. Upon elevated $[Ca^{2+}]_c$,

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this activity is complemented by the sarcoendoplasmic reticulum Ca^{2+}-ATPase (SERCA) that fills the ER/SR Ca^{2+} store and to a lesser extent, by the mitochondrial Ca^{2+} uniporter (mtCU). All these proteins sense and are activated by Ca^{2+}, and therefore any elevations in $[Ca^{2+}]_c$ stimulate removal of cytoplasmic Ca^{2+}, resulting in a homeostatic control of $[Ca^{2+}]_c$ (Fig. 1A, green arrows). Nevertheless, various cell stimuli such as membrane depolarization, extracellular signaling molecules, or intracellular messengers, promote an increase of $[Ca^{2+}]_c$ from 100nM to 1 μM or more. This increase results from either the influx of extracellular Ca^{2+} via the plasma membrane (PM) Ca^{2+} channels or the release of Ca^{2+} from internal stores mostly via the 1,4,5-triphosphate receptor (IP3R) and ryanodine receptor (RyR) from ER/SR (Fig. 1A, blue arrows). The $[Ca^{2+}]_c$ increase is usually steep, followed by a decay giving rise to $[Ca^{2+}]_c$ spikes or repetitive $[Ca^{2+}]_c$ oscillations, which are supported by multiple positive and negative feedback effects of Ca^{2+} favoring synchronized activation and rapid deactivation of the Ca^{2+} channels and by the homeostatic regulation of the Ca^{2+} removal mechanisms. The Ca^{2+}-regulated proteins present different thresholds for activity depending on their function. For example, PMCA and SERCA pumps have high affinities for Ca^{2+} and low pumping rate (≈30 and ≈10 Hz, respectively) (Juhaszova et al., 2000; Lytton et al., 1992), which make them suitable to respond to modest elevations in cytoplasmic Ca^{2+} levels and to reestablish the resting Ca^{2+} level. NCX and MCU, show a lower affinity for Ca^{2+} and greater transport rates (150 - 300 Hz for NCX, (Boyman et al., 2009)) and thus can limit larger $[Ca^{2+}]_c$ transients. Each cell type presents a unique combination of Ca^{2+} channels and pumps to create a cell type-and agonist-specific calcium signal that suits their physiological requirements (Berridge et al., 2000).

The low resting $[Ca^{2+}]_c$ and the calcium signal have to be tightly regulated because almost every aspect of cell function is controlled by Ca^{2+}, including secretion, gene expression, muscle contraction and metabolism, and any unregulated $[Ca^{2+}]$ elevations would cause cell injury or cell death (Fig. 1B) (Clapham, 2007; Hajnoczky et al., 2006; Neher and Sakaba, 2008). Furthermore, regulation of organelle-specific cell functions might depend on propagation of the $[Ca^{2+}]_c$ signal into specific organelles like the nucleus for gene regulatory events (Zhang et al., 2009) and mitochondrial matrix for oxidative metabolism (Griffiths and Rutter, 2009).

Molecular mechanisms of Ca^{2+} sensing

The information encoded in the calcium signal is deciphered by various intracellular Ca^{2+}-binding motifs. These motifs are present in the effector proteins, including Ca^{2+} channel proteins (i.e. IP3R and RyR) and proteins mediating Ca^{2+}-controlled cell functions (i.e. isocitrate dehydrogenase (ICDH) (Fig. 2A). Ca^{2+}-binding motifs are also present in specialized Ca^{2+}-sensing proteins, which couple changes in $[Ca^{2+}]$ to a wide variety of cellular functions depending on their localization, pattern of modulation, and the Ca^{2+} source. These proteins either simply associate with the effector proteins (e.g. calmodulin (CaM), troponin C) or display enzyme activity (e.g. calcineurin or calpain) to relay the effect of Ca^{2+}-binding to the effector proteins (Fig. 2B and C, respectively). CaM can also confer Ca^{2+}-sensitivity to enzymes like the Ca^{2+}/CaM-dependent protein kinase (CaMK) that phosphorylates many effectors of Ca^{2+} to alter their activity (Fig. 2D). Depending on the

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loop geometry of their Ca^{2+}-binding site(s), Ca^{2+}-binding proteins can be classified into three families: the EF-hand proteins, the annexins and the C2 domain proteins.

EF-hand proteins

The EF-hand denotes a Ca^{2+}-binding motif that contains a Ca^{2+}-coordinated loop that is flanked by two α-helices orientated almost perpendicular to one another. The bound Ca^{2+} ion is coordinated by 7 ligands (primarily carboxylate) in a pentagonal bipyramid arrangement (Strynadka and James, 1989). EF-hand domains are the most common Ca^{2+}-binding motifs found in proteins. This family of proteins presents a wide range of functions, which are as diverse as Ca^{2+} buffering in the cytoplasm, signal transduction between compartments and gene expression in the nucleus (Fig. 1B). The diversity of biological functions carried out by these proteins in a wide range of $[Ca^{2+}]$ is possible because Ca^{2+} binds to EF-hand domains with different affinities, extending from 10^{-6}M to 10^{-3}M (Gifford et al., 2007). Some Ca^{2+}-binding proteins with relatively high affinity behave as Ca^{2+}-buffer proteins, which modulate the shape and/or duration of Ca^{2+} signals and help maintain Ca^{2+} homeostasis. In contrast, Ca^{2+}-sensors having affinity constants ranging between 10^{-5}M and 10^{-7}M can detect and respond to a physiologically relevant change in intracellular $[Ca^{2+}]$. These differences in function correlate with differences in the conformational changes induced by Ca^{2+} binding. Ca^{2+} binding to EF-hands of Ca^{2+} sensor proteins induces a conformational change, characterized by a significant opening of their structure that permits their interaction with downstream targets (Zhang et al., 1995). On the contrary, Ca^{2+} buffer proteins stay in a 'closed' conformation upon Ca^{2+} binding that is similar to their Ca^{2+}-free state (Skelton et al., 1994).

A ubiquitously expressed and well-characterized protein specialized for Ca^{2+}-sensing is CaM. CaM has two globular domains, each containing a pair of EF-hand motifs, connected by a central helix. Activation by Ca^{2+} binding causes each of the EF-hand domains of CaM to undergo a significant opening of their structure. As a result, the hydrophobic binding sites within the central helix of CaM are exposed to interact with downstream targets (Zhang et al., 1995). Ca^{2+}-activated CaM (Ca^{2+}/CaM) interacts in a Ca^{2+}-dependent manner with either their target enzymes, leading to their own activation (e.g. CaMK and calcineurin), or the activation of their target proteins, resulting in the regulation of their function in a Ca^{2+}-dependent manner (e.g. Orai, Fig. 3B). The CaM-dependent activation of enzymes may occur by direct or sequential mechanisms (e. g. CaMK and calcineurin, respectively).

In the first case, CaM interaction and activation of target enzymes only occur under elevated $[Ca^{2+}]_c$, whereas in the sequential mechanism, partial Ca^{2+}-activation of CaM, under resting Ca^{2+} conditions, is enough to interact with target enzymes and form an inactive low affinity complex. For its activation, this complex requires further binding of Ca^{2+} to CaM's EF hands. This specific mechanism would provide a sensitive switch for control of enzyme activity within a narrow range of free $[Ca^{2+}]$ (Kincaid and Vaughan, 1986). In addition to Ca^{2+}/CaM interaction with downstream targets, Ca^{2+}-free CaM (apo-CaM) can also interact with target proteins in a reversible or irreversible manner and regulates their activities. Therefore, CaM interaction with its target proteins is not only facilitated by its Ca^{2+}-induced conformational change, but the interaction can also be mediated through Ca^{2+}-independent

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binding sites named IQ-motif. These motifs of sequence IQXXXRGXXXR provide binding sites for CaM and other proteins of the EF-hand family (Cheney and Mooseker, 1992).

Among the many downstream targets of CaM, CaMK enzymes are one of the best characterized (Swulius and Waxham, 2008). As a kinase enzyme, CaMK catalyze the transfer of phosphate from the gamma position of ATP to the hydroxyl group of Ser, Thr, or Tyr within protein substrates. Therefore, this CaM-dependent enzyme transduces the intracellular calcium signals into changes in the phosphorylation state and activity of target proteins. CaMK also performs autophosphorylation to increase its affinity for CaM, thus resulting in their association at low $[Ca^{2+}]_c$. The CaMK capacity to trap CaM enables these enzymes to detect the frequency of the calcium signals (Meyer et al., 1992). Depending on the downstream targets of CaMK, the members of this family can be classified into two classes: multifunctional kinases and substrate-specific kinases. Multifunctional kinases have multiple downstream targets (e.g. CaMKK, CaMKI, CaMKII and CaMKIV) and their activation can lead to signaling that affects many downstream pathways controlling a variety of cellular functions. In contrast, substrate-specific kinases have only one known downstream target (e.g. CaMKIII, phosphorylase kinase, and the myosin light chain kinases) and thereby, they usually have a specific function within the cell or tissue where they are expressed.

Calcineurin and calpain can directly bind and sense Ca^{2+} that affects their protein phosphatase and protease function, respectively. Calcineurin is regulated by Ca^{2+} both directly and via CaM. Calcineurin has been implicated in a wide variety of biological responses including lymphocyte activation, neuronal and muscle development (Schulz and Yutzey, 2004). On the other hand, calpain is uniquely regulated by Ca^{2+}-binding to its EF-hand domains. Members of the calpain family have been linked to various biological processes, including integrin-mediated cell migration, cytoskeletal remodeling, cell differentiation and apoptosis (Suzuki and Sorimachi, 1998).

Emerging literature highlights a sub-branch of the CaM family, the neuronal calcium sensor (NCS) proteins (Burgoyne, 2007). Some NCS proteins are uniquely expressed in neurons, while other members (such as NCS-1) are also expressed in other tissues (Kapp-Barnea et al., 2003). NCS proteins are implicated in the regulation of several neuronal functions. Tissue specific expression of Ca^{2+} sensing proteins like NCS can provide for selective control of specific pathways in different paradigms.

Annexins and C2 domain proteins

Annexins and C2 domains proteins present a unique architecture of their Ca^{2+}-binding sites that allow them to peripherally dock onto negatively charged membrane surfaces in their Ca^{2+}-bound conformation. As a result, these families are considered to provide a link between Ca^{2+}-signaling and membrane functions (Fig. 1B). The Ca^{2+}-binding sites of **annexins** don't present an EF-hand-type helix–loop–helix structure and only five of the seven coordination sites are provided by protein oxygen. The other two coordination sites are provided by water molecules, which can be replaced by phosphoryl groups when the annexin binds lipid (i.e. Ca^{2+}-and phospholipid-binding motif) (Swairjo et al., 1995).

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Knockout and knockdown approaches have revealed that multiples steps in the endocytosis and exocytosis process depend on annexin (Ali et al., 1989; Mayran et al., 2003). In particular, annexin 2 and 13 have been linked to endocytosis while annexin 1, 2 and 6 has been linked to exocytosis. The **C2 domain** is another Ca^{2+}-and phospholipid-binding motif, but in this case the core structure of the domain is based entirely on β-sheets rather than on α-helices (characteristic of the annexin structure) (Nalefski and Falke, 1996). Slight variations in the interconnecting loops residues of the β–sandwich core confer C2 domains with different abilities to respond to different Ca^{2+} concentrations and lipids. For instance, C2 domains of classical protein kinase C isoforms and synaptotagmins bind to the anionic headgroup of phosphatidylserine (Corbalan-Garcia et al., 1999; Fukuda et al., 1996) whereas the C2 domain of cPLA2 binds to the neutral phosphatidylcholine (Nalefski et al., 1998). This family of proteins is involved in membrane trafficking (e.g. Synaptotagmins and E-Synaptotagmins) and signal transduction (e.g. protein kinase C isoforms).

Cryo-EM determination of Ca^{2+} sensing motifs

Recent developments in cryo-EM have enabled high-resolution determination of structures that resisted x-ray crystallography. Cryo-EM technologies allowed some illumination on the structural aspects of Ca^{2+} sensing by ion channels like the RyR (Bai et al., 2016; des Georges et al., 2016; Efremov et al., 2015; Wei et al., 2016; Yan et al., 2015; Zalk et al., 2015), the IP3R (Bosanac et al., 2005; Fan et al., 2015; Seo et al., 2012) and the Ca^{2+}-activated K^+ (BK) channels (Hite et al., 2017; Russo et al., 2009). Studies using single-particle cryo-EM identified a pair of EF-hand domains at the central domain of RyR1 (4060 – 4134) (des Georges et al., 2016; Wei et al., 2016) and modulator binding sites for Ca^{2+}, ATP, and caffeine at the interdomain interfaces of the C-terminal domain (4957–5037) (des Georges et al., 2016). Although IP3R structure in its apo-state has been recently elucidated at near-atomic (4.7 A°) resolution (Fan et al., 2015), more studies are needed to define the molecular architecture of the domains that control channel gating. Up to date, the only information available is given by mapping the sequence conservation across the RyR and IP3R family. This analysis revealed that the Ca^{2+}-binding domain described at the C-terminal of RyR1 at the C-terminal is conserved, whereas the pair of EF-hands located at the central domain of RyR1 are absent in IP3R, thus suggesting that these EF-hands are not involved in Ca^{2+}-activation (des Georges et al., 2016). This hypothesis is supported by the fact that deletion or sequence-scrambling of EF-hand domains in RyR2 and RyR1 didn't affect the activation of the channel by Ca^{2+} (Fessenden et al., 2004; Guo et al., 2016). In addition, the study of BK channels in the Ca^{2+}-bound and Ca^{2+}-free states have revealed the molecular basis of channel gating by voltage and Ca^{2+}. At the level of Ca^{2+}-sensing, this channel presents a “gating ring” at the cytoplasm which is formed by four Ca^{2+}-sensors. Each sensor includes two regulators of K^+ conductance (RCK) that regulate the conductance of K^+ through the binding of two Ca^{2+} ions and a Mg^{2+} ion. Moreover, the central pore–gate domain (located in the transmembrane domain) appeared to be connected to both the voltage sensors, also located in the transmembrane domain, and to the Ca^{2+} sensors, located in the cytoplasm. Therefore, these data suggest a new shared pathway for channel activation (Hite et al., 2017; Tao et al., 2017).

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Localization and compartmentalization

Ca^{2+} regulates many different cellular functions. To achieve this versatility, the calcium signal displays a range of spatial and temporal patterns detected by various Ca^{2+} sensors differently. Although the bulk $[Ca^{2+}]_c$ peaks at around 1 mM, close to the open Ca^{2+} channels, $[Ca^{2+}]_c$ can reach 10-100 mM. These “nanodomains” provide meaningful signal for low affinity Ca^{2+} sensing motifs unresponsive to fluctuations in the global $[Ca^{2+}]_c$.

A major direction of recent progress on local Ca^{2+} sensing has been focused on detection of Ca^{2+} within organelles and at organellar interfaces (Fig. 3A). An example is the process known as store-operated Ca^{2+} entry (SOCE), whereby Ca^{2+} influx across the plasma membrane is activated in response to a decrease in the ER Ca^{2+} content (Fig. 3B). The main role of SOCE is to refill the intracellular Ca^{2+}-stores to maintain the primary source of intracellular Ca^{2+} mobilization and a favorable environment for protein folding in the ER lumen. Essential components of the molecular machinery responsible for SOCE have been recently discovered. Among them, STIM1 (and its STIM2 isoform) is the ER transmembrane protein responsible for sensing the changes in $[Ca^{2+}]_{ER}$ through a pair of Ca^{2+}-binding EF-hand domain that are exposed to the ER lumen (Liou et al., 2005; Roos et al., 2005; Zhang et al., 2005). Under resting conditions, STIM1 is found associated with SARAF, which prevents its spontaneous activation (Jha et al., 2013; Palty et al., 2012). Upon activation of Ca^{2+} release from the ER, Ca^{2+} level in the ER lumen drops, thus causing dissociation of Ca^{2+} from STIM1's EF-hands. Store depletion is also accompanied by the dissociation of SARAF from STIM1 (Albarran et al., 2016; Jha et al., 2013). As a result, STIM1 oligomerizes and translocates to specific regions of the ER close to the plasma membrane (named ER-PM junctions), where it interacts with and activates the plasma membrane Ca^{2+} channel Orai (Park et al., 2009). Ca^{2+}-influx through PM, such as that induced by SOCE, has been recently related to accumulation of extended synaptotagmin (E-Syt) 1 at ER-PM contact sites (Idevall-Hagren et al., 2015). Three E-Syts have been shown to participate in the ER-PM tethering via their C2 domains. E-Syt1 interacts with PM in a Ca^{2+}-dependent manner, whereas E-Syt2 and E-Syt3 interaction with PM only requires the presence of PI(4,5)P2 (Giordano et al., 2013). A recent addition in the mechanism of SOCE regulation is the discovery of CRACR2A, a cytoplasmic Ca^{2+} sensor that interacts with and stabilizes the STIM1-Orai complex at low $[Ca^{2+}]_c$ conditions (Srikanth et al., 2010). Increase in the $[Ca^{2+}]_c$ induces the dissociation of CRACR2A from the complex, resulting in the liberation of the Orai residues implicated in Ca^{2+}/CaM binding and thereby, SOCE inactivation (Mullins et al., 2009).

Recently identified local Ca^{2+} sensing mechanisms are also located at the mitochondria, where intermembrane space Ca^{2+} sensors control mitochondrial Ca^{2+} uptake (Fig. 3C) and mitochondrial surface targeted Ca^{2+}-sensors control mitochondrial motility and distribution along microtubules (Fig. 3D). Mitochondrial Ca^{2+} uptake via the mtCU is fundamental for energy metabolism and cell survival. The long-waited molecular composition of mtCU was finally revealed such that molecular details of the transport system can be studied, as well as its physiological relevance. The pore-forming component of the mtCU channel (MCU) is located in the inner mitochondrial membrane. MCU opening is tightly controlled by the EF-hand Ca^{2+}-sensing proteins MICU1 and MICU2, which are located in the intermembrane

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space that is rapidly equilibrated with the $[Ca^{2+}]_c$. At submicromolar $[Ca^{2+}]_c$ conditions, MICU1/2 is required to keep MCU closed (Csordas et al., 2013; Mallilankaraman et al., 2012; Patron et al., 2014). Loss of function mutation of the EF-hand doesn't interfere with MICU1/2-dependent closure of the MCU, indicating that binding of Ca^{2+} was not involved (Csordas et al., 2013). Releasing of ER Ca^{2+} via IP3R, which involves Ca^{2+}-mediated feedback loops and perhaps clustering of IP3Rs, results in an increase of $[Ca^{2+}]_c$ up to above 10 μM at the ER-mitochondrial interface. At high $[Ca^{2+}]_c$, Ca^{2+} likely binds to the EF hand domains of MICU1/2 inducing a conformational change that promotes MCU opening (Fig. 3C). High $[Ca^{2+}]_c$-induced rapid activation of the MCU seems to be required for effective sensing and decoding of short lasting $[Ca^{2+}]_c$ spikes and oscillations (Csordas et al., 2013). The MCU-mediated $[Ca^{2+}]_m$ increase activates the Ca^{2+}-sensitive dehydrogenases (PDH, α-KGDH and ICDH), glycerol-3-phosphate dehydrogenase (mtGPDH) as well as the ATPSynthase (Tarasov et al., 2012) (Fig. 1B) to enhance ATP production and in turn, meet energy demands. Notably, excessive Ca^{2+} uptake is sensed in the mitochondrial matrix to activate the permeability transition pore via cyclophilin D initiating a mitochondrial death pathway but the exact mechanism of Ca^{2+} sensing in this paradigm remains elusive (Baines et al., 2005; Basso et al., 2005).

Calcium signaling controls mitochondrial motility along the microtubules to support dynamic localization of mitochondria to the sites of $[Ca^{2+}]$ elevation, providing ATP production at the sites of energy demand. Ca^{2+} sensing is needed for this homeostatic distribution of the mitochondria (Yi et al., 2004). The mechanism for the Ca^{2+} effect on mitochondrial transport hasn't been completely elucidated. However, the role of two Ca^{2+}-sensing mitochondrial outer membrane proteins, Miro 1 and Miro 2 has been shown (Macaskill et al., 2009b; Saotome et al., 2008; Wang and Schwarz, 2009). These proteins interact with the adaptor proteins TRAK1/2 to anchor mitochondria to microtubular motor proteins kinesin, for anterograde movement, (MacAskill et al., 2009a; Wang and Schwarz, 2009) and dynein, for retrograde movement (Russo et al., 2009). At low $[Ca^{2+}]_c$, Miro1/2 facilitate mitochondrial movements along microtubules independent of their EF-hands. At high $[Ca^{2+}]_c$, functional EF-hand domains of Miro1/2 have been shown to be required to suppress mitochondrial movement (Macaskill et al., 2009b;

Saotome et al., 2008; Wang and Schwarz, 2009). For anterograde movement, two distinct mechanisms have been proposed to explain the Ca^{2+}-induced inhibition: (1) the dissociation of kinesin from TRAK1/2 (MacAskill et al., 2009a) or (2) the dissociation of kinesin from microtubules due to its interaction with Miro1/2's EF-hand domains (Wang and Schwarz, 2009). For retrograde movement, the mechanism responsible of Ca^{2+}-induced inhibition of mitochondrial movement is unknown.

Disease linked to genetic impairments of Ca^{2+} sensing proteins

Human mutations of a range of Ca^{2+} transporters and sensing proteins have been linked to disease long ago. These mutations cause perturbation of specific components of the Ca^{2+}-controlling and/or processing machinery in a tissue-specific or global manner, which leads to the impairment of Ca^{2+} homeostasis (Brini and Carafoli, 2009). Recent progress in clinical genetics has helped to identify new mutations and patients exhibiting mutation/

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polymorphism in Ca^{2+} sensing proteins. Here we focus on mutations of some proteins referred to in the previous sections.

Up to now, more than 300 disease mutations in RyR which cause either gain-of-function or loss-of-function have been identified. Most of these mutations are clustered in three different regions of RyR sequence, which are located in: N-terminal region (first ~600 amino acids), a central region (amino acids ~2100–2500), and the C-terminal area (amino acid ~3900–end). Mutations in the C-terminal area of RyR2 (including EF-hand and pore domains) have been recently related to Ca^{2+} sensing mechanisms (Jiang et al., 2004; Uehara et al., 2017). Three mutations in this area (N4104K, R4496C, and N4895D) have been shown to decrease the threshold for RyR2 activation by SR luminal Ca^{2+}, thus affecting overload-induced SR Ca^{2+} release (Jiang et al., 2004). A single mutation at K4750Q in RyR2 causes hypersensitization to activation by either $[Ca^{2+}]_c$ or SR luminal Ca^{2+} as well as loss of cytosolic Ca^{2+}/Mg^{2+}-mediated inactivation and leads to a very severe clinical phenotype (Sugiyasu et al., 2009; Uehara et al., 2017). Mutations in RyR2 are linked to catecholaminergic polymorphic ventricular tachycardia (Priori et al., 2001), whereas RyR1 mutations are associated with central core disease (Zhang et al., 1993) and malignant hyperthermia (MacLennan, 1992).

STIM1 mutations have also been reported, which can be classified as loss-of-function or gain-of-function mutations. In the first case, mutations in STIM1 cause almost complete loss of SOCE activity, although the protein expression is only moderately reduced. In contrast, gain-of-function mutations induce a continuous activation of SOCE that results in increased intracellular Ca^{2+} levels and therefore, impairment of Ca^{2+} homeostasis. In both cases, mutations can be located in the cytosolic C-terminus and interfere with intra- and intermolecular protein interaction with STIM1 and Orai1, or in the ER luminal Ca^{2+}-sensing domain. Among them, p.R429C mutation has been reported to interfere with SOCE activation at multiple steps, causing constitutive accumulation of STIM1 at the ER-PM associations without cytoplasmic oligomerization and interaction with ORAI1 required for ORAI1 activation (Maus et al., 2015). Loss-of-function mutations in STIM1 clinically manifest as severe combined immunodeficiencylike disease, autoimmunity, muscular hypotonia, and ectodermal dysplasia. The gain-of-function mutations in STIM1 have been associated with a wider spectrum of diseases ranging from non-syndromic tubular aggregate myopathy (TAM) to York platelet and Stormorken syndromes depending on the mutation site. In the case of non-syndromic TAM, most of the mutations causing this disease are located in the EF-hand domain of STIM1 (summarized in (Lacruz and Feske, 2015)).

Searching for the molecular composition of mtCU and studying how the components work together have allowed a molecular diagnosis of patients with unclassified dysfunction. Recently, two elegant studies have shown human MICU1 mutations leading to the loss of MICU1 protein (Lewis-Smith et al., 2016; Logan et al., 2014). In both cases, MICU1 deficiency caused abnormal mitochondrial Ca^{2+} handling, demonstrating the crucial role of Ca^{2+} sensing proteins in the regulation of mitochondrial Ca^{2+} uptake. More specifically, the patient cells display increased mitochondrial Ca^{2+} content (Logan et al., 2014). In murine models, mitochondrial Ca^{2+} overload and increased sensitivity to permeability transition have been also shown and been linked to pathogenesis (Antony et al., 2016; Liu et al., 2016). The MICU1 loss-of-function clinical phenotype is characterized by proximal myopathy,

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learning difficulties and a progressive extrapyramidal movement disorder (Logan et al., 2014) or fatigue and lethargy (Lewis-Smith et al., 2016).

Based on the clinical presentation of RyR, STIM1 and MICU1 mutations, a broad range of organ dysfunctions and human disorders are expected to be associated with mutations in Ca^{2+}-sensing proteins. However, in the case of the Ca^{2+}-sensing effector proteins, the mutations commonly alter more than just the Ca^{2+} sensitivity. Interestingly, several human mutations have also been documented in the specialized Ca^{2+} sensing protein, CaM and some of these mutations are confined to the C-domain's EF-hands and specifically alter the affinity for Ca^{2+} binding. The mutations were documented in infants who exhibited life-threatening ventricular arrhythmias combined variably with epilepsy and delayed neurodevelopment (Crotti et al., 2013). The severe multisystem impairments indicate the fundamental relevance of Ca^{2+} sensing for normal development and health.

Perspectives

The vast physiological relevance of the intracellular Ca^{2+} sensing toolkit is supported by the severe mouse phenotypes and human disorders associated with deletions/mutations of various Ca^{2+} sensing proteins. Interestingly, deletion of some Ca^{2+} sensing proteins like STIM1 and MICU1 has more severe consequences in mice than a loss-of-function mutation in human. This difference likely involves more effective adaptation in humans, the molecular basis of which remains to be explored. The main intracellular Ca^{2+} sensing motifs have been defined and the long sought Ca^{2+} sensing proteins regulating store-operated Ca^{2+} entry and the mitochondrial Ca^{2+} uniport were identified recently. However, due to the amino acid sequence diversity in EF-hands and other Ca^{2+} sensing motifs, it is likely that the Ca^{2+} sensing protein family will continue to broaden. Future progress is also expected on the tuning of the Ca^{2+} sensors by posttranslational modifications, including changes in the thiol redox state. Since the Ca^{2+} controlled elements often strategically positioned close to a Ca^{2+} source, it is important to measure their Ca^{2+} exposure, which has become feasible by linking genetically encoded fluorescent Ca^{2+} sensors to the protein of interest and recording the fluorescence with high spatial/temporal resolution imaging. Furthermore, while many proteins have been resistant to x-ray crystallography, very recent results indicate that the structural rearrangements caused by Ca^{2+} binding might be determined by single-particle cryo-EM and other emerging structural approaches at least for some ion channels. This information is expected to greatly facilitate the development of new pharmacological approaches for targeting impairments of the Ca^{2+}-regulation of cellular functions.

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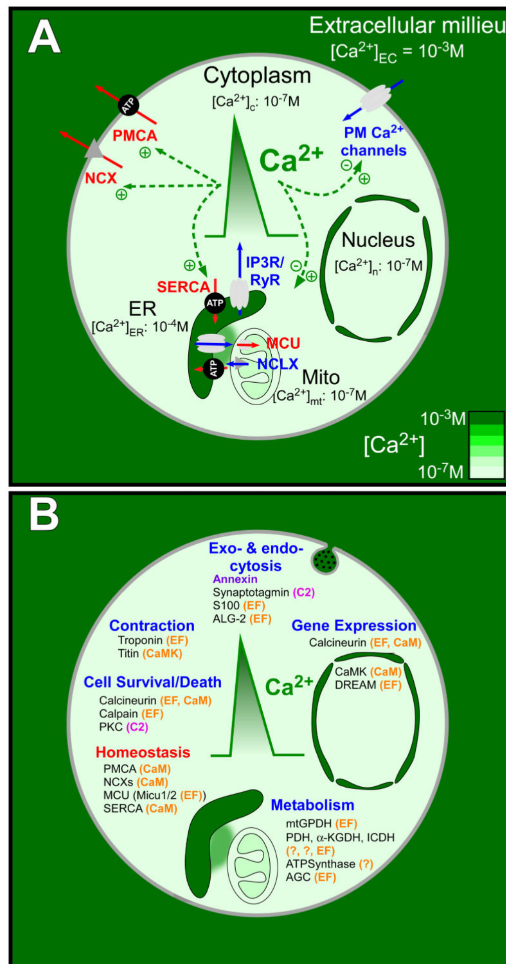

Figure 1. A schematic representation of the Ca^{2+}-regulated proteins involved in: cellular Ca^{2+} homeostasis and signaling

A) $[Ca^{2+}]$ in the different cellular compartments is indicated by a green scale ranging from 100nM (light green) to 1mM (dark green). The Ca^{2+}-transporting systems that increase $[Ca^{2+}]_c$ are highlighted in blue, and in red those which decrease $[Ca^{2+}]_c$. The green arrows indicate the positive and negative feedback effects of $[Ca^{2+}]_c$ on the Ca^{2+}-transporting systems. B) Cellular processes regulated by calcium signaling are listed in this scheme as well as the main Ca^{2+}- regulated proteins involved in each process. In parenthesis are indicated the Ca^{2+}-binding motifs of the Ca^{2+}-regulated proteins, which could belong to: the EF-hand proteins (EF – EF hand domains; CaM – Calmodulin), the annexins or the C2 motif proteins (C2).

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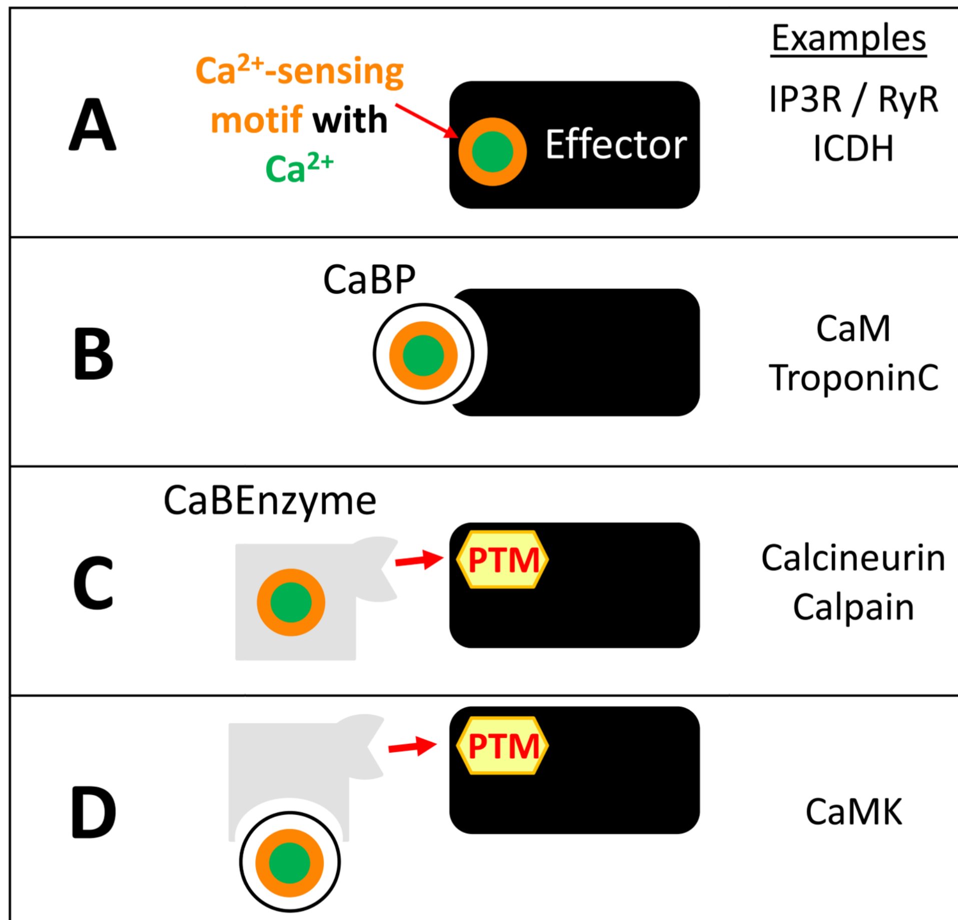

Figure 2. Different types of Ca^{2+}-dependent regulation of effector's function depending on the localization of Ca^{2+}-binding motif

Ca^{2+}-binding sites can be present in the effector proteins (A) and thereby regulate their function in a Ca^{2+}-dependent manner, or in specialized Ca^{2+}-sensing proteins (B – D). These proteins may regulate effector protein activity by Ca^{2+}-dependent association (i.e. Ca^{2+}-binding proteins, CaBP) (B) or by post-translation modifications (C – D). These modifications are displayed by enzymes that are regulated in a Ca^{2+}-dependent manner either because they have a Ca^{2+}-binding motif (i.e. Ca^{2+}-binding enzymes, CaBEnzyme) (C) or because they are associated with a CaBP (D).

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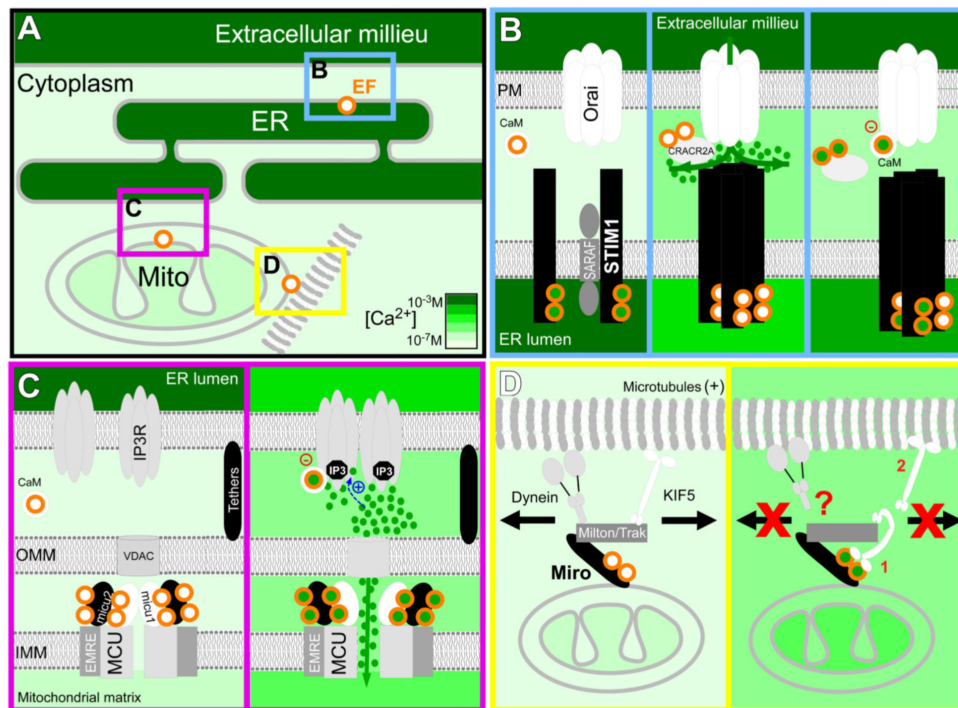

Figure 3. Local calcium signaling is mediated by compartmentalized Ca^{2+}-sensors within the cell

A) Scheme visualizing the cellular localization of Ca^{2+}-sensors that regulate local function such as store-operated Ca^{2+} entry (SOCE) (B), mitochondrial Ca^{2+} uptake (C) or mitochondrial motility (D). B) SOCE is regulated by STIM1 which senses the ER lumen Ca^{2+} content via its EF-hands. Upon ER Ca^{2+} depletion, STIM1 undergo a Ca^{2+}-regulated conformational change that promotes its oligomerization and activation of Orai Ca^{2+} channels. Increase of $[Ca^{2+}]_c$ suppresses Ca^{2+} influx by triggering CaM binding to Ora1. C) Mitochondrial Ca^{2+} uptake via MCU is regulated by the Ca^{2+}-sensing proteins MICU1 and MICU2. In resting conditions, MICUs interaction with MCU prevent mitochondrial Ca^{2+} uptake. Local Ca^{2+} release by IP3R promotes the MCU pore opening due to a Ca^{2+}-regulated conformational change of MICUs. D) Mitochondrial motility along the microtubules is controlled by the Ca^{2+}-sensing protein Miro. At low cytoplasmic $[Ca^{2+}]$, Miro facilitates the retrograde and anterograde movement of mitochondria through its interaction via Milton/Trak with dynein and kinesin (KIF5), respectively. Upon Ca^{2+}-binding to Miro's EF hands due to an increase in $[Ca^{2+}]_c$, mitochondrial motility is suppressed in both directions.