Trends in Cell Biology

CellPress REVIEWS

Review

Calcium Dynamics as a Machine for Decoding Signals

Carlotta Giorgi,[1] Alberto Danese,[1] Sonia Missiroli,[1] Simone Patergnani,[1] and Paolo Pinton[1,2,3,*]

Calcium (Ca^{2+}) is considered one of the most-important biological cations, because it is implicated in cell physiopathology and cell fate through a finely tuned signaling system. In support of this notion, Ca^{2+} is the primary driver of cell proliferation and cell growth; however, it is also intimately linked to cell death. Functional abnormalities or mutations in proteins that mediate Ca^{2+} homeostasis usually lead to a plethora of diseases and pathogenic states, including cancer, heart failure, diabetes, and neurodegenerative disease. In this review, we examine recent discoveries in the highly localized nature of Ca^{2+}-dependent signal transduction and its roles in cell fate, inflammasome activation, and synaptic transmission.

Highlights

Ca^{2+} homeostasis alterations lead to defects in cell cycle progression.

inositol 1,4,5-trisphosphate (IP3) receptors are a target of oncogenes and tumor suppressors.

Ca^{2+} signaling has an important but still controversial role in inflammasome activation.

Ca^{2+} signaling has a key role in synaptic activity and plasticity as well as in neurodegeneration.

The mitochondrial calcium uniporter (MCU) is not essential for homeostatic cardiac function.

Introduction

The regulation of Ca^{2+} signaling is crucial for cellular homeostasis. Ca^{2+} ions are the most-common second messengers of eukaryotic cells, decoding a variety of cellular and physiological processes, including muscle contraction, secretory events, cell cycle, differentiation, and gene transcription. Ca^{2+} signals are finely tuned using a ubiquitous broad group of gene products: channels, pumps, transporters, and binding proteins (Figure 1, Key Figure).

The Ca^{2+}-dependent signaling system is versatile and dynamic and can affect several Ca^{2+}-sensitive enzymes that convert changes in Ca^{2+} concentration ($[Ca^{2+}]$) into defined cell actions [1]. Fast responses to external stimuli are regulated by rapid and highly localized Ca^{2+} spikes similar to those that occur in synaptic connections [2]. By contrast, repetitive Ca^{2+} transients or waves control slower intracellular dynamics, such as smooth muscle contractility and cell proliferation [2]. Variations in mitochondrial $[Ca^{2+}]$ have pivotal roles in physiological processes (i.e., ATP production necessary for the correct energy state of the cell) and cell death events (i.e., the induction of apoptosis and the activation of autophagy) [3]. Indeed, excessive transfer of Ca^{2+} from the endoplasmic reticulum (ER) via inositol 1,4,5-trisphosphate (IP3) receptor channels (IP3Rs) to mitochondria leads to mitochondrial Ca^{2+} overload, the subsequent opening of the mitochondrial permeability transition pore (mPTP; Box 1), and release of proapoptotic factors [4] (Figure 1). Here, we focus on advances in understanding Ca^{2+} signaling behaviors and their role in cell fate, **inflammasome** (see Glossary) activation, and synaptic transmission.

Temporal and Spatial Signaling of Ca^{2+}

The concentration of free Ca^{2+} in the cytosol ($[Ca^{2+}]_c$) has to be maintained at the nanomolar range ($\sim10^{-7}$ M), approximately 10^4-fold lower than that of the extracellular milieu ($\sim10^{-3}$ M), through the consumption of a significant amount of ATP [1]. Maintaining a low $[Ca^{2+}]_c$ allows Ca^{2+} to act as a second messenger. Indeed, minimal changes in $[Ca^{2+}]_c$ are perceived as specific signals and, as a consequence of the low amount of mobilized Ca^{2+}, the ATP necessary to return to resting and/or unstimulated conditions is limited. Given the steep

[1]Department of Morphology, Surgery and Experimental Medicine, Section of Pathology, Oncology and Experimental Biology, Laboratory for Technologies of Advanced Therapies (LTTA), University of Ferrara, Ferrara, Italy
[2]Cecilia Hospital, GVM Care & Research, E.S: Health Science Foundation, Cotignola, Italy
[3]CNR Institute of Cell Biology and Neurobiology, Monterotondo, Italy

*Correspondence: paolo.pinton@unife.it (P. Pinton).

 https://doi.org/10.1016/j.tcb.2018.01.002
© 2018 Elsevier Ltd. All rights reserved.

CellPress REVIEWS

electrochemical gradient between the extracellular environment (or the intracellular stores) and the cytoplasm, increases in $[Ca^{2+}]_c$ can be elicited by both Ca^{2+} entry from the extracellular space through plasma membrane (PM) channels and Ca^{2+} release from intracellular stores [1] (Figure 1).

Cells manage this external pool of Ca^{2+} by activating various entry channels, such as voltage-gated calcium channels (VGCCs), which are dominant in excitable cells, and nonvoltage-gated channels, which are dominant in nonexcitable cells. VGCCs belong to the voltage-operated Ca^{2+} channel family and are activated by depolarizing membrane potentials, while nonvoltage-gated channels include ligand-gated channels, such as the P2X purinergic ionotropic receptor families [5], and transient receptor potential channels (TRPCs) [6].

The principal organelles involved in Ca^{2+} storage are the ER and the Golgi apparatus, which have a critical role both in the maintenance of low $[Ca^{2+}]_c$ in resting conditions and as a source of Ca^{2+} to be released by specific mediators [7]. Recently, lysosomes were suggested to participate in the regulation of Ca^{2+} homeostasis, acting as Ca^{2+} stores that respond to physiological second messengers [8] and provide bidirectional communication with the ER Ca^{2+} storage organelle [9]. Nicotinic acid adenine dinucleotide phosphate (NAADP) mobilizes localized Ca^{2+} signals from lysosome-related Ca^{2+} stores. The release pathway requires high $[Ca^{2+}]$ in the organelle and involves the two-pore channel (TPC) and the mucolipin (MCOLN) family of TRPCs [10]. The binding of physiological ligands to G protein- or phosphotyrosine-coupled receptors at the PM activates different isoforms of phospholipase C (PLC), which most often initiates the primary route for Ca^{2+} mobilization. In turn, PLC hydrolyzes the PM lipid phosphatidylinositol 4,5-bisphosphate to IP3 and diacylglycerol. IP3 mediates the opening of IP3Rs, which occur in three isoforms (IP3R1–3), resulting in the rapid release of Ca^{2+} into the cytoplasm and the mitochondrial compartment (Figure 1). The members of another family of ER channels involved in ER Ca^{2+} release, RyRs, are activated via a phenomenon known as Ca^{2+}-induced Ca^{2+} release (CICR) [11].

Mitochondria can accumulate high levels of Ca^{2+} that can be more than 20 times higher than those in the cytosol due to: (i) the presence of mitochondrial juxtapositions with ER membranes, termed mitochondria-associated membranes (MAMs) (Box 2); (ii) the presence of an electrochemical gradient (−180 mV, negative inside); and (iii) the existence of a Ca^{2+}-selective channel [termed the **mitochondrial calcium uniporter** (MCU) complex], which enables Ca^{2+} accumulation inside the matrix [12]. Interestingly, mitochondrial Ca^{2+} accumulation may also be regulated by the Ca^{2+}-regulated transcription factor cyclic AMP response element–binding protein (CREB), which binds the MCU promoter and stimulates its expression upon increases in cytosolic Ca^{2+} levels [13]. Voltage-dependent anion channels (VDACs), which are located on the outer mitochondrial membrane (OMM), are responsible for the rapid transfer of Ca^{2+} from ER–mitochondria apposition, and their function results in high Ca^{2+} microdomains in the mitochondrial intermembrane space [14].

Once the ER Ca^{2+} content is depleted, extracellular Ca^{2+} influxes cross membrane channels to restore the resting (unstimulated) conditions of the ER $[Ca^{2+}]$. This mechanism is called **store-operated Ca^{2+} entry** (SOCE), and includes the ER-inserted stromal interaction molecule-1 (STIM1) and the calcium release-activated calcium channel protein ORAI1, which physically interact at ER–PM junctions in a functional Ca^{2+} release-activated Ca^{2+} (CRAC) channel complex [15]. Furthermore, STIM1 and STIM2 proteins are required for Ca^{2+}-store-depletion-mediated Ca^{2+} influx, functioning as Ca^{2+} sensors that monitor the loading level of intracellular Ca^{2+} stores and promote Ca^{2+} influx via activation of the PM Ca^{2+} channel

Glossary

F-box proteins: contain a protein motif comprising approximately 50 amino acids that functions as a protein–protein interaction site. F-box proteins are substrate receptors of the SKP1-Cullin 1-F-box protein (SCF) E3 ubiquitin ligase and have important roles in several physiological processes and activities. In humans, there are 69 SCF ligases, each utilizing a different F-box protein subunit. F-box proteins are classified into three subfamilies (FBXW, FBXL, and FBXO) depending on the presence of specific domains other than the F-box motif, which is important for binding to the SKP1 adaptor. Importantly, the FBXL subfamily is characterized by the presence of leucine-rich repeats.

Inflammasomes: multiprotein signaling complexes that operate as platforms for the activation of inflammatory caspases and the maturation of a set of proinflammatory cytokines. Inflammasome formation is triggered by a range of substances that emerge during infection, tissue damage, or metabolic imbalances. Once the protein complexes form, inflammasomes activate caspase-1, which proteolytically activates the proinflammatory cytokines interleukin-1β and interleukin-18. In addition, inflammasome activation causes a rapid, proinflammatory form of cell death termed pyroptosis.

Mitochondrial calcium uniporter (MCU) complex: located at the inner mitochondrial membrane and includes the channel-forming subunit MCU (also known as CCDC109A) and MCU regulatory subunit b (MCUb; also known as CCDC109B). EMRE (also known as C22orf32) and MICU1 (previously known as CBARA1 and EFHA3) are essential MCU regulators. Other important regulators are MICU2 (also known as EFHA1) and MICU3 (also known as EFHA2).

Sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA): a pump that transports Ca^{2+} from the cytosol to the SR/ER and Golgi apparatus. The different isoforms (SERCA1, SERCA2a, SERCA2b, and SERCA3) are species and tissue specific. Ca^{2+} homeostasis and SERCA2 activity

ORAI1 [16]. Importantly, **sarco/endoplasmic reticulum Ca^{2+}-ATPase** (SERCA) pumps situated on the ER as well as SPCA (on the Golgi apparatus) remove Ca^{2+} from the cytoplasm to re-establish basal Ca^{2+} levels in intracellular stores; simultaneously, the PM Ca^{2+}-ATPase (PMCA) contributes to the transport of Ca^{2+} from the cytosolic to extracellular spaces [7] (Figure 1).

Regulation of Cell Fate

Ca^{2+} participates in essential molecular processes that mediate cell survival and death, including defense and programmed cell death mechanisms, such as cell cycle (Figure 2), apoptosis, and autophagy [17] (Figure 3).

Cell Cycle

Ca^{2+} influx, from both intra- and extracellular sources, regulates cell cycle progression and apoptosis in a variety of cell types and several developmental stages [17]. A moderate increase in $[Ca^{2+}]_c$ and in the levels of reactive oxygen species (ROS) is essential for cell cycle progression in meiosis; an increase above the physiological threshold may lead to metaphase-II cell cycle arrest in oocytes. However, a massive increase in $[Ca^{2+}]_c$ generates oxidative stress, which induces Fas ligand- and mitochondria-mediated apoptosis [18] (Figure 2).

Disorders of cell cycle control may result from differences in $[Ca^{2+}]$ caused by various mechanisms. For example, if IP3R-mediated Ca^{2+} transfer to a mitochondrial signal is nonfunctional or perturbed, normal cells restrict their entry into the cell cycle. Interestingly, cancer cells bypass Ca^{2+} checkpoints and undergo necrotic collapse during cytokinesis after complete abolishment of IP3R activity [19].

Ca^{2+} has a role in mitotic chromosome condensation after nuclear envelope breakdown (NEB). Ca^{2+} is known to increase and shift from storage organelles to chromatin during the mitotic phase [20]. Low $[Ca^{2+}]$ disrupts the structure of mitotic chromosomes in a concentration-dependent manner. Condensin I is a scaffold protein that is essential for this rearrangement process. Similar to Ca^{2+}, condensin I intervenes in a late phase of chromosome condensation; this process is mostly achieved before condensin I and Ca^{2+} bind to chromosomes. Condensin-depleted cells display decondensed chromosomes. It has been hypothesized that Ca^{2+} then assists condensin I in promoting chromosome compaction; low $[Ca^{2+}]$ disrupts mitotic chromosome organization and causes a mitotic delay [20] (Figure 2).

Moreover, Ca^{2+} performs an essential role in T cell activation by regulating entry into the cell cycle through calcineurin and nuclear factor of activated T cells (NFAT). In particular, an increase in $[Ca^{2+}]_c$ induces a conformational change in calmodulin (CaM), which binds Ca^{2+} and promotes its ability to interact with, and activate, the protein phosphatase, calcineurin. Calcineurin dephosphorylates the cytoplasmic subunits of NFAT transcription complexes (NFATc), which translocate into the nucleus and regulate genes that are involved in the cell cycle, apoptosis, angiogenesis, and metastasis [21]. The generation of coordinated yet spatially distinct subcellular Ca^{2+} signals activates different transcription factor isoforms, such as NFAT proteins. This interplay increases the bandwidth of excitation–transcription coupling and recruits different gene expression profiles [22].

Recently, it was proposed that the G1/S cell cycle transition is controlled by SOCE [23]. Indeed, inhibition of the STIM1–ORAI1 interaction during SOCE upregulates the expression of cyclin E. This high cyclin E concentration results in cell cycle arrest and increased autophagy. Thus, Ca^{2+} may be an important factor in the regulation of the G1/S transition [23] (Figure 2).

represent a nodal point linking vascular remodeling and cell survival.
Store-operated Ca^{2+} entry (SOCE): a major route of Ca^{2+} entry in nonexcitable cells. SOCE is mediated by the STIM and ORAI proteins and has recently been implicated in cancer cell proliferation, metastasis, and tumor neovascularization, as well as in antitumor immunity. Enhanced expression of ORAI1 and STIM1 as well as enhanced SOCE have been reported in therapy-resistant ovarian carcinoma cells and in colorectal, cervical, liver, lung, and clear cell renal cancers.

Cell Press REVIEWS

Key Figure

Schematic Model of Intracellular Calcium (Ca^{2+}) Homeostasis

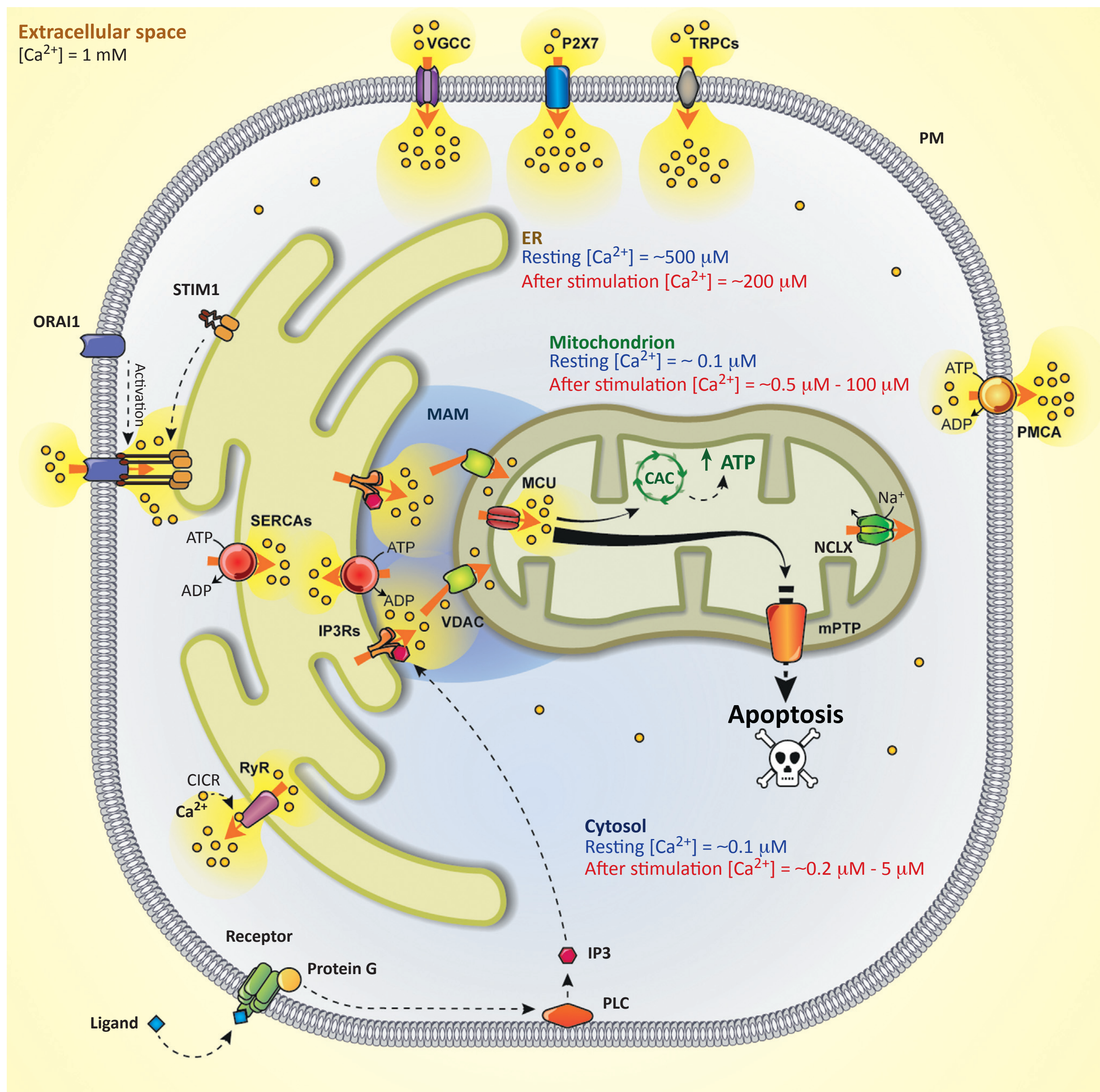


Figure 1. The Ca^{2+} concentration [Ca^{2+}] inside cells under resting conditions is maintained at a low concentration (Ca^{2+} levels are indicated in blue). Ca^{2+} enters the cell through different channels [voltage-gated calcium channels (VGCC), P2X7, and transient receptor potential channels (TRPCs)] that are located on the plasma

(Figure legend continued on the bottom of the next page.)

Cell Death

Several stimuli regulate the apoptotic process; among these, Ca^{2+} signaling has a key role in triggering apoptosis in a variety of cell types [24]. VDAC not only controls metabolic and energy crosstalk between the mitochondria and the rest of the cell, but also participates in apoptosis through the release of mitochondrial proapoptotic proteins to the cytosol and interaction with the antiapoptotic proteins Bcl-2 and Bcl-xL [25]. However, several studies suggest that Ca^{2+} dynamics at the ER are a main regulator of apoptosis (Figure 3) [83,84]. Indeed, a pool of p53 is localized at the ER–MAM, where it acts as a cell death inducer by regulating SERCA activities [26]. This interaction enhances Ca^{2+} accumulation in the ER under stress conditions and serves to rapidly overload the mitochondrial matrix with Ca^{2+}, which is the first step in the induction of apoptosis (Figure 3). Mutated or nonfunctional p53 in tumors impedes this proapoptotic mechanism and contributes to the progression of disease [26].

Numerous studies have also identified several oncogenes and tumor suppressors that act as specific regulators of the IP3R3-mediated Ca^{2+} flux to mitochondria. For example, promyelocytic leukemia protein (PML), a tumor suppressor associated with an extensive range of malignancies [27], is essential for the AKT- and protein phosphatase 2A (PP2A)-dependent modulation of IP3R-mediated Ca^{2+} release from the ER and, thus, apoptosis [28] (Figure 3). The proto-oncogene AKT inhibits IP3R type III and protects the cell from apoptosis, decreasing Ca^{2+} transfer to mitochondria [29]. This finding is also supported by the reticular localization of mTORC2, the complex responsible for AKT activation [30]. This AKT-mediated reduction in Ca^{2+} release via IP3R3 is regulated by ER-localized PTEN in a protein phosphatase-dependent manner. PTEN, which is a tumor suppressor that regulates AKT activation and is commonly mutated or deleted in human cancers, directly interacts with IP3R, counteracting the reduced IP3R-dependent Ca^{2+} release mediated by AKT phosphorylation [31]. Once activated, IP3Rs are continuously degraded, and this degradation appears to enable cells to adapt to the persistent activation of IP3-dependent signaling. The ubiquitin-proteasome pathway is one of the major signaling routes regulating IP3R degradation. Recently, the **F-box protein** FBXL2 was found to promote the ubiquitylation and degradation of IP3R3, limiting Ca^{2+} mobilization from the ER to the mitochondria and, thus, protecting cells from Ca^{2+}-dependent apoptosis [32]. Interestingly, PTEN competes with FBXL2 for binding to IP3R3, thereby protecting it from FBXL2-mediated degradation.

IP3R3 stabilization restores the sensitivity to apoptosis induced by photodynamic therapy (PDT) in tumors with low or no PTEN expression [32]. This study provides a proof-of-principle that inhibiting IP3R3 degradation in PTEN-deregulated cancers is a valid therapeutic strategy (Figure 3). Similarly, IP3R3 is bound and deubiquitinated by BRCA1-associated protein 1 (BAP1) at the ER [33]. As a consequence, the Ca^{2+} flux from the ER is enhanced, and the apoptotic machinery is activated (Figure 3). Thus, a high incidence of cancer, which is typical for BAP1-mutated individuals, results from the combination of nuclear (critical to preserve the genome stability) and cytosolic (indispensable for Ca^{2+}-dependent apoptosis) activities [33].

membrane. The binding of ligands to plasma membrane G-protein-coupled receptors activates phospholipase C (PLC) to promote the generation of inositol 1,4,5-trisphosphate (IP3) and the release of Ca^{2+} from the endoplasmic reticulum (ER) and the Golgi apparatus into the cytosol (the Ca^{2+} levels reached in stimulated cells are indicated in red). Mitochondria are prone to take up great quantities of Ca^{2+} due to their interaction with the ER through contact sites defining hotspot Ca^{2+} signaling units (MAMs). After agonist stimulation, Ca^{2+} is released from the ER to mitochondria, where it triggers an increase in cellular metabolism and ATP production. By contrast, the mitochondrial Ca^{2+} overload induced by apoptotic stimuli or ER stressors sensitizes the mitochondria to apoptosis, leading to mitochondrial permeability transition pore (mPTP) opening. Ca^{2+} returns to its resting levels through the concerted action of channels and pumps, such as plasma membrane Ca^{2+}-ATPase (PMCA) and the Na^+/Ca^{2+} exchanger (NCX), which permit ion extrusion in the extracellular milieu. Sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA) re-establishes basal Ca^{2+} levels in intracellular stores. STIM1 and ORAI1 are the major proteins responsible for the SOCE mechanism (see the main text for further details). Abbreviations: IP3Rs, IP3 receptors; MCU, mitochondrial Ca^{2+} uniporter; RyR, ryanodine receptor; VDAC, voltage-dependent anion channel.

CellPress REVIEWS

Box 1. Mitochondrial Permeability Transition Pore

The mitochondrial permeability transition pore (mPTP) has a key role during MPT, a cellular phenomenon leading to an irreversible increase in the permeability of the inner mitochondrial membranes (IMM) with consequent mitochondrial depolarization, Ca^{2+} release, and matrix swelling. Together, these features result in cell death through apoptosis or necrosis, depending on the biological setting [4]. MPT occurs during a variety of conditions that share an increase in mitochondrial Ca^{2+} or reactive oxygen species (ROS) levels that trigger mPTP formation. The induction of excessive Ca^{2+} entry into the cells via excitotoxicity is a key factor during neuronal cell death [74]. MPT has a key role during heart attack and stroke, and excessive increases in $[Ca^{2+}]_m$ contribute to MPT induction after ischemia/reperfusion injury [75]. Finally, because chemotherapy-resistant tumor cells exhibit reduced Ca^{2+} mobilization from intracellular storage depots, a strong relationship between mPTP and cancer has been proposed [76].

The exact molecular composition of mPTP remains unknown, but various models have been proposed. Currently, the mitochondrial F1/FO ATP synthase [Complex V (CV)] has been proposed as a strong candidate for mPTP [4]. A recent study reported that an appropriate C-ring conformation is required for MPT induction once CV dimers have dissociated [77]. In parallel, it has been suggested that the β subunit of CV is important as an mPTP trigger. Accordingly, mutation of the β subunit affects Ca^{2+}-ATPase activity and increases resistance to Ca^{2+}-dependent mPTP cell death [78]. Finally, during Ca^{2+}-induced MPT, the C subunit (a hydrophobic protein with lipid-like properties) was found to associate with polyphosphate [polyP and polyhydroxybutyrate (PHB)], thus promoting the generation of a water-permeable channel [79]. These major observations suggest that, under appropriate conditions, a major deformation of the CV complex occurs, starting from F1 portions and transmitted to FO, where a pore is generated. Despite this knowledge, the effective contribution of c-subunits to mPTP formation remains elusive. Additionally, a screening approach has identified the mitochondrial protease SPG7 as a major requirement for MPT [80]. Although its molecular contribution to mPTP formation remains unknown, it is reasonable to speculate that the proteolytic activity of SPG7 is required for ATPase rearrangement during MPT. Notably, the recent genetic manipulation of key components of CV in a specific cell type indicates its role in MPT [81,82]. Thus, further investigations are still needed to determine whether any or all of these subunits are potential components of the mPTP.

Box 2. Mitochondria-Associated Membranes

Communication between the ER and mitochondria has been proven essential for several cellular processes. One mechanism by which this interplay is achieved is by close juxtaposition between these organelles via MAMs.

The physical interaction between the mitochondria and ER represents approximately 20% of the total mitochondrial network, which does not include membrane fusion, but is mediated through protein bridges [57,58].

Formation of these contact sites appears to be required for some key cellular events. Until now, various roles of the MAM fraction have been described as being related to their distinctive lipid and protein composition, and, based on this protein composition, MAMs were found to be crucial for specific cellular processes [57].

Among these MAM-dependent processes, the most significant are the transport of Ca^{2+} [due to the presence of IP3R, sigma-1 receptor (Sig1R), PML, and ryanodine receptor]; the regulation of morphology, dynamics, and functions of mitochondria (including mitochondria-shaping proteins and the chaperone proteins mitofusin 1 and 2); the formation of autophagosomes (VAPB-PTPIP51 tethering); autophagy, apoptosis, and programmed cell death (i.e., Bcl-2 and HCLS1-binding protein 3); the import of phosphatidylserine into mitochondria from the ER for decarboxylation to phosphatidylethanolamine; inflammation [involving adaptor ASC, thioredoxin-interacting protein (TXNIP) and NLRP3]; ER stress [ER resident protein 44 (ERp44) and 75-kDa glucose-regulated protein (GRP75)]; lipid synthesis and trafficking (including FACL1, FACL4, SERAC1, PSS-1, and PSS-2); ROS production (through p66shc and Ero1-Lα); and cell survival.

Given that MAMs have such important roles in all these essential physiological cellular processes, it is not surprising that perturbations in MAM composition lead to disease [57]. In recent years, several proteins have been linked to neuronal diseases, such as movement disorders (Parkinson's disease), neurodegenerative diseases (schizophrenia, dementia, and seizures) and genetic disorders (Huntington's disease). Therefore, targeting MAM function and structure might be a novel strategy for treating such diseases. However, further studies are required to reveal the as yet unelucidated molecular mechanisms.

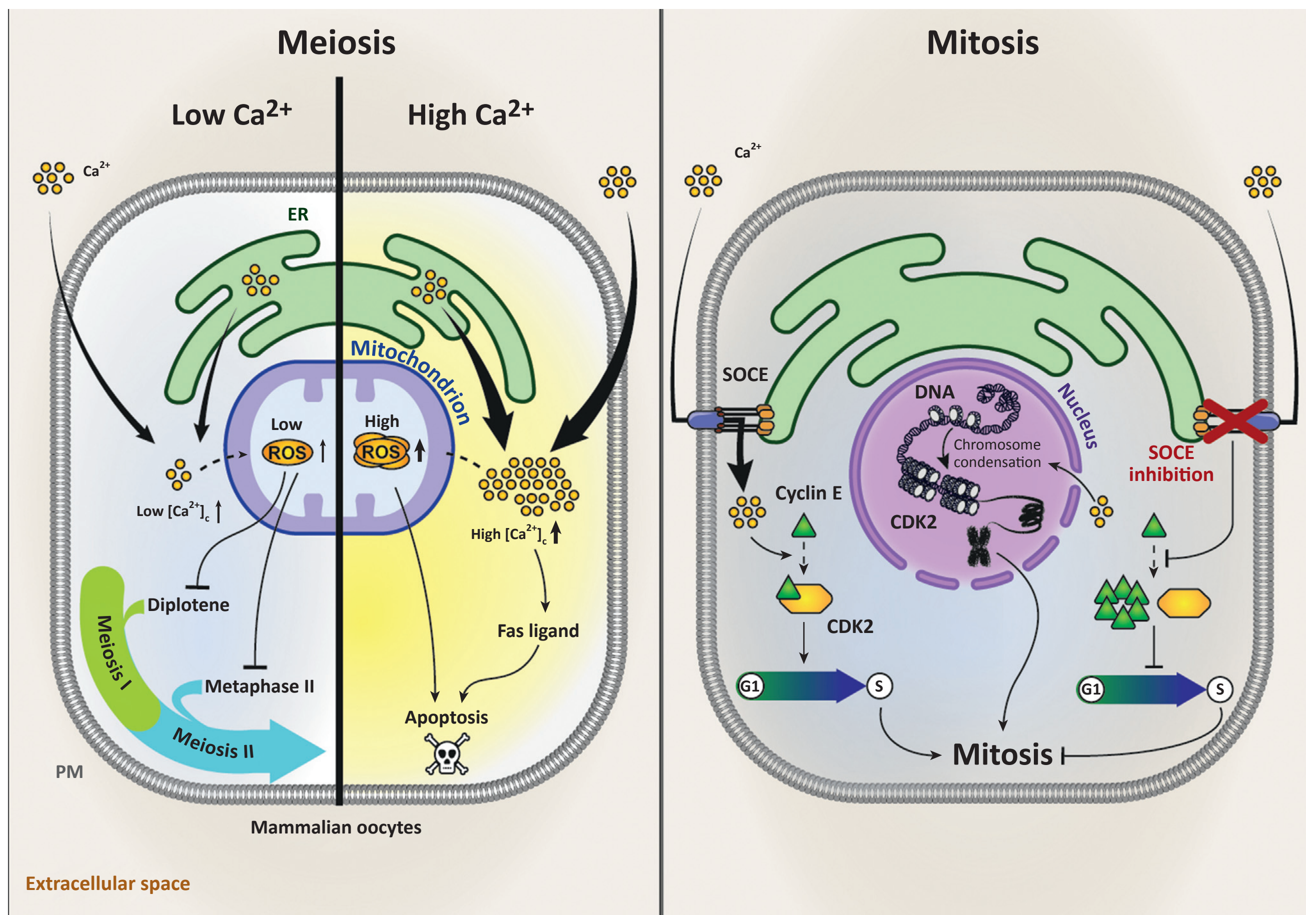


Figure 2. Extracellular calcium concentration ([Ca^{2+}]) and cellular [Ca^{2+}] ([Ca^{2+}]$_c$) Are Key Regulators of Cell Cycle Progression, Arrest, and Apoptosis. Intra- and extracellular Ca^{2+} stores are essential for cell cycle progression. Abbreviations: CDK2, cyclin-dependent kinase 2; ER, endoplasmic reticulum; SOCE, store-operated Ca^{2+} entry.

In addition, IP3R1 has been observed as a tumor suppressor target, physically and functionally interacting with BRCA1 and exerting a proapoptotic function [34].

The Ca^{2+} signaling cascade also triggers neuronal cell death via a process called excitotoxicity, in which excessive glutamate is released from neurons and glial cells and consequently induces a massive and rapid translocation of external Ca^{2+} into cerebral tissues through the activation of ROC and VGCC channels [35] (Figure 4). Following Ca^{2+} overload, most neuronal cells undergo apoptosis upon assembly of the mPTP. Alternatively, Ca^{2+} may activate Ca^{2+}-binding calpain proteases, thus promoting the degradation of structural and enzymatic proteins, which in turn induce cell death. Apoptosis also occurs after a prolonged period of subtle changes in Ca^{2+} levels, which gradually impair the function of Ca^{2+} channels and sensors residing in the PM, ER, and mitochondria.

Such Ca^{2+}-dependent alterations reflect different unfavorable conditions affecting the brain environment. The most-striking event is the activation of the neuronal cell death throughout ischemic injury. Accordingly, elevated Ca^{2+} levels have been associated with ischemic

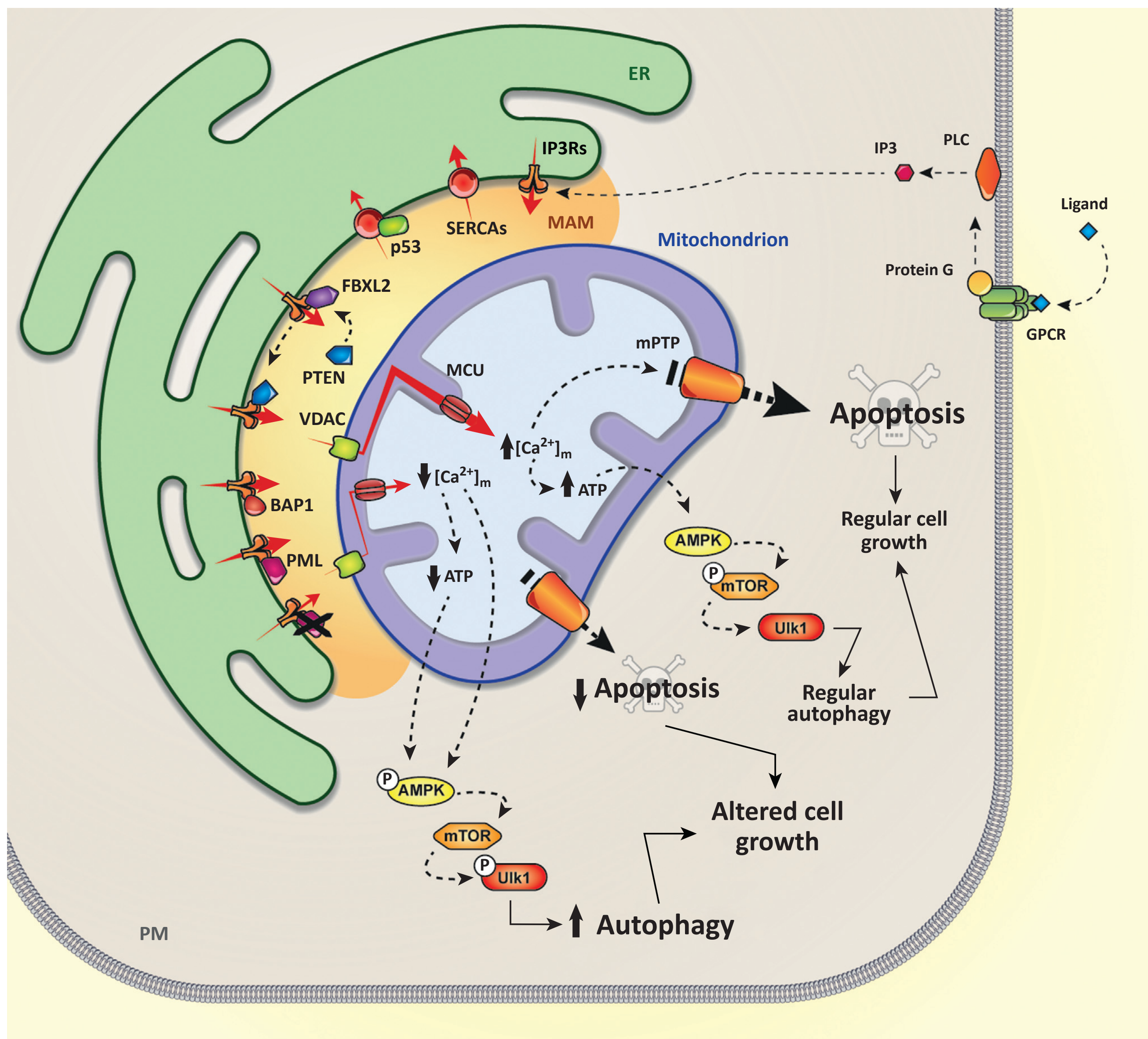
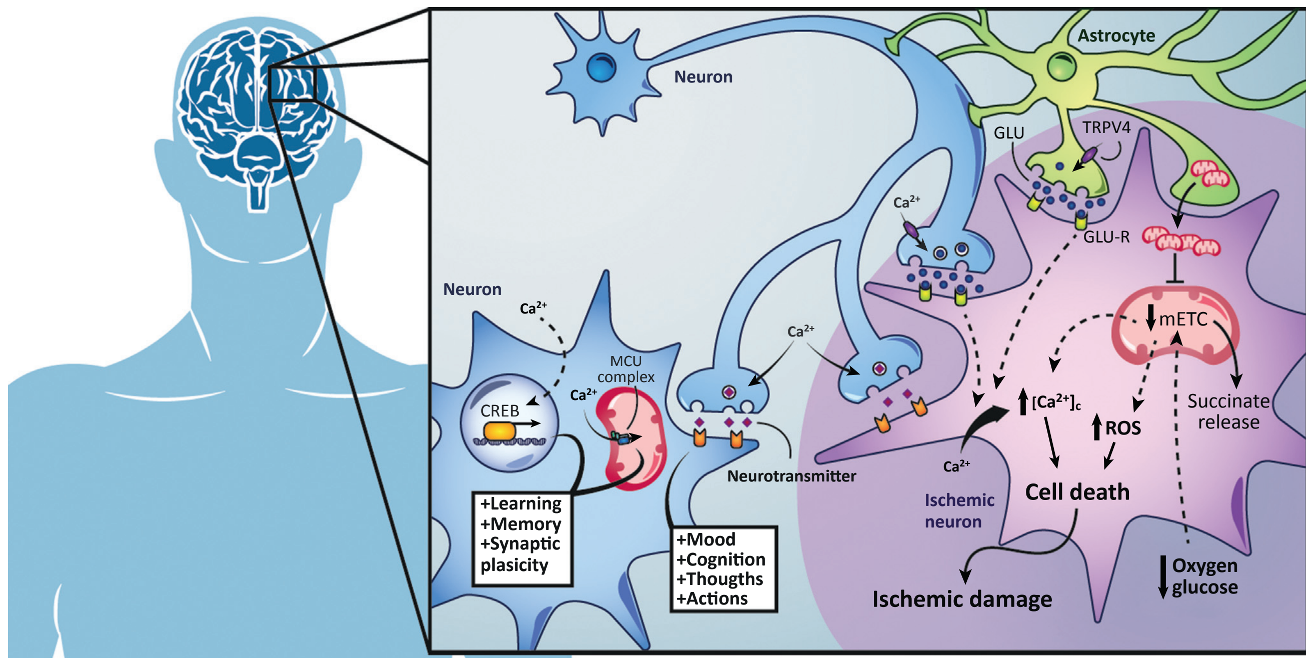


Figure 3. The Role of Calcium (Ca^{2+}) Signals in Triggering Apoptosis and Cancer. Ca^{2+} dynamics occurring at the endoplasmic reticulum (ER), mitochondria-associated membrane (MAMs), and mitochondria have a key role in cell death processes. Abbreviations: AMPK, AMP-activated protein kinase; BAP1, BRCA1-associated protein 1; FBXL2, F-box and leucine-rich repeat protein 2; GPCR, G-protein-coupled receptors; IP3, inositol 1,4,5-trisphosphate; IP3R, IP3 receptor; MCU, mitochondrial calcium uniporter; mPTP, mitochondrial permeability transition pore; mTOR, mechanistic target of rapamycin; PLC, phospholipase C; PM, plasma membrane; PML, promyelocytic leukemia protein; PTEN, phosphatase and tensin homolog; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; VDAC, voltage-dependent anion channel.

stroke [36]. A recent study suggested that Ca^{2+}/calmodulin-dependent protein kinase kinases (CaMKK)-β (activated by elevation of intracellular Ca^{2+} levels) are a target for reducing long-term disability after ischemic stroke [37]. Similarly, the Ca^{2+}-permeable cation channel transient receptor vanilloid 4 (TRPV4) contributes to the Ca^{2+} and glutamate dynamics occurring during neurodegeneration after stroke [38] (Figure 4). However, increased $[Ca^{2+}]$ may also result in

Cell Press REVIEWS



Trends in Cell Biology

Figure 4. Schematic Cartoon of Calcium (Ca^{2+}) Signaling in Brain Circuits. In neurons, Ca^{2+} waves regulate activity-dependent gene expression and accomplish learning, memory, and actions through several Ca^{2+}-dependent proteins. Abbreviations: CREB, cAMP response element binding; GLU-R, glutamate receptor; ROS, reactive oxygen species; MCU, mitochondrial calcium uniporter; mETC, mitochondrial electron transport chain; TRPV4, transient receptor potential vanilloid 4.

smaller cerebral infarct volumes and better clinical outcomes [39]. Consequently, the pathophysiological role of Ca^{2+} during ischemic stroke and the molecular mechanisms involved remain unknown. A clearer picture regarding the Ca^{2+} dynamics occurring during ischemia may be found by considering the mitochondrial environment. As demonstrated, CD38 and cADP ribose trigger the entry of astrocytic mitochondria into adjacent neurons during an ischemic attack. As a result, this mitochondrial–neuroglial crosstalk provides bioenergetic and metabolic support for damaged neurons and improves neurorecovery and neuroprotective mechanisms after stroke injury [40]. In addition, loss-of-function or altered expression of subunits and intermediates of the electron transport chain (ETC) occur during stroke [41] (Figure 4). During oxygen-glucose deprivation, neurons release succinate (a substrate for the ETC complex II), which affects mitochondrial integrity and triggers cell death through ROS production and cellular Ca^{2+} accumulation (Figure 4). These discoveries present novel therapeutic approaches for stroke treatment. Using a specific molecule, it is possible to counteract succinate-mediated cell death by preserving the proper functioning of the mitochondrial population [42].

Autophagy

Autophagy is the process by which cellular material is delivered to lysosomes for degradation and recycling. Several studies have underlined the importance of Ca^{2+} signaling for autophagy. In particular, Ca^{2+} ions have been shown to have an important role at the ER–mitochondrial and ER–lysosomal interfaces [43]. During starvation, Ca^{2+} is released from the lysosome through MCOLN1, thereby inducing the activation of transcription factor EB (TFEB) dephosphorylation and the phosphatase calcineurin. In such conditions, TFEB translocates to the nucleus and

CellPress REVIEWS

activates the transcription of lysosomal and/or autophagic pathway gene expression, thus supporting the role of the lysosome as a signaling hub for both Ca^{2+} and autophagy [44].

The core of autophagic machinery is also regulated by IP3R3, IP3R1, and their capacity to regulate Ca^{2+} dynamics (Figure 3). An impairment of IP3Rs-mediated Ca^{2+} release leads to a reduction in mitochondrial Ca^{2+} uptake, which compromises oxidative phosphorylation and stimulates autophagy [45]. This event results in the activation of AMP-activated protein kinase (AMPK), which subsequently activates prosurvival autophagy. In this process, PML appears to have key role, whereby it coordinates Ca^{2+} transfer from the ER to mitochondria via a molecular mechanism involving the AMPK-mammalian target of rapamycin-UNC-51-like kinase 1 (AMPK-MTOR-ULK1) network (Figure 3) [46]. However, during starvation, the upregulation of Ca^{2+}-binding proteins (CaBPs), together with the direct binding of Beclin 1 to the ligand-binding domain of IP3Rs, underpins the sensitization of Ca^{2+} signaling through IP3Rs, leading to autophagy stimulation [47]. Considering that Ca^{2+}-driven oxidative phosphorylation is a primary energy source for the normal function of several cell types, future studies may find a role for the IP3R-dependent autophagic mechanism in other human diseases.

Ca^{2+} in Excitable Cells: Synaptic Transmission and Contraction

Ca^{2+} signaling routes have a pivotal role as a second messenger of electrical signaling in excitable cells [2]. In neural populations, the consequences of excessive Ca^{2+} signals vary according to their magnitude and duration and the type of neuron affected. For example, synaptic transmission, a chemical event that is regulated via the release, diffusion, and receptor binding of neurotransmitter molecules, is a consequence of elevated $[Ca^{2+}]_c$ levels via release from the ER or extracellular Ca^{2+} influx. Interestingly, it was proposed that the cooperative gating of clustered Ca^{2+} channels (~eight) generates a more-persistent and greater Ca^{2+} influx, thereby facilitating Ca^{2+} entry into neurons [48]. Mitochondria also have a primary role in Ca^{2+} signaling during synaptic transmission. Dysregulation of the components required for $[Ca^{2+}]_m$ entry via the MCU complex (comprising MCU and several regulators, including MICU1 and MCUR1 [12]) results in the inhibition of Ca^{2+} signaling and Ca^{2+}-dependent processes (Figure 4). Loss-of-function of MICU1 was recently found in a cohort study of subjects with brain disorders [49], a phenotype determined by excessive mitochondrial Ca^{2+} loading, which caused cytoplasmic Ca^{2+} buffering.

The role of Ca^{2+} in neuronal cells is not limited to synaptic transmission. Intracellular Ca^{2+} waves permit the modulation of numerous signaling cascades in neuronal cells upon the activation of Ca^{2+}-dependent proteins, such as CaMKKs, CREB, and mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERKs). For example, increases in $[Ca^{2+}]_c$ stimulate Ca^{2+}-dependent adenylyl cyclase and protein kinase A (PKA) activities, thereby promoting the activation of CREB, a critical event that regulates learning and memory as well as neuronal gene expression and synaptic plasticity [50] (Figure 4). A recent study demonstrated that Ca^{2+}-dependent events are particularly important for excitation–transcription (E-T) coupling in interneurons. Given that the activity of interneurons is of critical significance for learning, memory, and plasticity, this finding may help to elucidate the biochemical mechanisms that connect interneuron activity to neuronal activity and behavioral phenotypes [51].

In addition to the function of Ca^{2+} in neuronal cells, Ca^{2+} channel activation and subsequent Ca^{2+} entry are necessary events for hormone secretion [52], muscle development, homeostasis, regeneration, and muscle contraction in smooth muscle and cardiac cells [53,54]. Recent studies have shown that loss of MICU1 also has a critical role in cell survival and tissue regeneration in these cells [55]. Accordingly, MICU1 = null mice exhibit severe ataxia and

CellPress REVIEWS

muscle weakness correlated with excessive resting mitochondrial Ca^{2+} levels [56]. Interestingly, this phenotype was similar to that found in a fibroblast cell line derived from patients expressing loss-of-function mutations in MICU1 [49]. Deletion of MCUR1 in vascular endothelial cells resulted in decreased MCU-mediated $[Ca^{2+}]_m$ uptake and impaired mitochondrial bioenergetics, cell proliferation, and migration [57]. Loss of MCU abrogated Ca^{2+} uptake, which protected against cell death in an ischemia-reperfusion mouse model and resulted in a lack of contractile responsiveness. Most importantly, the primary function of the MCU complex in the heart is to regulate mitochondrial Ca^{2+} uptake to meet the energetic demand necessary for contractile activity; these findings suggest that MCU is dispensable for homeostatic cardiac function [58]. Consistent with this hypothesis, deletion of the channel responsible for mitochondrial Ca^{2+} influx did not result in major cardiac phenotypes. Conversely, deletion of the $Na^+/Ca^{2+}/Li^+$ (NCLX) channel (the primary mechanism for mitochondrial Ca^{2+} extrusion in excitable cells) resulted in a lethal phenotype characterized by severe myocardial dysfunction and fulminant heart failure due to excessive Ca^{2+} overload, increased ROS production, and necrotic cell death [59].

The Ca^{2+} signaling affecting cardiac muscle may also be determined by epigenetic mechanisms, such as histone modifications, DNA methylation, and miRNA. Lack of histone deacetylase (HDAC)-1/-2 inhibits fetal Ca^{2+} signaling, and HDAC genes are indispensable for cardiac morphogenesis and contractility [60]. Using high-throughput functional screening of the human miRNAome, a series of miRNAs regulating Ca^{2+} signaling in cardiac muscle were identified by their interaction with SERCA2a mRNA. The most potent miRNA was miR-25, which was upregulated in myocardial samples from patients with severe heart failure [61]. In addition, mir-133a regulates cardiac hypertrophy by controlling IP3R-2 levels (the predominant isoform found in the heart) [62]. Similarly, hypertrophy may be caused by Ca^{2+} fluxes arising from endothelin-1-stimulated IP3Rs-2 opening. $[Ca^{2+}]$ increases activate the calcineurin (CnA)/NFAT pathway to promote hypertrophic signaling [63]. Despite this evidence, an IP3R-2 = knockout mouse model showed no significant difference in cardiac function or hypertrophy [64]. Further information is needed to understand the effective role of IP3R-2 as well as the involvement of other IP3Rs isoforms in healthy and diseased heart [64].

Inflammasome Activation

NOD-like receptor protein 3 (NLRP3) is the most extensively studied protein of the inflammasome family of caspase-1-activating complexes, which are activated by diverse stimuli during infection and metabolic disease [65]. This protein has a critical role in the initiation and progression of several diseases, such as neurological disorders [65]. Ca^{2+} signaling has been proposed as a key regulator in the proximal steps upstream of NLRP3 inflammasome activation [66] (Figure 5).

The Ca^{2+}-sensing receptor (CaSR) activates NLRP3 by increasing intracellular Ca^{2+} through the modulation of PLC activity and the induction of Ca^{2+} release from ER stores [67]. Additionally, CaSR stimulation results in decreased cellular cAMP concentration, which independently activates the NLRP3 inflammasome. Interestingly, in autoinflammatory diseases known as cryopyrin-associated periodic syndromes (CAPS), the inhibition of intracellular Ca^{2+} signaling pathways blocks lipopolysaccharide (LPS)-mediated NLRP3 inflammasome activation in the peripheral blood mononuclear cells (PBMCs) of patients with CAPS [67]. By contrast, another study showed that the cAMP pathway is not involved in inflammasome activation [68]. cAMP induction by the direct stimulation of adenylyl cyclases with forskolin did not induce interleukin-1β (IL-1β) release, and the adenylyl cyclase inhibitor SQ22536 had no effect on inflammasome stimulation in monocytes [68]. In addition, increased extracellular Ca^{2+} was

Cell Press REVIEWS

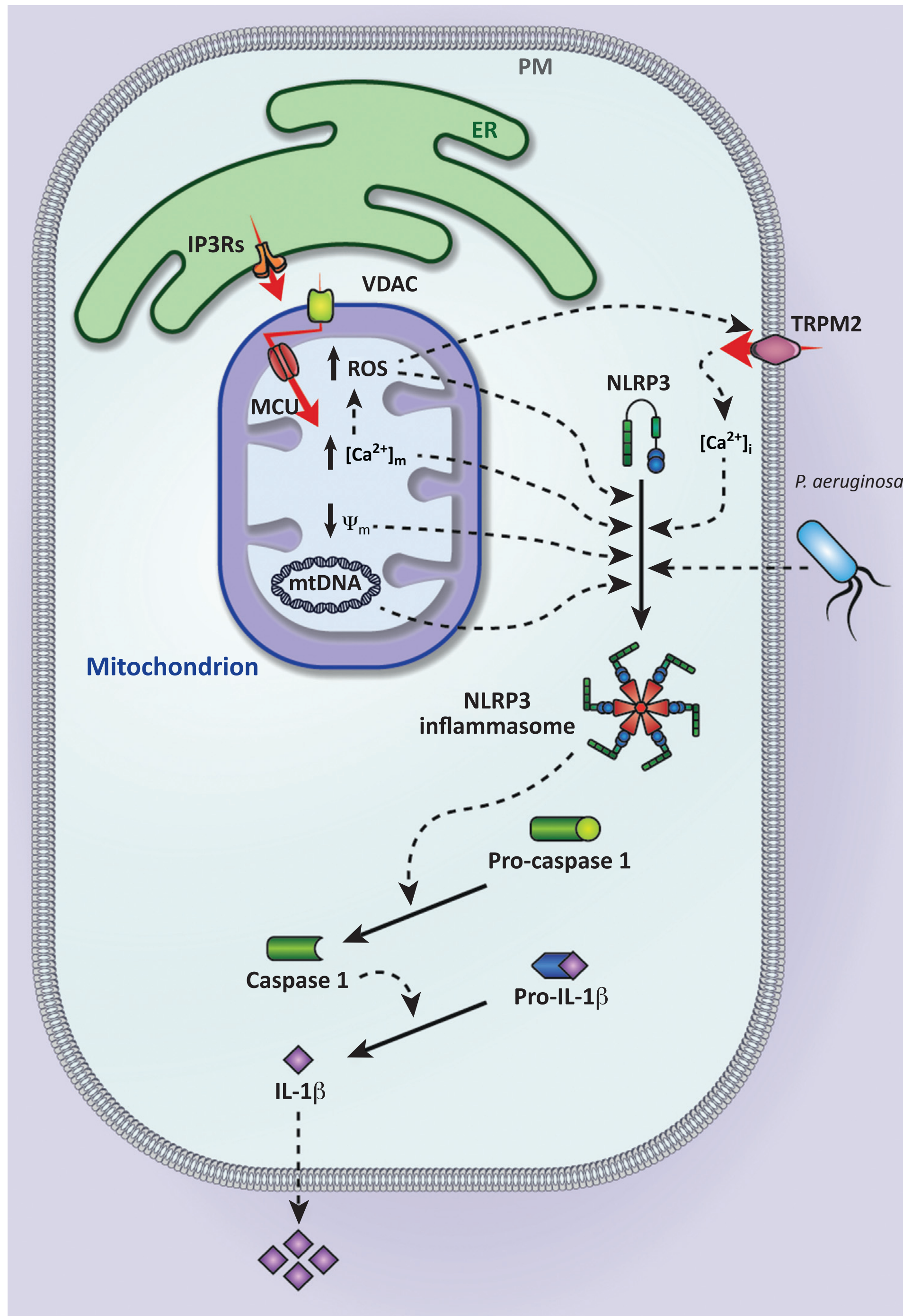


Figure 5. Mechanisms of Inflammasome Activation through Calcium (Ca^{2+}) Mobilization. Ca^{2+} signaling has been proposed to trigger mitochondrial dysfunction, including increased mitochondrial reactive oxygen species (mtROS) and oxidized mitochondrial (mt)DNA and is implicated in NOD-like receptor protein 3 (NLRP3) inflammasome activation. Abbreviations: CASR, calcium-sensing receptor; ER, endoplasmic reticulum; IP3Rs, inositol 1,4,5-trisphosphate receptors; IL-1β, interleukin-1β; MCU, mitochondrial Ca^{2+} uniporter; *P. aeruginosa*, *Pseudomonas aeruginosa;* PLC, phospholipase C; PM, plasma membrane; TRPM2, transient receptor potential melastatin 2.

CellPress REVIEWS

shown to activate the NLRP3 inflammasome via the stimulation of GPRC6A, a member of family C of the G-protein-coupled receptors, and via the phosphatidylinositol/Ca^{2+} pathway (Figure 5). Inflammatory responses can be inhibited by antagonists of CaSR, which is significantly reduced in monocytes from GPRC6A$^{-/-}$ mice [68]. Alternatively, in response to ATP and other stimuli, Ca^{2+} mobilization from the ER and influx from the extracellular space induces mitochondrial damage, including increased mitochondrial ROS (mtROS) production, loss of membrane potential, and the release of mitochondrial DNA into the cytosol, thus activating the NLRP3 inflammasome [69] (Figure 5). Importantly, the inhibition of Ca^{2+} signaling blocks NLRP3 inflammasome activation without affecting the activation of the absent in melanoma 2 (AIM2) and NLR family CARD domain-containing protein 4 (NLRC4) inflammasomes [67,69]. ROS released from damaged mitochondria trigger a Ca^{2+} influx via the PM cation channel transient receptor potential melastatin 2 (TRPM2) [70]. Blocking TRPM2-mediated Ca^{2+} mobilization drastically impaired caspase-1 activation and did not affect mitochondrial ROS production. These observations identify a novel role for TRPM2 in linking oxidative stress to Ca^{2+} mobilization, which ultimately results in NLRP3 inflammasome activation [70] (Figure 5).

The role of Ca^{2+} in inflammasome induction and NLRP3 recruitment has also been observed in cystic fibrosis (CF) [71]. Mutations in cystic fibrosis transmembrane conductance regulator (CFTR) aberrantly influence Ca^{2+} signaling, mitochondrial physiology, and the levels of *Pseudomonas aeruginosa*-dependent NLRP3-inflammasome activation. The reintroduction of wild-type CFTR or treatment with VX-809 (a CFTR corrector that partially restores CFTR function) abrogated *P. aeruginosa*-dependent mitochondrial dysfunctions and NLRP3 recruitment. It has also been suggested that a specific Ca^{2+} channel links these unfavorable phenomena. Indeed, the amplitude of *P. aeruginosa*-dependent NLRP3 activation is affected by MCU [71] (Figure 5).

Despite these findings, the role of Ca^{2+} signaling in NLRP3 activation remains controversial. A comparison of the contribution of decreased cytosolic K^+ concentrations and increased cytosolic Ca^{2+} in NLRP3 activation [72] revealed that: (i) increased $[Ca^{2+}]_c$ is not a necessary or sufficient signal for NLRP3 inflammasome activation by endogenous ATP-gated P2X7 receptor channels, the bacterial ionophore nigericin, or LLME-induced lysosomal disruption; (ii) agonists for three Ca^{2+}-mobilizing G-protein-coupled receptors (FPR, P2Y2R, and CaSR) are ineffective as robust activators of NLRP3 signaling when directly compared with the K^+ efflux agonists; and (iii) BAPTA blocks NLRP3 activation independently of its expected inhibitory effects on Ca^{2+} signaling [72]. Thus, further studies are needed to fully explore the biological significance of Ca^{2+} signaling in NLRP3 inflammasome activation.

Concluding Remarks

Ca^{2+} represents an essential signal for every aspect of cellular life. Each Ca^{2+} wave is a specific and unique code that is indispensable for the transfer of important information based on its precise amplitude, frequency, timing, and duration. Living cells have evolved to invest much of their energy to respond to changes in $[Ca^{2+}]$ by developing a complex intracellular Ca^{2+} signaling network. However, a cell should utilize Ca^{2+} with care. Slight remodeling of the amplitude or the temporal and spatial control of Ca^{2+} signals can trigger deleterious processes that contribute to several pathogenic states, such as cancer, inflammation, heart failure, and neurodegeneration. Thus, it is essential to extend our knowledge of the numerous pathways by which Ca^{2+} regulates life and death decisions. To address this challenge, it is necessary to refine the conventional methods used to assess Ca^{2+} dynamics. Among them, Ca^{2+} fluorescent indicators represent the most-feasible means of visualizing Ca^{2+} dynamics [73]. These indicators permit the measurement of Ca^{2+} changes in subcellular to multicellular

Outstanding Questions

Do intracellular Ca^{2+} fluctuations regulate entry into a subsequent cell cycle phase? Conversely, does the succession of cell cycle phases modulate intracellular Ca^{2+} concentration?

Considering the new emerging role of Ca^{2+} in inflammasome activation, would it be useful to understand the mechanism involved in the $[Ca^{2+}]_c$ changes in intracellular compartments during inflammation?

Could Ca^{2+} signaling be considered a target to counteract several diseases associated with inflammasome activation?

Is it conceivable to develop new drugs that modulate Ca^{2+} content inside the cell to promote or prevent cell death *in vivo*?

Is it plausible that the degradation or modulation of a specific Ca^{2+} uniporter subunit could prevent or induce Ca^{2+}-mediated cell death?

Could the development of new instrumentation, methodologies, and probes for the study of Ca^{2+} signaling be applied to clinical research and diagnostics?

environments, as well as at high speed or as time-lapse images. However, most of these indicators have limited applications for *in vivo* research and do not represent reliable diagnostic tools. Despite several methodological improvements, such as the development of two-photon and super-resolution microscopy, we remain unable to trace Ca^{2} dynamics over a period of months or following a specific curative treatment. Only after addressing these challenges will we be able to develop novel targets and treatments for chronic human diseases (see Outstanding Questions).

Acknowledgments

P.P. is grateful to Camilla degli Scrovegni for continuous support. The authors thank Luigi Sbano for helping with the preparation of the figures. P.P. is supported by the Italian Ministry of Education, University and Research; the Italian Ministry of Health; Telethon (GGP15219/B); the Italian Association for Cancer Research (AIRC: IG-18624); and by local funds from the University of Ferrara. C.G. is supported by local funds from the University of Ferrara, the Italian Association for Cancer Research (AIRC: IG-19803), the Italian Ministry of Health, and by a Fondazione Cariplo grant.

References

1. Clapham, D.E. (2007) Calcium signaling. *Cell* 131, 1047–1058
2. Santulli, G. and Marks, A.R. (2015) Essential roles of intracellular calcium release channels in muscle, brain, metabolism, and aging. *Curr. Mol. Pharmacol.* 8, 206–222
3. Bhosale, G. *et al.* (2015) Calcium signaling as a mediator of cell energy demand and a trigger to cell death. *Ann. N. Y. Acad. Sci.* 1350, 107–116
4. Morciano, G. *et al.* (2015) Molecular identity of the mitochondrial permeability transition pore and its role in ischemia-reperfusion injury. *J. Mol. Cell Cardiol.* 78, 142–153
5. Bartlett, R. *et al.* (2014) The P2X7 receptor channel: recent developments and the use of P2X7 antagonists in models of disease. *Pharmacol. Rev.* 66, 638–675
6. Mulier, M. *et al.* (2017) TRP channel pores and local calcium signals. *Cell Calcium* 66, 19–24
7. Vandecaetsbeek, I. *et al.* (2011) The Ca2+ pumps of the endoplasmic reticulum and Golgi apparatus. *Cold Spring Harb. Perspect. Biol.* 3, a004184
8. Galione, A. (2015) A primer of NAADP-mediated Ca(2+) signalling: from sea urchin eggs to mammalian cells. *Cell Calcium* 58, 27–47
9. Morgan, A.J. *et al.* (2013) Bidirectional Ca(2)(+) signaling occurs between the endoplasmic reticulum and acidic organelles. *J. Cell Biol.* 200, 789–805
10. Raffaello, A. *et al.* (2016) Calcium at the center of cell signaling: interplay between endoplasmic reticulum, mitochondria, and lysosomes. *Trends Biochem. Sci.* 41, 1035–1049
11. Walweel, K. and Laver, D.R. (2015) Mechanisms of SR calcium release in healthy and failing human hearts. *Biophys. Rev.* 7, 33–41
12. Marchi, S. and Pinton, P. (2014) The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications. *J. Physiol.* 592, 829–839
13. Shanmughapriya, S. *et al.* (2015) Ca2+ signals regulate mitochondrial metabolism by stimulating CREB-mediated expression of the mitochondrial Ca2+ uniporter gene MCU. *Sci. Signal.* 8, ra23
14. Caterino, M. *et al.* (2017) Protein-protein interaction networks as a new perspective to evaluate distinct functional roles of voltage-dependent anion channel isoforms. *Mol. Biosyst.* 13, 2466–2476
15. Stathopulos, P.B. *et al.* (2013) STIM1/Orai1 coiled-coil interplay in the regulation of store-operated calcium entry. *Nat. Commun.* 4, 2963
16. Brandman, O. *et al.* (2007) STIM2 is a feedback regulator that stabilizes basal cytosolic and endoplasmic reticulum Ca2+ levels. *Cell* 131, 1327–1339
17. Humeau, J. *et al.* (2017) Calcium signaling and cell cycle: progression or death. *Cell Calcium* Published online July 25, 2017. http://dx.doi.org/10.1016/j.ceca.2017.07.006
18. Tiwari, M. *et al.* (2017) Calcium signaling during meiotic cell cycle regulation and apoptosis in mammalian oocytes. *J. Cell Physiol.* 232, 976–981
19. Cardenas, C. *et al.* (2016) Selective vulnerability of cancer cells by inhibition of Ca(2+) transfer from endoplasmic reticulum to mitochondria. *Cell Rep.* 14, 2313–2324
20. Phengchat, R. *et al.* (2016) Calcium ions function as a booster of chromosome condensation. *Sci. Rep.* 6, 38281
21. Mognol, G.P. *et al.* (2016) Cell cycle and apoptosis regulation by NFAT transcription factors: new roles for an old player. *Cell Death Dis.* 7, e2199
22. Kar, P. *et al.* (2016) Control of NFAT isoform activation and NFAT-dependent gene expression through two coincident and spatially segregated intracellular Ca2+ signals. *Mol. Cell* 64, 746–759
23. Chen, Y.W. *et al.* (2016) The STIM1-Orai1 pathway of store-operated Ca2+ entry controls the checkpoint in cell cycle G1/S transition. *Sci. Rep.* 6, 22142
24. Danese, A. *et al.* (2017) Calcium regulates cell death in cancer: roles of the mitochondria and mitochondria-associated membranes (MAMs). *Biochim. Biophys. Acta* 1858, 615–627
25. Shoshan-Barmatz, V. *et al.* (2017) VDAC1 functions in Ca2+ homeostasis and cell life and death in health and disease. *Cell Calcium* 69, 81–100
26. Giorgi, C. *et al.* (2015) p53 at the endoplasmic reticulum regulates apoptosis in a Ca2+-dependent manner. *Proc. Natl. Acad. Sci. U. S. A.* 112, 1779–1784
27. Galluzzi, L. (2016) Novel insights into PML-dependent oncosuppression. *Trends Cell Biol.* 26, 889–890
28. Giorgi, C. *et al.* (2010) PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. *Science* 330, 1247–1251
29. Marchi, S. *et al.* (2012) Selective modulation of subtype III IP(3)R by Akt regulates ER Ca(2)(+) release and apoptosis. *Cell Death Dis.* 3, e304
30. Boulbes, D.R. *et al.* (2011) Endoplasmic reticulum is a main localization site of mTORC2. *Biochem. Biophys. Res. Commun.* 413, 46–52
31. Bononi, A. *et al.* (2013) Identification of PTEN at the ER and MAMs and its regulation of Ca(2+) signaling and apoptosis in a protein phosphatase-dependent manner. *Cell Death Differ.* 20, 1631–1643
32. Kuchay, S. *et al.* (2017) PTEN counteracts FBXL2 to promote IP3R3- and Ca2+-mediated apoptosis limiting tumour growth. *Nature* 546, 554–558

33. Bononi, A. *et al.* (2017) BAP1 regulates IP3R3-mediated Ca2+ flux to mitochondria suppressing cell transformation. *Nature* 546, 549–553
34. Hedgepeth, S.C. *et al.* (2015) The BRCA1 tumor suppressor binds to inositol 1,4,5-trisphosphate receptors to stimulate apoptotic calcium release. *J. Biol. Chem.* 290, 7304–7313
35. Bano, D. and Ankarcrona, M. (2017) Beyond the critical point: an overview of excitotoxicity, calcium overload and the downstream consequences. *Neurosci. Lett.* Published online August 24, 2017. http://dx.doi.org/10.1016/j.neulet.2017.08.048
36. Chung, J.W. *et al.* (2015) Elevated calcium after acute ischemic stroke: association with a poor short-term outcome and long-term mortality. *J. Stroke* 17, 54–59
37. Liu, L. *et al.* (2016) Calcium/calmodulin-dependent protein kinase kinase beta is neuroprotective in stroke in aged mice. *Eur. J. Neurosci.* 44, 2139–2146
38. Rakers, C. *et al.* (2017) TRPV4 channels contribute to calcium transients in astrocytes and neurons during peri-infarct depolarizations in a stroke model. *Glia* 65, 1550–1561
39. Ovbiagele, B. *et al.* (2006) Are elevated admission calcium levels associated with better outcomes after ischemic stroke? *Neurology* 67, 170–173
40. Hayakawa, K. *et al.* (2016) Transfer of mitochondria from astrocytes to neurons after stroke. *Nature* 535, 551–555
41. Schwarz, K. *et al.* (2014) The breathing heart – mitochondrial respiratory chain dysfunction in cardiac disease. *Int. J. Cardiol.* 171, 134–143
42. Wu, B. *et al.* (2017) Succinate-induced neuronal mitochondrial fission and hexokinase II malfunction in ischemic stroke: therapeutical effects of kaempferol. *Biochim. Biophys. Acta* 1863, 2307–2318
43. Perera, R.M. and Zoncu, R. (2016) The lysosome as a regulatory hub. *Annu. Rev. Cell Dev. Biol.* 32, 223–253
44. Medina, D.L. *et al.* (2015) Lysosomal calcium signalling regulates autophagy through calcineurin and TFEB. *Nat. Cell Biol.* 17, 288–299
45. Cardenas, C. *et al.* (2010) Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca2+ transfer to mitochondria. *Cell* 142, 270–283
46. Missiroli, S. *et al.* (2016) PML at mitochondria-associated membranes is critical for the repression of autophagy and cancer development. *Cell Rep.* 16, 2415–2427
47. Decuypere, J.P. *et al.* (2011) Ins(1,4,5)P3 receptor-mediated Ca2+ signaling and autophagy induction are interrelated. *Autophagy* 7, 1472–1489
48. Moreno, C.M. *et al.* (2016) Ca(2+) entry into neurons is facilitated by cooperative gating of clustered CaV1.3 channels. *Elife* 5, e15744
49. Logan, C.V. *et al.* (2014) Loss-of-function mutations in MICU1 cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling. *Nat. Genet.* 46, 188–193
50. Li, B. *et al.* (2016) Sequential ionic and conformational signaling by calcium channels drives neuronal gene expression. *Science* 351, 863–867
51. Cohen, S.M. *et al.* (2016) Excitation-transcription coupling in parvalbumin-positive interneurons employs a novel CaM kinase-dependent pathway distinct from excitatory neurons. *Neuron* 90, 292–307
52. Sabourin, J. *et al.* (2015) Store-operated Ca2+ entry mediated by Orai1 and TRPC1 participates to insulin secretion in rat beta-Cells. *J. Biol. Chem.* 290, 30530–30539
53. Tu, M.K. *et al.* (2016) Calcium signaling in skeletal muscle development, maintenance and regeneration. *Cell Calcium* 59, 91–97
54. Bers, D.M. (2014) Cardiac sarcoplasmic reticulum calcium leak: basis and roles in cardiac dysfunction. *Annu. Rev. Physiol.* 76, 107–127
55. Antony, A.N. *et al.* (2016) MICU1 regulation of mitochondrial Ca(2+) uptake dictates survival and tissue regeneration. *Nat. Commun.* 7, 10955
56. Liu, J.C. *et al.* (2016) MICU1 serves as a molecular gatekeeper to prevent in vivo mitochondrial calcium overload. *Cell Rep.* 16, 1561–1573
57. Tomar, D. *et al.* (2016) MCUR1 is a scaffold factor for the MCU complex function and promotes mitochondrial bioenergetics. *Cell Rep.* 15, 1673–1685
58. Luongo, T.S. *et al.* (2015) The mitochondrial calcium uniporter matches energetic supply with cardiac workload during stress and modulates permeability transition. *Cell Rep.* 12, 23–34
59. Luongo, T.S. *et al.* (2017) The mitochondrial Na+/Ca2+ exchanger is essential for Ca2+ homeostasis and viability. *Nature* 545, 93–97
60. Montgomery, R.L. *et al.* (2007) Histone deacetylases 1 and 2 redundantly regulate cardiac morphogenesis, growth, and contractility. *Genes. Dev.* 21, 1790–1802
61. Wahlquist, C. *et al.* (2014) Inhibition of miR-25 improves cardiac contractility in the failing heart. *Nature* 508, 531–535
62. Drawnel, F.M. *et al.* (2012) Mutual antagonism between IP(3)RII and miRNA-133a regulates calcium signals and cardiac hypertrophy. *J. Cell Biol.* 199, 783–798
63. Higazi, D.R. *et al.* (2009) Endothelin-1-stimulated InsP3-induced Ca2+ release is a nexus for hypertrophic signaling in cardiac myocytes. *Mol. Cell* 33, 472–482
64. Garcia, M.I. and Boehning, D. (2017) Cardiac inositol 1,4,5-trisphosphate receptors. *Biochim. Biophys. Acta* 1864, 907–914
65. Guo, H. *et al.* (2015) Inflammasomes: mechanism of action, role in disease, and therapeutics. *Nat. Med.* 21, 677–687
66. Horng, T. (2014) Calcium signaling and mitochondrial destabilization in the triggering of the NLRP3 inflammasome. *Trends Immunol.* 35, 253–261
67. Lee, G.S. *et al.* (2012) The calcium-sensing receptor regulates the NLRP3 inflammasome through Ca2+ and cAMP. *Nature* 492, 123–127
68. Rossol, M. *et al.* (2012) Extracellular Ca2+ is a danger signal activating the NLRP3 inflammasome through G protein-coupled calcium sensing receptors. *Nat. Commun.* 3, 1329
69. Murakami, T. *et al.* (2012) Critical role for calcium mobilization in activation of the NLRP3 inflammasome. *Proc. Natl. Acad. Sci. U. S. A.* 109, 11282–11287
70. Zhong, Z. *et al.* (2013) TRPM2 links oxidative stress to NLRP3 inflammasome activation. *Nat. Commun.* 4, 1611
71. Rimessi, A. *et al.* (2015) Mitochondrial Ca2+-dependent NLRP3 activation exacerbates the pseudomonas aeruginosa-driven inflammatory response in cystic fibrosis. *Nat. Commun.* 6, 6201
72. Katsnelson, M.A. *et al.* (2015) K+ efflux agonists induce NLRP3 inflammasome activation independently of Ca2+ signaling. *J. Immunol.* 194, 3937–3952
73. Bootman, M.D. *et al.* (2013) Ca2+-sensitive fluorescent dyes and intracellular Ca2+ imaging. *Cold Spring Harb. Protoc.* 2013, 83–99
74. Liu, X. *et al.* (2015) Transient mitochondrial permeability transition mediates excitotoxicity in glutamate-sensitive NSC34D motor neuron-like cells. *Exp. Neurol.* 271, 122–130
75. Morciano, G. *et al.* (2017) Mechanistic role of mPTP in ischemia-reperfusion injury. *Adv. Exp. Med. Biol.* 982, 169–189
76. Bonora, M. and Pinton, P. (2014) The mitochondrial permeability transition pore and cancer: molecular mechanisms involved in cell death. *Front. Oncol.* 4, 302
77. Bonora, M. *et al.* (2017) Mitochondrial permeability transition involves dissociation of F1FO ATP synthase dimers and C-ring conformation. *EMBO Rep.* 18, 1077–1089
78. Giorgio, V. *et al.* (2017) Ca2+ binding to F-ATP synthase beta subunit triggers the mitochondrial permeability transition. *EMBO Rep.* 18, 1065–1076
79. Elustondo, P.A. *et al.* (2016) Mitochondrial permeability transition pore induction is linked to formation of the complex of ATPase C-subunit, polyhydroxybutyrate and inorganic polyphosphate. *Cell Death Discov.* 2, 16070

80. Shanmughapriya, S. *et al.* (2015) SPG7 Is an essential and conserved component of the mitochondrial permeability transition pore. *Mol. Cell* 60, 47–62
81. He, J. *et al.* (2017) Persistence of the mitochondrial permeability transition in the absence of subunit c of human ATP synthase. *Proc. Natl. Acad. Sci. U. S. A.* 114, 3409–3414
82. He, J. *et al.* (2017) Permeability transition in human mitochondria persists in the absence of peripheral stalk subunits of ATP synthase. *Proc. Natl. Acad. Sci. U. S. A.* 114, 9086–9091
83. Bittremieux, M. *et al.* (2016) ER functions of oncogenes and tumor suppressors: Modulators of intracellular Ca(2+) signaling. *Biochim. Biophys. Acta* 1863, 1364–1378
84. Marchi, S. *et al.* (2018) Mitochondrial and endoplasmic reticulum calcium homeostasis and cell death. *Cell. Calcium* 69, 62–72