Biochimica et Biophysica Acta 1833 (2013) 2542–2559

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

Review

The endoplasmic reticulum and junctional membrane communication during calcium signaling☆

Andy K.M. Lam, Antony Galione *

Department of Pharmacology, University of Oxford, Mansfield Road, Oxford OX1 3QT, UK

ARTICLE INFO

Article history:
Received 18 February 2013
Received in revised form 3 June 2013
Accepted 3 June 2013
Available online 13 June 2013

Keywords:
Endoplasmic reticulum
Calcium
Inositol trisphosphate
Cyclic ADP-ribose
NAADP
Organelle

ABSTRACT

The endoplasmic reticulum is a major organelle in all eukaryotic cells which performs multiple functions including protein and lipid synthesis and sorting, drug metabolism, and Ca^{2+} storage and release. The endoplasmic reticulum, and its specialized muscle counterpart the sarcoplasmic reticulum, is the largest and most extensive of Ca^{2+} storage organelle in eukaryotic cells, often occupying in excess of 10% of the cell volume. There are three major components of Ca^{2+} storage organelles which mediate their major functions: Ca^{2+} uptake, mediated by pumps and exchangers; storage enhanced by luminal Ca^{2+} binding proteins, and Ca^{2+} mobilization mediated by specific ion channels. Ca^{2+} mobilization from the endoplasmic reticulum plays a central role in Ca^{2+} signaling. Through Ca^{2+} release channels in its membrane, the pervading and plastic structure of the endoplasmic reticulum allows Ca^{2+} release to be rapidly targeted to specific cytoplasmic sites across the whole cell. That several endoplasmic reticulum Ca^{2+} release channels are also activated by Ca^{2+} itself, contributes to endoplasmic reticulum membrane excitability which is the principal basis for generating spatio-temporal complex cellular Ca^{2+} signals, allowing specific processes to be regulated by this universal messenger. In addition, the endoplasmic reticulum forms discrete junctions with the plasma membrane and membranes of organelles such as mitochondria and lysosomes, forming nanodomains at their interfaces that play critical roles in Ca^{2+} signaling during key cellular processes such as cellular bioenergetics, apoptosis and autophagy. At these junctions key Ca^{2+} transport and regulatory processes come into play, and a recurring theme in this review is the often tortuous paths in identifying these mechanisms unequivocally. This article is part of a Special Issue entitled: Functional and structural diversity of endoplasmic reticulum.

1. Introduction

Ca^{2+} ions are the most commonly employed signal transduction element in intracellular signaling [1]. In contrast with other intracellular messengers, Ca^{2+} is neither synthesized or metabolized. Instead its storage and circulation are controlled in cells by the regulation of channels, pumps and exchangers, which act to determine spatially and temporally the precise concentrations in the cytoplasm and other cellular compartments. Ca^{2+} ions are highly buffered in the cytoplasm, with individual free Ca^{2+} ions on average diffusing less than 1 μm over a few μs before being captured by binding proteins or translocated by pumps or transporters [2]. Ca^{2+} ions are thus inherently highly localized messengers, and Ca^{2+} entry across the plasma membrane will only affect sub-plasma membrane targets. In contrast, Ca^{2+} storage organelles may be positioned throughout the cell to target Ca^{2+} precisely to cytoplasmic Ca^{2+} effector proteins throughout the cell. It is important to consider Ca^{2+} changes on both sides of the organellar membrane. Increased Ca^{2+} loading of stores may itself be an important luminal trigger for opening of Ca^{2+} release channels from within [3]. Release of Ca^{2+} into the cytoplasm from the ER will also lower luminal Ca^{2+} concentrations and this is in itself a signal for capacitative calcium entry, and may modulate the other major functions of the ER given its multi-tasking roles in protein synthesis and folding, at least over prolonged periods of Ca^{2+} signaling [4]. Chronic ER Ca^{2+} depletion, for example, in Darier's disease due to mutations in SERCA2a pumps, results in abnormalities in protein synthesis and targeting in keratinocytes which rely exclusively on this pump isoform [5].

The ER plays a central role in cellular Ca^{2+} homeostasis and signaling. First it acts as an important component of the cell's Ca^{2+} buffering systems, which act to maintain cytoplasmic free Ca^{2+} concentrations at extremely low levels, typically around 100 nM. Ca^{2+} pumps and exchangers in the ER membrane are critical for this role, which allow the sequestration of Ca^{2+} into the lumen yielding luminal free Ca^{2+} concentrations as high as 100–500 μM, as direct measurements using luminally-targeted Ca^{2+} probes have indicated. This role was first discovered over fifty years ago, when vesicles of fragmented SR were found to cause the relaxation of permeabilized muscle fibers through ATP-dependent Ca^{2+} uptake [6]. Some years later, the family of proteins

☆ This article is part of a Special Issue entitled: Functional and structural diversity of endoplasmic reticulum.

* Corresponding author. Tel.: +44 1865 271633; fax: +44 1865 271853.
E-mail addresses: andy.lam@pharm.ox.ac.uk (A.K.M. Lam), antony.galione@pharm.ox.ac.uk (A. Galione).

0167-4889/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbamcr.2013.06.004

responsible, the sarco-endoplasmic reticulum Ca^{2+} ATPases (SERCA pumps) were purified, sequenced and cloned [7]. They were found to be inhibited by the plant sesquiterpene lactone, thapsigargin, which has been a major tool in the study of ER Ca^{2+} storage and the consequences of perturbing it [8]. The role of the SR/ER as a source of Ca^{2+} signals in cellular processes was again led by studies of striated muscle, with the discovery of Ca^{2+}-induced Ca^{2+} release (CICR) from the SR [9,10]. The role of the ER as a source of Ca^{2+} for cell signaling was noted with the finding that intracellular pools of Ca^{2+} play a major role in stimulus-secretion coupling in exocrine glands [11]. This was extended with the discovery that inositol 1,4,5 trisphosphate (IP_3) mobilized Ca^{2+} from non-mitochondrial stores in permeabilized pancreatic acinar cells [12], and the identification of specific IP_3-gated calcium channels (IP_3 receptors, IP_3Rs) expressed in ER membranes [13].

Studies on the Ca^{2+} release channels of the ER, showed that IP_3Rs in common with RyRs are also activated by Ca^{2+} in addition to IP_3 [14], and that RyRs can be activated by another Ca^{2+} mobilizing intracellular messenger, cyclic ADP-ribose (cADPR) [15]. These messengers serve to modulate the process of CICR mediated by these intracellular channels, allowing the ER network to function as an excitable system displaying regenerative propagating cytoplasmic Ca^{2+} waves. For this reason, the ER, when discussed in terms of neuronal Ca^{2+} signaling, has been termed a "neuron within a neuron' [16], with the chemical excitability of this intracellular membrane network showing several striking parallels with the electrical excitability of the neuronal plasma membrane. Indeed, isolated SR vesicles, when reconstituted into an agarose matrix, can be demonstrated to support regenerative Ca^{2+} waves [17]. This analogy can be further extended with the nervous system by using the term "Ca^{2+} synapses" to describe the close appositions of ER regions with either the plasma membrane or organelles such as mitochondria or lysosomes. Such ER junctions, sites of Ca^{2+} transfer, are emerging as key intracellular microdomains for Ca^{2+} signaling [18] (Fig. 1). They are now considered crucial for generating, coordinating and integrating intracellular Ca^{2+} signals which are a major focus of this review article, along with the molecular components of Ca^{2+} and ion signaling, which are often concentrated at these sites.

2. Morphology and architecture of the ER

The ER was described and named by Keith Porter in his pioneering studies of cell ultrastructure by the then emerging technique of electron microscopy in the 1940s and 1950s [19]. Given the multifarious roles of the ER in cells, the size of this organelle varies between cells depending on their specializations. In terms of its role in Ca^{2+} signaling, it was perhaps not surprising that studies of striated muscle led the way, with the SR, given its specialized role in excitation–contraction coupling, identified as Ca^{2+}-accumulating structures in skeletal muscle by visualizing calcium oxalate accumulation by electron microscopy [20].

2.1. Structure and heterogeneity of Ca^{2+} stores

The ER is a continuous membranous network with distinct morphologies in all nucleated eukaryotic cells. In the case of rough ER, which is particularly well developed in cells that secrete proteins, it appears as flattened sacs or cisternae, where membranes are studded with ribosomes at sites of protein synthesis of integral membrane proteins or those destined for vesicular trafficking including exocytosis. Smooth ER often appears as comprised of more tubular structures, and is a site of lipid synthesis, and plays a major role in Ca^{2+} storage and release. The nuclear envelope is contiguous with the ER, but may also act as a discrete Ca^{2+} store in the perinuclear region of the cell. Under physiological conditions, the ER network has been shown to be a continuous structure [21,22], and may function as a connected Ca^{2+} store. In a series of elegant experiments, Petersen and colleagues showed that Ca^{2+} diffuses more rapidly in the ER lumen of pancreatic acinar cells than it does in the cytoplasm on account of lower luminal buffering. Taking advantage of the polarized nature of acinar cells, they showed that Ca^{2+} uptake by the ER in the basolateral domain could refill fingers of ER in the secretory pole via a lumen rather than a cytoplasmic route, a process they termed "tunneling" [23]. However, functionally in some cells the ER may operate as a series of discrete heterogeneous Ca^{2+} stores. Heterogeneity of the ER in terms of Ca^{2+} signaling is due to the spatial distribution

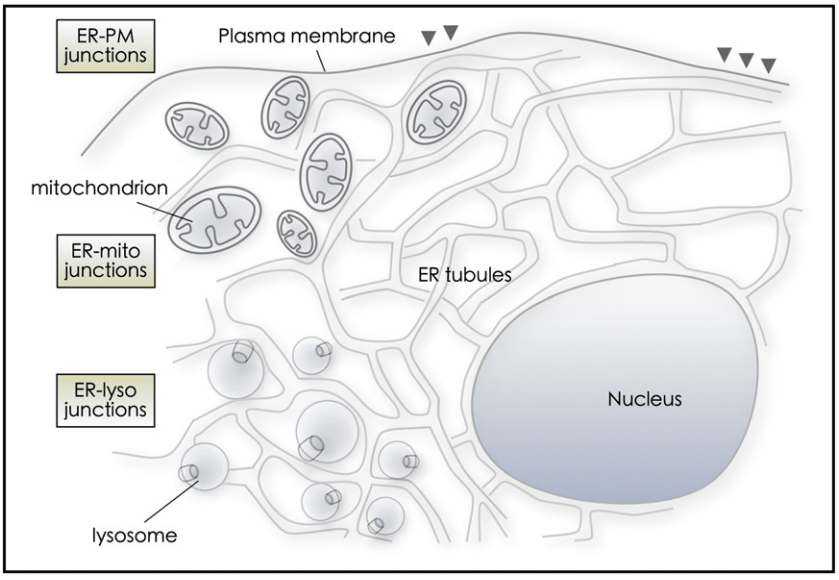

Fig. 1. The ER and junctional membrane communication during Ca^{2+} signaling. Schematic depicting the nanometer-scale junctions formed between the ER and the plasma membrane, mitochondria and lysosomes. The elaborated tubular system of the ER allows multiple parallel, dynamic interactions with organellar membranes, allowing for real-time information exchange using Ca^{2+} as a signaling messenger. The emerging view is that the ER processes and integrates the multiple inputs from various interacting organelles to elicit a resultant Ca^{2+} response of a particular phenotype which may also be modulated in real-time. Supporting this view, loss or malfunction of any of the organellar inputs can result in defects in eliciting the 'intended' or 'default' Ca^{2+} phenotype. For example, Ca^{2+} oscillations ('default') are switched to a sustained low amplitude Ca^{2+} elevation when mitochondrial Ca^{2+} release is inhibited [56] or complete cessation of oscillatory Ca^{2+} transients when SOCE is inhibited [148].

of Ca^{2+} channels, Ca^{2+} binding proteins and pumps/transporters. In astrocytes and smooth muscle cells the major Ca^{2+} release channels of the ER/SR, IP_3R and RyRs are found to segregate to different regions [24]. Such discrete localization of Ca^{2+} release channels can manifest itself functionally in smooth muscle in a striking way, with Ca^{2+} release via RyRs situated in SR under the sarcolemma stimulating openings of Ca^{2+}-activated potassium channels leading to relaxation, whereas Ca^{2+} release from IP_3-sensitive stores situated in the cell's interior leads to myosin light chain kinase activation and hence contraction [25].

Returning to the SR of striated muscle, Ca^{2+} release channels (RyRs) and SERCA Ca^{2+} pumps also appear to be segregated, with specific regions of this organelle separately effecting Ca^{2+} release and uptake [26]. SR is often used to refer to the entire ER in striated and smooth myocytes. However, some authors, correctly in our view, use the term SR when describing the extensive network of smooth reticular membranes that surround myofilaments and closely abut the t-tubule-invaginations of the plasma membrane (sarcolemma) whose primary role is to store and release Ca^{2+} during excitation–contraction coupling. In contrast, the ER proper in these cells, especially in the peri-nuclear regions, is a site of protein synthesis (rough ER) and cell growth (including hypertrophy), and may play a major role in cellular stress such as the unfolded protein response [27]. This division may be too dogmatic since there is some evidence that protein synthesis, for example, may also occur in the SR.

IP_3Rs are not distributed evenly in the ER but may form clusters that may be preformed [28,29] or induced by IP_3 itself [30,31], and these sites may act as hotspots for IP_3-evoked Ca^{2+} release resulting in the formation of strategically positioned Ca^{2+} signaling microdomains for signaling [32]. However, the mechanisms responsible for the crucial localization of Ca^{2+} signaling proteins are poorly understood. Regional variations in organelle Ca^{2+} buffering proteins are also an important source of store heterogeneity. The abundance of calsequestrin, the major luminal Ca^{2+} binding protein in the SR, and its ER counterpart, calreticulin has been demonstrated, which will serve to create non-uniformity in luminal Ca^{2+} [33]. For example, calsequestrin is very abundant in terminal cisternae, the site of major SR Ca^{2+} release during excitation-contracting coupling in striated muscle, but almost absent in longitudinal SR. Calsequestrin has been found to oligomerize to form dense core structures that could act to provide islands of discrete Ca^{2+} rich pools within the SR [34]. Regional variations in luminal Ca^{2+} are important for signaling events, since the major SR/ER Ca^{2+} release channels are all sensitized to cytoplasmic messengers by increased luminal Ca^{2+} levels. Ca^{2+} release by RyRs in striated muscle may cause local depletion of luminal Ca^{2+} visualized with luminally targeted Ca^{2+} indicators (see Section 3) as SCRAPS, discrete regions of low Ca^{2+} in the SR [35]. This phenomenon may help to terminate CICR during excitation–contraction coupling. Local reductions in ER Ca^{2+} may be important for activating specific regional ER–plasma membrane communication sites or puncta for capacitative Ca^{2+} entry (see Section 6.2). Elevation of Ca^{2+} and activation of PKC have been suggested to induce ER fragmentation [36], and fragmentation due to cell permeabilization protocols may explain the quantal nature of IP_3-evoked Ca^{2+} release, whereby the release of Ca^{2+} by sub-optimal concentrations of IP_3 is graded [37].

2.2. Dynamics and plasticity

Although the traditional view of the ER is a fairly static rigid tubular network, this belies the ability of this organelle as a continuously flexing and dynamic reticular structure. A particularly noteworthy example of the plasticity of the ER comes from the study of the maturation of oocytes to eggs competent for fertilization. During the maturation process the ER elaborates particularly in cortical regions where it envelopes cortical granules [38]. The cortical ER is relatively static while internal ER displays more mobile and flexing cisternae. Generally the formation of cortical ER is associated with a higher sensitivity to Ca^{2+} mobilizing messengers and Ca^{2+} excitability, to ensure that a robust Ca^{2+} wave is generated upon fertilization. After fertilization the ER is again reorganized with the result that it now resembles that of the immature egg.

2.3. ER specializations

In addition to the major subdomains of the ER, the rough and smooth ER and the nuclear envelope, several other specialized regions have been proposed to impact upon Ca^{2+} signaling. The cisternal organelle is a neuronal Ca^{2+} storage organelle which closely abuts the plasma membrane at the axon's initial segment [39], and is predominantly found in association with GABAergic synapses in for example, pyramidal cells. It is rich in IP_3Rs, SERCA pumps and calreticulin, and has been proposed to regulate membrane excitability, possibly coupling surface membrane depolarization to Ca^{2+} release which may in turn regulate $GABA_A$ receptor signaling, and thus is an important modulator of action potential initiation.

The spine apparatus is a membrane-bound organelle consisting of stacked smooth ER situated in dendritic spines, and proposed to play a key role in the modulation of synaptic plasticity [40]. These organelles colocalize with the actin-binding protein, thrombospondin [41]. Whether IP_3R and RyRs are present on spine apparatus is not known, however, the SA colocalizes with an intracellular Ca^{2+} pool within the dendritic spine that impacts on mechanisms underlying synaptic plasticity. IP_3R activation is associated with depression of synaptic strength and RyR activation with potentiation.

Annulate lamellae (AL) are cytoplasmic organelles and a subdomain of the ER. They are rich in nucleoporins and are well studied in the context of nuclear envelope breakdown during the cell cycle. ALs have been reported to deliver IP_3Rs to the cortical ER patches of eggs to enhance the Ca^{2+} excitability of the ER during fertilization as described in Section 2.2 [42]. Conversely, IP_3Rs in AL membranes appear to have a lower sensitivity to IP_3, but the mechanism for this is unclear [43].

The nuclear envelope is a relatively well characterized Ca^{2+} store and in recent years Ca^{2+} release from this sub-domain of the ER has been proposed to modulate nuclear pore permeability and hence mRNA transport between nucleus and cytoplasm [44], as well as selective control of nuclear events [45]. The discovery of deep invaginations in the nuclear envelope [46] which express IP_3Rs [47] and RyRs [48] termed the nucleoplasmic reticulum allow for focal Ca^{2+} delivery deep within the nucleus. This has been demonstrated by highly localized two-photon uncaging of IP_3, and this membrane system also shows excitability in that they can support Ca^{2+} waves [47]. It is proposed that such localized Ca^{2+} release may selectively activate specific processes such as the activation of nuclear calcium-regulated transcription factors.

3. Monitoring ER luminal Ca^{2+} levels

Understanding ER Ca^{2+} responses under different pathophysiological conditions requires direct monitoring of ER luminal Ca^{2+} levels, which can only be indirectly surmised from monitoring cytoplasmic Ca^{2+} signals. In most cases, cytosolic Ca^{2+} transients only crudely correlate in time with ER luminal Ca^{2+} changes and cannot be used as surrogates for luminal Ca^{2+} changes despite their ER origin [49,50]. It is therefore imperative to directly monitor in real time $[Ca^{2+}]_{ER}$ when precise measurement is desired. Luminal measurements of Ca^{2+} report free concentrations in the range of 500 μM–1 mM [51]. A variety of approaches based on fluorescent imaging has been employed to report ER luminal Ca^{2+} dynamics in real time. These methods rely on fluorescent Ca^{2+} sensors that are selectively targeted to the ER lumen and are either genetically encoded, i.e. fluorescent protein-based, or synthetic, with appropriate K_ds for luminal Ca^{2+}.

Genetically encoded Ca^{2+} indicators (GECIs) are fluorescent protein(s) concatenated with biological Ca^{2+} sensors [52–55]. Ca^{2+} binding either causes an increase in fluorescent intensity or change in FRET efficiency between the two fluorescent proteins, which can be converted into [Ca^{2+}] [54]. An important advantage of GECIs is that they can be specifically targeted to distinct subcellular compartments such as the nucleus, ER, mitochondria or the plasma membrane. ER-targeted GECIs commonly contain an N-terminal ER signal sequence and a C-terminal KDEL motif to enable their targeting and retention in the ER lumen. The most commonly used ER-targeted GECIs are the YC4.3ER (K_d = 0.8 & 700 μM) [52], D1ER (K_d = 0.8 & 60 μM) [50] and the split cameleon YC7.3ER (K_d = 130 μM) [56]. These sensors are all FRET-based and hence are ratiometric, allowing the precise measurement of ER luminal Ca^{2+} level. However, these FRET-based sensors all suffer from a narrow dynamic range and slow response kinetics, implying that small and/or fast changes in Ca^{2+} may not be readily detected, although D1ER appears to be capable of resolving relatively fast Ca^{2+} transients occurring at 5 Hz in skeletal myocytes [57]. Interestingly, a recent study reported the engineering of a Ca^{2+}-binding site on the green fluorescent protein (GFP), named CatchER, that allows it to act as a low affinity Ca^{2+} sensor itself [58]. Using a kinetically well-defined Ca^{2+} transient, CatchER appeared to exhibit a 2.5-fold faster response kinetics and a considerably larger fluorescent change when compared to that reported by D1ER [58].

Ca^{2+} indicator dyes are fluorescent derivatives based on the specific Ca^{2+} chelators EGTA and/or BAPTA [59,60]. Ca^{2+} dyes are rendered permeant to biological membranes by esterification with an acetoxymethyl group and are retained in the cell through the activity of endogenous esterases [61]. Cellular esterases are ubiquitous and can be found in the cytosol and other subcellular compartments including the ER lumen. Hence, loading is not selective for the cytoplasmic compartment and dye compartmentalization is often a concern during cytoplasmic Ca^{2+} measurements, which can affect both the dynamics and kinetics of the intended signal [62]. However, this normally undesirable property can be harnessed to yield, depending on the dye properties, selective accumulation in organelles such as the mitochondria and the ER lumen under favorable conditions, for example at 37 °C [62,63]. Unless the fluorescence intensity of the dye is low at resting Ca^{2+} levels, the dye molecules accumulated in the cytosol often have to be dialyzed out through either cell permeabilization using mild detergents such as saponin/digitonin or equivalents [51,64,65], or through a patch pipette in the whole-cell configuration [49,51,66,67]. By combining simultaneous dye loading from the same patch pipette (an indicator with different spectral properties), simultaneous recording of ER luminal Ca^{2+}, cytosolic Ca^{2+} and electrophysiological changes has been successfully employed to investigate the temporal and causal relationship between organellar Ca^{2+} dynamics, and has engendered some important and otherwise overlooked insights [49,67].

The most commonly used low affinity Ca^{2+} indicators for ER luminal Ca^{2+} measurement are magFura-2 (K_d = 25–50 μM) and Fluo-5N (K_d = 90 μM). Fluo-5N is probably unique among most other indicators given its very appropriate K_d for measuring $[Ca^{2+}]_{ER}$ and its low resting fluorescence, which allows it to report selectively fluorescent signals originated from the ER lumen with little contamination by cytosolic signals [63]. A recent study has reported an additional modifying approach which successfully increased the selectivity of ER luminal loading of Fluo-5N. These authors generated stable cell lines that express an ER-targeted rat carboxylesterase, which enhanced esterase activity selectivity in the ER lumen, and shortened the loading period to achieve selective loading [68]. Using this approach, they demonstrated ER Ca^{2+} changes in response to typical agonists without significant contamination by cytosolic signals [68]. This novel approach should be of interest given its ease of use and that it preserves the high dynamic range and fast on/off kinetics typical of synthetic Ca^{2+} indicator dyes.

4. ER channels

4.1. Ca^{2+} release channels

Various ion channels are known to participate in Ca^{2+} release from the ER in response to agonist stimulation. The two major channel families are the IP_3Rs [69] and RyRs [70], although the TRP channel TRPP2 (polycystin 2) may also function as an ER Ca^{2+} release channel [71,72]. A hallmark of all these ER resident channels is that these channels all display Ca^{2+} sensitivity and are all non-selective cation channels that also permeate Ca^{2+}. This Ca^{2+} sensitivity underlies the phenomenon known as CICR which serves to amplify local Ca^{2+} release into global Ca^{2+} signals and/or propagating Ca^{2+} waves that invade the whole cytoplasm [73]. This is analogous to action potential propagation mechanisms on the plasma membrane of excitable cells, although orders of magnitude slower, and therefore, although the ER is probably not electrically excitable itself, it provides an excitable medium that underlies the generation of global Ca^{2+} transients when triggered by intracellular messengers such as IP_3, cyclic ADP-ribose (cADPR) and Ca^{2+} itself.

4.2. The IP_3 receptor channel

The IP_3R was first identified as the cerebellar P_{400} protein and subsequently shown to display IP_3 binding activity and mediate Ca^{2+} release from microsomes [13,74,75]. It is a large conductance non-selective cation channel that also conducts K^+ and other mono/divalent ions with a $P_{divalent}$:P_K of ~3.2–11, although slight Cl^- permeability is also detectable [69,76]. Since there is no K^+ gradient across the ER membrane, Ca^{2+} are the main ions that pass through IP_3R channels. A recent study has measured the unitary Ca^{2+} conductance of single IP_3Rs (rat, type 3), taking advantage of the presence of native IP_3Rs in the nuclear envelope that can be patch clamped more readily than other parts of the ER, and shown to be ~11 pS [77]. The channels also exhibit block by permeant divalent ions at high concentrations [77,78]. Given that negligible membrane potential is present across the ER membrane (see below), Ca^{2+} release from the ER is driven solely by the steep chemical gradient maintained by the SERCA pump. The IP_3R has also been suggested to mediate its own K^+ countercurrent to oppose membrane depolarization by Ca^{2+} fluxes which would otherwise inhibit further Ca^{2+} release ([79], see below).

IP_3Rs are sensitive to cytosolic Ca^{2+}, although this may vary depending on isoform [69]. Channel activity is regulated by both Ca^{2+} and IP_3 as co-agonists [69,80]. The IP_3R displays biphasic Ca^{2+} sensitivity, being activated in the range of submicromolar and inhibited in the range of micromolar at moderate concentrations of IP_3 [14,69,81]. IP_3 binding broadens the concentration window where Ca^{2+} is activatory by removing tonic inhibition by resting Ca^{2+} levels and above [69,81]. Elevation of cytosolic IP_3 concentrations thus sensitize individual IP_3R clusters to activation by resting Ca^{2+} and beyond certain thresholds, Ca^{2+} transients from individual clusters coalesce into a global Ca^{2+} transient and/or a propagating Ca^{2+} wave [82]. Excess Ca^{2+} inhibits the IP_3R, a property that is believed to be important in the generation of Ca^{2+} oscillations, among other mechanisms [83].

The IP_3R has also been reported to be sensitive to luminal Ca^{2+}, which was initiated by the observation of 'quantal' events [84,85]. This property was, however, not observed in bilayer studies where Ca^{2+} concentration on both sides of the membrane can be accurately controlled [86], and may reflect ER heterogeneity rather than an intrinsic property of the channels themselves as described above (Section 2.1). A recent study using carefully clamped voltage and [Ca^{2+}] on both sides of the ER membrane suggested that luminal Ca^{2+} may act via a feed-through mechanism depending biphasically

on cytosolic Ca^{2+} levels [87]. However, this notion remains a subject of debate at present.

The IP_3R is a homo- or hetero-tetrameric ~1000 kDa protein complex formed by four IP_3R subunits [88]. This huge protein complex is essentially a large cytoplasmic structure connected to much more modestly sized transmembrane channel domains with bulbous domains projected into the cytosol serving as important signaling scaffolds for modulation and integration of a wide range of cellular signals. Each subunit has six transmembrane helices located at the C-terminus and has a large cytosolic N-terminal domain that contains multiple regulatory domains including the IP_3 binding core [88]. Atomic resolution structures have informed our ideas about the gating of the channels by IP_3, with many favoring the "clam-shell" model for conformational changes [80], originally invoked for the gating of ionotropic glutamate receptors [89]. The clam-shell-like IP_3 binding core binds IP_3 and is regulated by the N-terminal suppressor domain [88,90]. Interactions with IP_3 have been proposed to pull the alpha and beta-domains together, causing the clam-shell to close, and allosteric changes are propagated to the pore region via the N-terminal suppressor domain [80]. IP_3 binding is also regulated by an IP_3 receptor interacting protein IRBIT which acts as a pseudoligand by competing with free IP_3 thereby decreasing the apparent affinity of IP_3 [91,92]. Thus, IP_3 sensitivity *per se* is tightly regulated depending on the IP_3 receptor isoform and the endogenous levels of IRBIT. In addition, the activity of the IP_3 receptor can be modulated by its phosphorylation state which is regulated by the coordinated action of various serine/threonine kinases including PKA [93], Akt/PKB [94], PKC [95], PKG [96,97], the tyrosine kinase Lyn [98], Fyn [99] and the serine/threonine phosphatases PP1 [100] and PP2A [93]. In most cases, the kinase itself is associated with the IP_3R cytosolic domain via their respective adaptor proteins for example IRAG for PKG and BANK for Lyn, allowing rapid activity modulation in response to stimulation.

4.3. Ryanodine receptors

The ryanodine receptor channel (RyR) was first identified as the skeletal/cardiac SR Ca^{2+} release channel that shows high affinity binding to the plant alkaloid ryanodine [101,102]. Individual RyR isoforms (RyR1–3) as for IP_3Rs form homotetramers and have tissue-specific expression where skeletal muscles express high level of RyR1, cardiac muscle expresses high levels of RyR2 while RyR3 expression can be found at low levels in a wide range of tissues [103]. In general, all isoforms are large conductance non-selective cation channels that also conduct K^+ and other mono/divalent ions with a $P_{divalent}:P_K$ of ~6:1 [104–106]. There is little selectivity within the monovalent and divalent ion groups [104–106]. The unitary Ca^{2+} current of RyR2 under quasi-physiological ionic conditions has been estimated and measured to be around 1 pA [107,108]. This compares with an estimate of <0.5 pA for the IP_3R [69]. Such high unitary Ca^{2+} currents of the RyR are well-suited to mediate rapid and efficient Ca^{2+} release in striated muscle cells during excitation–contraction coupling (ECC) to sufficiently saturate Ca^{2+} effector targets such as troponins [109]. On the other hand, its non-selectivity among ions suggests that the conducting properties of the RyRs are prone to modulation by changes in cellular ion concentrations, for example, in those observed under pathological conditions.

RyRs are gated by cytosolic Ca^{2+}. In general, all RyRs are activated in the range of 1–10 μM $[Ca^{2+}]_{cyto}$, while the individual isoforms display different low affinity Ca^{2+} inhibition ([70] and the references therein). RyR1 is completely inhibited when $[Ca^{2+}]_{cyto}$ approaches 1 mM, while RyR2 and RyR3 are only partially inhibited [70]. The physiological significance of the partial low affinity inhibition of the RyR2 and RyR3 is yet to be understood, as supraphysiologically high $[Ca^{2+}]_{cyto} > 1$ mM is probably never realized inside the cell, although local concentrations within tens of nanometers away from the channel mouth may attain $[Ca^{2+}]$ of >100 μM [110–113]. Some studies have also observed heterogeneity within the single RyR1 populations, while RyR2 and RyR3 appear to be more homogeneous [70]. In addition, the RyRs are also sensitive to regulation by various cytosolic ligands such as Mg^{2+} [114,115], ATP [115,116] and the Ca^{2+} mobilizing messenger, cyclic ADP-ribose [15,117,118].

RyRs are also sensitive to luminal Ca^{2+} in the range of 0.1–10 mM. For RyR2, increasing luminal Ca^{2+} appeared to increase the maximal open probability elicited by cytosolic Ca^{2+} [119], while for RyR1, the effect of luminal Ca^{2+} appeared to be agonist-dependent and not apparent when cytosolic Ca^{2+} is the sole agonist [120]. This difference in sensitivity to luminal Ca^{2+} between RyR1 and RyR2 appears to correlate with the different probability of the occurrence of Ca^{2+} sparks [121], spontaneous local Ca^{2+} transients generated by small numbers of clustered RyRs, in skeletal and cardiac myocytes, more frequently observed in the latter. The sensitivity to luminal Ca^{2+} is an intrinsic property of the RyR as indicated by single channel studies using RyR1/2 chimeras [121]. This is also an intrinsic property of the luminal portion of the RyR as this does not require a Ca^{2+} current flowing to the cytosolic side, is independent of the identity of the charge carrier and independent of cytosolic buffering by fast chelators [119,120,122].

RyRs form homotetrameric channels of ~2000 kDa together with a growing list of protein binding partners, notably calmodulin, FKBP12 and FKBP12.6 from the cytosolic side, and junctin, triadin and calsequestrin from the transmembrane and luminal side [70,103]. Each subunit contains 4–10 transmembrane helices at the C-terminus, leaving an enormous N-terminal cytosolic domain, as also seen for IP_3Rs, that can be regulated by the aforementioned cytosolic ligands and modulators. One important feature of the enormous N-terminal cytosolic portion is that the N-terminal domain interacts with the central domain to stabilize the closed RyR, and loss of such interaction, known as domain unzipping, contributes to physiological activation as well as mutational and non-mutational mechanisms in the pathogenesis of a range of RyR-mediated myopathies [123,124]. Of these regulatory modulators, the FKBP immunophilins have attracted considerable interest as removal of these proteins from the RyR has been shown to destabilize the channels [125,126], although much debate exists [70]. In addition, the activity of the RyRs is also modulated through their phosphorylation and redox state [124]. Phosphorylation by PKA and CaMKII represents a major mechanism of sympathetic modulation of SR excitability which in part underlies the positive chronotropic and inotropic effects observed during an adrenergic response [127]. Deregulation of phosphorylation, however, can contribute to arrhythmogenesis and the progression to heart failure [124].

4.4. ER/SR membrane potential and counter-ion channels

Like other biological membranes, the resting potential of the ER/SR is governed by electrochemical mechanisms. Isolated SR vesicles exhibit a large K^+-selective cation conductance in the range of 0.1 S/cm^2 that constitutes the major ionic conductance of the SR [128]. The SR membrane potential is thus clamped at the Nernst potential of K^+, which is 0 mV as the $[K^+]$ in the cytosol and the SR lumen are similar [129]. This conductance is mediated by a K^+-selective cation channel that has also been observed in single channel recordings which has a similar ionic selectivity and sensitivity to blockade by quaternary ammonium derivatives and Cs^+ [130–132]. Given its large conductance, the SR membrane potential will always be shunted near 0 mV and is only minimally depolarized during Ca^{2+} release. In addition, the SR membrane is also known to be permeable to Cl^- and H^+ [133], although their physiological significance is relatively less well understood.

For long-lasting Ca^{2+} release (comparable to or longer than the sub-millisecond charging time of the ER/SR membrane) to occur, counter-ion channels must operate in parallel to prevent Ca^{2+} from charging up the ER/SR membrane [79]. As mentioned above, the

SR K^+-selective cation conductance is believed to be the predominant countercurrent mechanism in the ER/SR. However, substituting Cs^+ for K^+, which does not permeate the putative SR K^+ channels, Ca^{2+} release is still supported [134], suggesting the existence of additional countercurrent mechanisms. One such mechanism has been proposed by a recent study, which suggested that the RyRs and likely IP_3Rs may mediate their own countercurrent given their ionic non-selectivity and the physiological K^+ and Mg^{2+} gradients [79]. This proposal is consistent with genetic evidence where in mice whose all SR K^+ channels have been knocked out (see below), robust caffeine-induced Ca^{2+} transient can still be observed [135]. However, the likely scenario is that different countercurrent mechanisms operate in a cooperative manner under physiological conditions to ensure the Ca^{2+} release ability of the ER/SR if one or the other mechanisms fail.

The SR K^+ channel has recently been shown to be formed by a transmembrane protein named TRIC channels (TRIC-A and -B, Trimeric Intracellular Cation), formerly known as mitsugumin (Japanese for junction) 33 [135]. These proteins are ubiquitously expressed and are particularly enriched in heart and skeletal muscle [135], consistent with the presence of the large K^+ conductance observed in isolated SR vesicles and the particular need of a counter-K^+ conductance in striated myocytes. When expressed heterologously, purified TRIC-A and -B channels appeared to satisfy the permeation properties of the SR K^+ channels, being non-selective among K^+ and Na^+ while restricting permeation by Ca^{2+} and Mg^{2+} [135]. The identification of the two TRIC channel isoforms has subsequently revealed that the SR K^+ conductances may in fact be due to the stochastic openings of both TRIC-A and TRIC-B channels, rather than by a single channel with multiple subconductance states [136]. Further, TRIC-A and TRIC-B channels appear to be gated by different stimuli, with TRIC-A being voltage-sensitive and TRIC-B tending to be opened at micromolar $[Ca^{2+}]_{cyto}$ and closed at high micromolar $[Ca^{2+}]_{SR}$ [136]. The different gating and conductance properties of the TRIC channel isoforms may underlie their tissue-specific roles observed in TRIC single knockout mice, for example TRIC-A is implicated in the control of vascular tone [137], while TRIC-B appears to play an important role in perinatal lung development [138].

4.5. Calcium leak pathways

In addition to regulated Ca^{2+} release pathways in the ER, there are also leak pathways [139]. These can be revealed, for example, by Ca^{2+} loss from ER stores when SERCA pumps are inhibited by thapsigargin. Two types Ca^{2+} release pathways have been proposed. The first are primary Ca^{2+} leak channels, the second are mechanisms by which normally physiologically regulated Ca^{2+} release channels are deregulated by interactions with, or loss of, modulator proteins. A number of channel proteins have been implicated in basal Ca^{2+} release pathways including translocons [140], the protein pores associated with ribosomes in rough ER, pannexins which may form hemichannels in the ER [141], and presenilins, whose mutations are associated with decreased Ca^{2+} leak from the ER [142] and neurodegenerative diseases. Examples of signaling proteins interacting and affecting leak through Ca^{2+} release channels include the anti-apoptotic protein Bcl-x1 which interacts with IP_3Rs to enhance openings [143], and FKBP proteins whose dissociation from RyRs destabilizes them and renders them leaky [125]. Presenilins have also been proposed to exert their effects by direct interaction and modulation of IP_3R openings too [144,145].

5. ER junctions in cells

The membrane of the ER abuts and forms discrete junctions with other membranes in the cell, both with the plasma membrane, and with intracellular membranes of various organelles [146]. These junctions were first noted from studies of electron micrographs, but in recent years progress has been made in our understanding of the molecular components of these junctions, as well as a growing appreciation of their functional roles. An important principle is that the membranes do not fuse, but require specialized proteins in apposing membranes to form stable yet often dynamic contacts. These key tethering proteins are diverse in structure and may specifically mediate membrane interactions depending on the origin of the membrane that interacts with that of the ER [147]. Such junctions occur both in excitable and non-excitable cells, but as described below, the key components may differ depending on cell type reflecting functional specializations, for example in the control of Ca^{2+} entry across the plasma membrane. Two major functions of these junctions have emerged. The first is transfer of lipids, and the second, the focus of this review, is the transfer of ions, and in particular Ca^{2+}. Such junctions are pivotal for the generation and coordination of Ca^{2+} signals throughout the cell, and are a major way in which organelles communicate to coordinate their various functions. These sites not only serve as conduits for Ca^{2+} transfer between compartments, controlling the amount of Ca^{2+} in various junctional spaces as well as organelle lumena, but also provide a local environment whereby junctional Ca^{2+} levels may control feedback mechanisms to modulate organellar communication. Junctions occur between the ER and all organelles of the cell, in addition to the plasma membrane. The three ER junctions that have been investigated prominently are those with the plasma membrane, the mitochondrion and the lysosome, and will be discussed here since they also are the most relevant for Ca^{2+} signaling.

6. ER–plasma membrane junctions

Functional interactions exist between the SR/ER and the plasma membrane (PM). Cross-talk between the two membranes is bidirectional: Ca^{2+} influx through the PM can trigger Ca^{2+} release from the SR/ER by CICR [70,127,148], while Ca^{2+} depletion in the SR/ER lumen can signal to activate further Ca^{2+} influx [149,150]. Localized spontaneous Ca^{2+} transients originating from the ER/SR can also modulate PM excitability in excitable cells [25,151]. These events are effected either via direct protein–protein interactions and/or via changes in local ion concentrations, for example, by modulating the activity of Ca^{2+} regulated channels, which occur at specialized ER–PM junctions where the required protein machinery is localized/assembled. In the case of ER–PM junctions, the proteins that tether the membranes together include the machinery for actual Ca^{2+} transfer and regulation rather than specialized structural tethering proteins as in the case of mitochondria or lysosome–ER interactions. However, very recently three new families of ER–PM tethering proteins have been reported from yeast proteomic analyses [152]. The other key aspect is the stability of dynamic nature of such interactions. In the case of PM–SR interactions, there is stable tethering which has important structural determinant of striated myocytes [153], whereas PM–ER interactions in capacitative Ca^{2+} entry are dynamic and organized during the Ca^{2+} signaling events [154].

6.1. Dyads and triads

Physical or functional interactions between plasma membrane (sarcolemma) and SR proteins provide a mechanistic basis for excitation–contraction coupling in both skeletal and cardiac muscle cells, resulting in concerted Ca^{2+} release from the SR. The former involves direct protein–protein interaction between the sarcolemmal L-type voltage-gated Ca^{2+} channels (VGCC) and the SR-located skeletal muscle RyR1, while the latter relies on Ca^{2+}-mediated coupling between L-type VGCC and the SR-located cardiac RyR2. Depolarization induces conformational changes in the skeletal muscle L-type VGCC which is propagated to the SR RyR1 through direct physical contact, while Ca^{2+} influx through the cardiac L-type VGCC leads to Ca^{2+} activation of the SR RyR2 for CICR, although RyR1 can also be

activated by Ca^{2+} [70,127,155]. Importantly, these events occur in specialized junctions formed between the sacrolemmal T-tubule system and junctional SR called the dyads or triads reflecting the number of participating membranes. These specialized junctions have been revealed by electron microscopy, where the two membranes were shown to be as close as 12 nm [156]. Immunolabeling studies also revealed the selective presence of L-type VGCC in T-tubular membranes apposed to junctional SR membranes [157], and that RyR1 and RyR2 are concentrated in junctional SR membranes as electron-dense structures known as 'feet' [158]. Dual labeling experiments further showed that these channels selectively colocalize [159,160]. Together with the closely apposed sacrolemmal and SR membranes, L-type VGCCs and the RyRs have been suggested to function as a 'couplon', with each couplon containing arrays of channel clusters [161,162]. Dyads appear to regulate the Ca^{2+} phenotype of cardiomyocytes, as subcellular regions of neonatal ventricular myocytes and atrial myocytes lacking these junctions appeared to have a slower onset of Ca^{2+} transient in response to an electrical pulse and hence altered excitation–contraction coupling [163]. Therefore, these observations together suggest that precise organization of signaling components is employed for the precise spatiotemporal transduction of sarcolemmal excitability into SR Ca^{2+} permeability essential for the proper function of striated muscles.

6.2. STIM–Orai ER–PM junctions

Ca^{2+} release from the ER lowers the concentration of free Ca^{2+} within the ER lumen, which leads to Ca^{2+} influx through the PM. This phenomenon has been termed capacitive or store-operated Ca^{2+} entry (SOCE), and the degree of SOCE depends on the degree of Ca^{2+} depletion in the ER. Recently, significant advances have been made concerning the identity of the molecular machinery involved in this process (Fig. 2). As originally proposed, such a phenomenon must involve a system consisting a Ca^{2+} sensor that senses the fall in ER luminal Ca^{2+} levels which in turn activates a PM-localized Ca^{2+}-permeable channel that allows for subsequent Ca^{2+} influx [164]. Using siRNA screens, STIM1 and STIM2 have been identified as ER luminal Ca^{2+} sensors that cluster and translocate toward the PM to activate SOCE [165–167]. The nature of the Ca^{2+} influx pathway has long been debated, with initial focus on members of the transient receptor channels (TRPs) [168]. However, the PM-localized Ca^{2+}-permeable channel was subsequently identified, also by employing siRNA screens, as Orai1-3 proteins, which, appear to recapitulate all the biophysical hallmarks of the I_{CRAC} channels believed to mediate SOCE [169–171]. These two classes of protein appear to be the two essential components that mediate SOCE, where oligomerized STIM cytosolic domains bind directly to gate Orai channels, allowing Ca^{2+} influx [149,172].

Activation of Orai1-mediated SOCE requires the translocation of oligomerized STIM molecules to pre-existing ER/PM junctions without global movements of the ER [173]. Such ER/PM junctions have been observed using electron microscopy, where STIM molecules were enriched after store-depletion [173,174]. The two membranes were found to be ~17 nm apart, a distance that may be spanned by the STIM cytosolic domain in an extended, active conformation [175]. The translocation of STIM toward the PM has been resolved using time-lapsed TIRF microscopy, and the half-time of the translocation process was reported to be 20–52 s [165,173], which paralleled or just preceded the onset of I_{CRAC} as monitored using simultaneous patch-clamp recordings [174]. As STIM molecules translocate to form punctate structures at ER/PM junctions, Orai channels were also redistributed from diffusely distributed clusters into punctate structures [174,176,177] that overlapped with STIM where localized SOCE occurred [174]. Interestingly, STIM translocation appears to be dynamic and can occur as a 'transient'. Such translocation 'transients' to ER/PM junctions have been observed using time-lapsed TIRF microscopy, which coincided with the decay phase of each Ca^{2+} transient during Ca^{2+} oscillations [148]. Such dynamic behavior of translocation was not observed when extracellular Ca^{2+} was removed upon which Ca^{2+} oscillations ceased [148], indicating the importance of store-refilling in maintaining the integrity of particular Ca^{2+} phenotypes during agonist stimulation.

Co-clustering of STIM1 and Orai depends on the direct association of STIM1 with Orai, phosphoinositides (PIs) and other protein components. The STIM1 cytosolic domain contains an alpha-helical domain that is required for Orai binding and gating termed SOAR or CAD (residues 344–442) [177,178]. SOAR/CAD appears to be an important

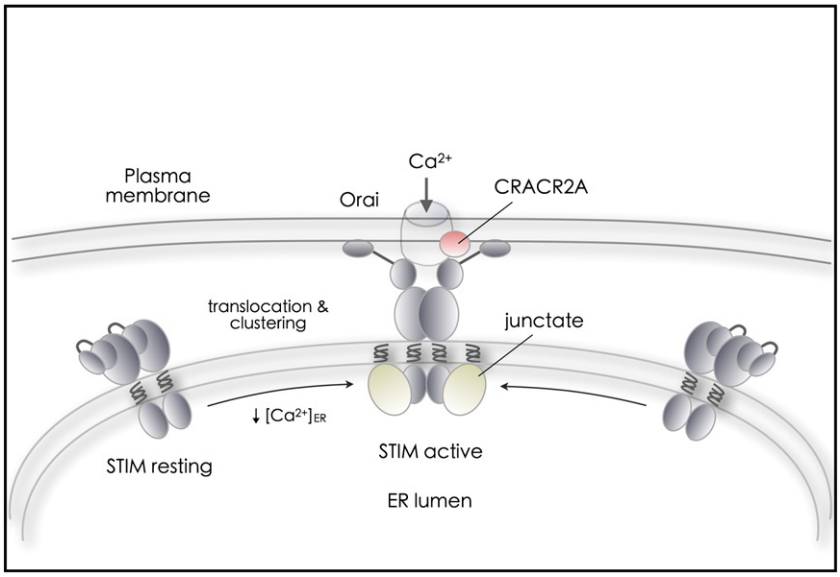

Fig. 2. ER–PM junction and the SOCE complex. STIM and Orai proteins constitute respectively the ER luminal Ca^{2+} sensor and the Ca^{2+} influx pathway required for SOCE. Upon store depletion, resting STIM dimers sense the drop in the luminal Ca^{2+} level and adopt an extended, active conformation. Active STIM dimers translocate to pre-existing ER–PM junctions in puncta, which redistributes Orai into discrete clusters. Through the removal of an autoinhibitory mechanism within the SOAR/CAD region of STIM [304], oligomerized SOAR/CAD directly activates Orai channels on the plasma membrane. Activation of Orai channels leads to selective Ca^{2+} influx, which can be sustained or temporally coincident with individual Ca^{2+} transients during Ca^{2+} oscillations. The assembly and disassembly of the SOCE complex is coordinately regulated by junctate, CRACR2A/B and SARAF (not shown) and is stabilized by phosphoinositide interaction with the poly-lysine domain of STIM. These interacting proteins likely function to accelerate the assembly/disassembly of the SOCE complex and consequently sharpen the transient of SOCE complex formation, which may facilitate the onset of SOCE while reducing the risk of store over-refilling.

requirement for the assembly of the SOCE complex at ER/PM junctions as deletion of which abolished store depletion-induced clustering of Orai and dramatically reduced SOCE [177,178]. Overexpression of this domain alone also caused clustering of Orai as indicated from size-exclusion chromatography [177]. However, clustering of Orai appeared to be independent of gating as the C437G mutation within the SOAR/CAD region preserved the ability of STIM1 to cluster Orai while dramatically abolished SOCE activity [177], suggesting additional gating effects of this domain.

The STIM–Orai complex is additionally regulated by Ca^{2+} through the interaction with Ca^{2+}-binding proteins. Two EF hand Ca^{2+}-binding proteins CRACR2A/B and junctate have been implicated in the clustering process. The ER luminal Ca^{2+}-binding junctate senses the decrease in luminal Ca^{2+} level and acts as a Ca^{2+}-dependent switch that binds STIM1 upon store depletion, which facilitates STIM1 recruitment toward junctional ER [179]. Constitutive SOCE has been observed upon overexpression of the EF hand junctate^{EFmut} mutant without store depletion [179], suggesting that junctate may induce the activation of STIM1 despite that STIM1 is in its Ca^{2+}-bound resting state. At the cytosolic face, CRACR2A/B act as Ca^{2+}-dependent regulators of the SOCE complex. Ca^{2+} binding dissociates CRACR2A/B from Orai1 and STIM1. Mutations in the EF hand (CRACR2A^{EFmut}) induced constitutive clustering of STIM1, suggesting that Ca^{2+} binding may serve to provide negative feedback which may force the disassembly of the SOCE complex [180], mostly likely after Ca^{2+} influx has been initiated. An additional regulator SARAF, an ER transmembrane protein, has recently been identified. SARAF interacts with STIM at the cytosolic face and negatively modulates basal SOCE activity, showing activity reminiscent of Ca^{2+}-dependent inactivation that is not observed when the fast chelator BAPTA is present [181]. Together, it can be seen that the assembly and disassembly of the SOCE complex is accelerated through the action of the above protein regulators, which may allow for better temporal coordination with cytosolic Ca^{2+} transients during Ca^{2+} oscillations such as those observed in [148], while minimizing the risk of store over-refilling.

In addition to protein–protein interactions, the C-terminal polylysine stretch of STIM1 also binds PIs [182]. Mutations of which (STIM1ΔK) or acute PI depletion prevented store depletion-induced STIM1 translocation or SOCE activity, suggesting that plasmalemmal PIs may facilitate translocation of activated STIM1 [177,183], likely through electrostatic attraction given the complementary charge of the polylysine domain. The relative importance of PIs may depend on the endogenous levels of Orai, as Orai overexpression alone can completely rescue the translocation defect of STIM1ΔK [177,179]. Thus, under physiological conditions, each of the above interactions likely contributes in a cooperative manner to coordinate the assembly and disassembly of the SOCE complex in a timely fashion.

Mitochondria, in particular, have been demonstrated to play a key role in capacitive Ca^{2+} entry by taking up Ca^{2+} released from the ER and thus contributing to lowering of luminal ER Ca^{2+} concentrations. In addition, they are also important in modulating Ca^{2+} concentrations due to Ca^{2+} entry via CRAC channels that would otherwise feedback and inactivate IP_3Rs and CRAC channels themselves [184]. These effects of mitochondria are further discussed below.

7. ER–mitochondria junctions

Mitochondria have long been known to play an important role in Ca^{2+} homeostasis where they can take up Ca^{2+} transiently followed by subsequent Ca^{2+} release [185]. Their inner membranes express a plethora of Ca^{2+} transporters, and they store Ca^{2+} in their matrix whose levels may regulate key bioenergetic functions. In addition, they act as important Ca^{2+} buffering mechanisms that particularly impact at regions of close apposition to the ER. In one study, it has been estimated that between 25 and 50% of the Ca^{2+} release from the ER/SR is taken up by mitochondria depending on cell type and the Ca^{2+} release channel involved [186].

7.1. Mitochondria and Ca^{2+} homeostasis

Isolated mitochondria have long been known to take up Ca^{2+} when supported by exogenous electron transport chain (ETC) substrates [187]. Mitochondrial matrix Ca^{2+} concentrations are crucial for regulating the many Ca^{2+} sensitive enzymes of the tricarboxylic acid (TCA) cycle [188] and for regulating cell death mechanisms [189]. In addition, activation or inhibition of Ca^{2+} uptake by mitochondria strongly modulates Ca^{2+} signaling mediated by Ca^{2+} release from the ER [190,191], presumably by affecting Ca^{2+} feedback inhibition on IP_3Rs illustrating the importance of mitochondria in shaping Ca^{2+} signals from adjacent structures. Mitochondrial Ca^{2+} transients were first observed using a mitochondrially targeted aequorin variant which directly demonstrated that $[Ca^{2+}]$ in the matrix ($[Ca^{2+}]_{mito}$) can reach tens of micromolars or higher during intracellular Ca^{2+} release from the ER [192–194]. As also observed for isolated ER/SR vesicles, isolated mitochondria can in vitro act as an excitable Ca^{2+} signaling network supporting Ca^{2+} waves of Ca^{2+} release [195]. Recently, key molecular components of Ca^{2+} uptake and release by mitochondria have been identified. In terms of Ca^{2+} transport mechanisms, there are key differences between this organelle and the ER. First, unlike the ER, there is a large electrical gradient across the inner membrane of the mitochondrion ($\Delta\Psi$), negative inside, which acts as a driving force for the uptake of Ca^{2+} [185]. In contrast to the ER, Ca^{2+} uptake appears to be principally mediated by a channel, whereas Ca^{2+} release occurs by the action of ion exchangers [196]. The concerted activity of both mechanisms leads to prodigious cycling of Ca^{2+} across the inner mitochondrial membrane [197]. Another difference is that buffering of Ca^{2+} in the mitochondrial matrix is largely due to formation of inorganic precipitates such as calcium phosphate in a labile gel, rather than due to Ca^{2+} binding proteins, which serve to keep free Ca^{2+} remarkably low [185]. Ca^{2+} uptake by the mitochondria is mediated by mitochondrial Ca^{2+} uniporter (MCU) activity on the inner mitochondrial membrane [198], while the outer mitochondrial membrane is permeant to low molecular weight substrates including ions. Given the low apparent affinity for Ca^{2+} of the MCU [198] and that cytosolic $[Ca^{2+}]$ seldom reaches the functional $[Ca^{2+}]$ range of the MCU, extra mechanistic features must exist for mitochondrial Ca^{2+} uptake to occur under physiological stimulation. This apparent paradox is generally solved by hypothesizing the existence of Ca^{2+} nano/microdomains with high local $[Ca^{2+}]$ at the Ca^{2+} source and the strategic localization of the Ca^{2+} effector within such domain. In this case, the MCU is the effector that must be located within nanometers from the Ca^{2+} source, ER IP_3 receptors or RyRs. Consistent with this hypothesis, mitochondria have been shown to be in close contact with the ER, ~20 to 100 nm apart, in a number of independent studies [199–201].

7.2. Mitochondrial Ca^{2+} signaling proteins

As highlighted above, Ca^{2+} uptake into the mitochondrial matrix is mediated through the Ca^{2+}-selective channel MCU on the inner mitochondrial membrane, and recently its molecular identity and that of regulators have been reported. A hallmark of MCU transport activity is its sensitivity to high affinity blockade by the polycation ruthenium red [198,202], which has been used to indicate the involvement of the MCU, despite the compound's non-selectivity. The MCU has a K_m for Ca^{2+} of 20 mM and mediates inwardly rectifying Ca^{2+} currents [198], ensuring unidirectional Ca^{2+} influx into the matrix under physiological conditions, i.e. within the IP_3 receptor Ca^{2+} nano/microdomain. Such a current–voltage (I–V) relation is also consistent with the requirement of ETC substrates [187], which ensures a highly hyperpolarized inner membrane potential for efficient Ca^{2+}

uptake. The molecular identity of this putative MCU has been contentious, with a number of candidates, proposed such as the mitochondrial uncoupling proteins, UCP2 and UCP3 [203,204]. However, the search appears to have ended with more recent candidates discovered through *in silico* genomic search, which revealed a transmembrane protein with two predicted transmembrane helices and a highly acidic putative pore loop [205,206]. This protein likely forms the pore-forming subunit that permeates Ca^{2+} as mutation of acidic residues in the putative pore-loop abolished observable single channel currents in bilayer and largely reduced mitochondrial Ca^{2+} uptake [205]. One of these mutations also rendered considerable resistance to ruthenium red blockade [206], consistent with the voltage-dependent blocking action of ruthenium red [198].

The MCU appears to exist in a macromolecular complex of ~300–400 kDa and is regulated by the Ca^{2+}-binding EF hand protein MICU1 [206,207]. Like MCU, shRNA silencing of MICU1 largely reduced mitochondrial Ca^{2+} uptake and is thus considered to be a key component of the MCU complex [207]. It was, however, not considered to be the pore-forming subunit as MICU1 has only one transmembrane. Through rescue experiments, the EF hand was found to be crucial to the regulatory effect of MICU1 [207]. This observation suggests that mitochondrial Ca^{2+} uptake, in addition to Ca^{2+} binding-unbinding at the MCU pore, may also be regulated by Ca^{2+}. However, a recent study reported that MICU1 may negatively regulate mitochondrial Ca^{2+} uptake by elevating the threshold for Ca^{2+} uptake [208]. Thus, although MICU1 appears to regulate the activity of the MCU, its precise role remains controversial. In addition, a protein termed MCUR1 has also been identified and found to regulate MCU-mediated Ca^{2+} uptake into mitochondria too [209].

An additional pathway for mitochondrial Ca^{2+} efflux is through the permeability transition pore (PTP) [210]. However, the molecular identity of this pore is unknown. The voltage-dependent anion channel (VDAC) and adenine nucleotide translocase may regulate or influence pore activity rather than being components as originally envisaged [211]. This conductance pathway seems to have low and high conductance states. The pathway allows rapid Ca^{2+} reduction of matrix Ca^{2+} preventing Ca^{2+} overload, but also causes the dissipation of the mitochondrial membrane potential. In the high conductance state it may lead to cell death and is the major pathway for release of activators of intrinsic apoptotic pathways such a cytochrome c.

A number of mitochondrial Ca^{2+} ion exchanger proteins have also been identified at the molecular level. These include a Na^{+}/Ca^{2+} exchanger (NCLX) [212] proposed to play a role in the exit of Ca^{2+} from the mitochondrial matrix, and Letm1, proposed as a Ca^{2+}/H^{+} transporter [213], but perhaps more prominent in regulating mitochondrial K^{+} and volume through K^{+}/H^{+} mechanisms [214].

Mitochondrial Ca^{2+} transport mechanisms serve to shape Ca^{2+} signals originating from the ER, but also have direct consequences for mitochondrial physiology [215]. ER–mitochondrial Ca^{2+} transfer plays an important role in cell energetics and survival including the control of apoptosis and necrosis [216]. As mentioned above, Ca^{2+} has long been known to stimulate mitochondrial respiration by activating matrix dehydrogenases [217–219]. Increased ATP production may allow increased activity of ER SERCA pumps to take up Ca^{2+}. In cardiac myocytes, elementary RyR openings in the SR drive local and transient changes in matrix Ca^{2+} concentrations termed "marks" have been visualized [220], which may in turn regulate cardiac excitability through local control of Ca^{2+}. A recent study has further shown that basal 'spontaneous' Ca^{2+} transients originated from the ER are essential in driving the basal metabolic rate of the mitochondria [221]. Blocking these transients and/or mitochondrial Ca^{2+} uptake inhibits substrate flux into the TCA cycle and activates AMPK-mediated pro-survival mechanisms [221], illustrating the importance of constitutive Ca^{2+} transfer at mitochondrial associated membrane (MAM). On the other hand, excess Ca^{2+} accumulation in the matrix can induce a process crucial to the initiation of apoptosis known as mitochondrial permeability transition [216,222]. Indeed, a decrease in the ER Ca^{2+} level or Ca^{2+} release rate and consequent decrease in ER–mitochondrial Ca^{2+} transfer appear to be correlated with the decrease in the sensitivity to various pro-apoptotic stimuli [94,223]. These may have important implications in the pathogenesis of apoptosis-related diseases such as autoimmune diseases, cancer and neurodegeneration [94,224–228].

7.3. Structural basis of ER–mitochondrial contacts

Juxtaposed ER and mitochondria are held by protein contacts (Fig. 3) [199]. These contacts appeared to be formed by the homo- and hetero-typic interactions between mitofusins (Mfn), a dynamin-related outer mitochondrial protein previously known to participate in mitochondrial fusion [229]. Through elegant rescue experiments using Mfn-double knockout fibroblasts, Mfn2 on the ER and Mfn1 or Mfn2 on the outer mitochondrial membrane were shown to be crucial to the formation of ER–mitochondria contacts [200]. These contacts are likely subjected to dynamic regulation, for example, by the Mfn-interacting protein trichoplein/mitostatin, which seems to negatively regulate ER–mitochondria contacts formed by Mfns [230]. Consistent with the Ca^{2+} nano/microdomain hypothesis, Mfn2 knockout fibroblasts, which had loosened ER–mitochondria contacts, exhibited defects in mitochondrial Ca^{2+} uptake upon agonist stimulation [200].

Beyond structural associations, the ER–mitochondria interface is also a specialized platform for inter-organellar communication. In addition to specific protein machinery that is required for proper contacts, a large number of proteins with diverse functionalities are also specifically enriched in these membrane domains. In particular, these membrane domains can be enriched through sucrose gradient centrifugation and are known as mitochondria-associated membranes (MAMs), separate from the ER and mitochondrial fractions [231].

MAMs, in addition to lipid synthases, are enriched with protein machinery that mediates ER–mitochondrial Ca^{2+} transfer. There, ER IP_3 receptors and the outer mitochondrial voltage-dependent anion channel (VDAC), together with the 75 kDa glucose-regulated protein (grp75) and additional protein regulators (see below), were found to be contained in a >1200 kDa macromolecular complex, which presumably facilitates Ca^{2+} shuttling between the two organelles by concentrating VDAC within the IP_3 receptor Ca^{2+} nano/microdomain [232]. Signals may also be additionally conveyed from the IP_3R N-terminal domain to the VDAC complex via grp75 [232], although the underlying molecular details have yet to be elucidated. At the luminal end of IP_3R, there are also molecular interactions with other protein regulators, for example, the sigma-1 receptor (Sig1R), an ER transmembrane chaperone targeted by many psychotropic drugs. Sig1R chaperone activity is mediated by its ER luminal domain and is unleashed by the removal of Ca^{2+} [233]. Thus, upon Ca^{2+} release, local depletion of ER luminal Ca^{2+} is expected to enhance its chaperone activity and indeed this was found to attenuate ubiquitination of the IP_3R thereby preventing the degradation of MAM-associated IP_3Rs [233]. Sig1R appears to have additional functions through yet uncharacterized mechanisms in supporting Ca^{2+} transfer at MAMs since its removal causes a reduction in the magnitude of agonist-induced mitochondrial Ca^{2+} transient [233]. Further regulation has been shown to involve the IP_3 R cytosolic domain which appears to be coordinately regulated by the tumor suppressor protein promyelocytic leukemia (PML) and Akt/PKB. PML, Akt and the protein phosphatase 2A (PP2A) which exist in the same >1200 kDa protein complex (see above), regulate the phosphorylation state of the IP_3 receptor, thereby modulating the efficiency of mitochondrial Ca^{2+} uptake and consequently the extent of pro-apoptotic signaling [94]. Thus, it is clear that in addition to the intimate spatial relationship between the ER and the mitochondria, tightly controlled molecular regulation of the Ca^{2+} transfer machinery

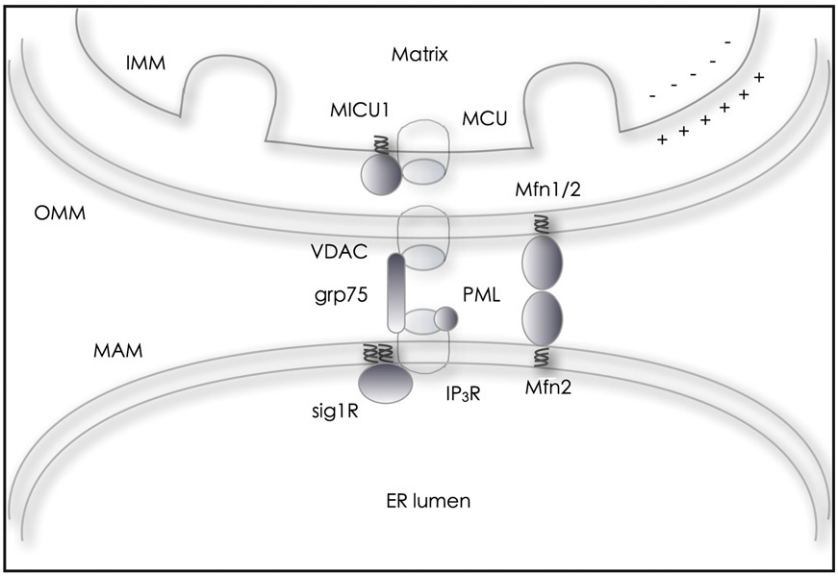

Fig. 3. ER–mitochondria junction. The ER and the mitochondria form a specialized Ca^{2+} shuttling junction that contributes to cellular Ca^{2+} dynamics. Tethering between the ER and mitochondria is mediated by *trans*-interaction between Mfns, which ensures a high efficiency of ER–mitochondrial Ca^{2+} transfer. ER–mitochondria junctions are enriched with Ca^{2+} signaling proteins including channels and their modulators. At these junctions, IP_3 receptors form a macromolecular complex with the outer mitochondrial VDAC to allow for efficient shuttling of Ca^{2+} into the intermembrane space. Mitochondrial Ca^{2+} uptake is mediated by the MCU on the inner mitochondrial membrane, which mediates unidirectional Ca^{2+} influx into the mitochondrial matrix. ER–mitochondrial Ca^{2+} transfer depends also on the steep electrochemical gradient generated by the electron transport chain and is subjected to coordinated regulation by protein regulators of IP_3Rs including PML and Sig1R, and phosphorylation state of IP_3Rs. This is further fine-tuned by Ca^{2+}-dependent regulation of MCU activity through the EF hand protein MICU1, although the precise action of MICU1 and the underlying molecular details are currently unclear.

also occurs within the MAMs. It is also expected that additional regulators exist, further fine-tuning ER–mitochondrial Ca^{2+} transfer.

Recent studies have provided quantitative measurements of the MAM Ca^{2+} microdomain and have come to reasonably good agreement. Two studies employed a GECI (with appropriate K_d) specifically targeted to the outer mitochondrial membrane (OMM) which would be expected to report precisely local $[Ca^{2+}]_{MAM}$. Using this approach, in a series of elegant experiments, Ca^{2+} hotspots were visualized in intact cells upon agonist stimulation and estimated the absolute $[Ca^{2+}]$ to be between 4 μM and 16 μM [234]. In a parallel approach, an EGTA-resistant Ca^{2+} increase was visualized using the OMM-targeted GECI in permeabilized cells under high buffering conditions (100 μM EGTA/40 μM Ca^{2+}) and estimated the $[Ca^{2+}]$ to be >4 μM [235]. EGTA is a slow Ca^{2+} chelator which allows the isolation of local Ca^{2+} events [112,113], illustrating the localized nature of this Ca^{2+} nano/microdomain. By controlling the distance between the ER and the mitochondria using recombinant protein tethers, demonstration that the localized Ca^{2+} domain in the MAMs is significantly compromised if the distance between the ER and the mitochondria is <7 nm was established [235]. These observations may suggest that an optimal gap width may be required for the proper enrichment of MAM proteins and architecture, for example to accommodate the large IP_3R cytosolic domain [235].

8. ER–lysosome junctions

Lysosomes and other acidic stores are emerging multifunctional organelles that play a number of important roles in the cell [236] including the generation and modulation of cytosolic Ca^{2+} signals. Although traditionally being viewed solely as a terminal acceptor of endosomal and/or autophagic cargoes, increasing evidence has pointed to lysosomes as a Ca^{2+} storage organelle being capable of releasing and buffering Ca^{2+}, likely through specific permeation pathways on their delimiting membranes [237,238]. Although by comparison with the ER, endolysosomal stores may be small, they nevertheless can exert a powerful influence on Ca^{2+} signaling in several ways critically dependent on their cellular location. Local Ca^{2+} release has been demonstrated by the targeting of Ca^{2+} reporter proteins to lysosomal membranes on their cytoplasmic face [239,240]. Mechanisms for controlling the positioning of lysosomes in cells are poorly understood. However, it is dependent on the cell's nutritional status [241,242] and involves the activity and membrane targeting of the mammalian target of rapamycin (mTOR complex), a serine/threonine protein kinase and the master regulator of cell growth, autophagy and cell survival in cells [243]. In the presence of nutrients, lysosomes are peripherally located, whereas in starvation, lysosomes migrate to the perinuclear region [241]. As well as regulating autophagic fluxes, lysosomal positioning is likely to affect their role in Ca^{2+} signaling and homeostasis. Endolysosomal luminal Ca^{2+} and Ca^{2+} release and have been proposed to be critical in endolysosomal fusion and trafficking [244,245], as well as triggering Ca^{2+} signaling events involving the ER [237,238]. The concept of endolysosomes as Ca^{2+} storage organelles playing a central role in Ca^{2+} signaling has been advanced by the finding that a specific Ca^{2+} mobilizing messenger, nicotinic acid adenine dinucleotide phosphate (NAADP) uniquely targets endolysosomes [237].

8.1. Lysosomes as Ca^{2+} signaling organelles

Endolysosomal Ca^{2+} and its release, along with luminal pH regulation are key factors in controlling the multifarious roles of this acidic membrane system, including vesicular trafficking, macromolecular degradation, autophagy and the myriad of functions associated with these organelles in health and disease [237,246]. Lumenal $[Ca^{2+}]$of lysosomes have been found to be in the range of 200–500 μM [247,248], not dissimilar to measurements of Ca^{2+} the ER lumen. Endosomes and lysosomes are highly acidic organelles, with the vacuolar proton pump ($V\text{-}H^+$-ATPase) playing the major role in acidification, with chloride channels such as CLC5 and CLC7 [249], and cation channels [250] implicated as acting as counter-ion channels to regulate acidification throughout the endosomal network. Lysosomes have also been found to have a modest membrane potential across their membrane of around 20 mV, lumen positive [251].

Recent focus on endolysosomal Ca^{2+} release channels has shown that members of the TRP superfamily are expressed in the membranes of the endolysosomal system, including TRPMLs (mucoplins), two-pore channels (TPCs), and TRPM2 [252]. All three have been suggested as

targets for the Ca^{2+} mobilizing messenger, NAADP [253]. In contrast, little is known about how Ca^{2+} is taken up or sequestered in the endolysosomal system. Ca^{2+} uptake is thought to be largely luminal proton-dependent and impaired in Niemann–Pick C disease, a lysosomal storage disease [248]. In platelets, SERCA3 pumps have also been implicated in Ca^{2+} sequestration by acidic organelles, known as dense granules. In other acidic stores, such as the acidocalcisomes of parasitic protozoa, polyphosphate matrices may have a role in luminal Ca^{2+} buffering [254]. In mammalian lysosomes luminal Ca^{2+} buffering mechanisms are unknown.

Endolysosomal Ca^{2+} and its release are key factors in the multifarious roles of this membrane system including vesicular trafficking, macromolecular degradation, autophagy and the myriad of functions associated with these organelles in health and disease [237,246].

8.2. Functional lysosomal–ER interactions

Lysosomal Ca^{2+} release evoked by the potent Ca^{2+} mobilizing, NAADP [255] can provide a Ca^{2+} trigger necessary for the excitation of the ER by CICR in response to various agonists acting at cell surface receptors [256–261], much like CICR that occurs in the dyads/triads of striated muscle (Fig. 4).

NAADP was initially identified as an NADP derivative that mobilizes Ca^{2+} via a distinct mechanism/receptor from that of IP_3 and cADPR) and targets non-ER Ca^{2+} stores in sea urchin egg homogenates [262,263]. Subsequently, NAADP was shown to release Ca^{2+} from reserve granules of sea urchin eggs, lysosome-like organelles, that could be inhibited by GPN which selectively disrupts lysosomes [264]. In echinoderm eggs, NAADP evokes a Ca^{2+} wave that propagates across the egg [265–267]. However, such Ca^{2+} wave becomes observably non-regenerative when ER Ca^{2+} release by both IP_3Rs and RyRs is inhibited, revealing the buffering effect exerted by the egg cytoplasm [265]. A recent study from our laboratory has furthered these studies, where we observed that concomitantly with NAADP-induced Ca^{2+} release, a luminal alkalization of acidic stores is seen [268]. Using organelle pH as a reporter of NAADP action, it was shown that Ca^{2+} may also be transferred from ER to lysosomes during IP_3 or cADPR signaling, which causes NAADP-dependent Ca^{2+} release [269]. This may be due to both local Ca^{2+}-dependent NAADP production, or luminal Ca^{2+} sensitization of NAADP-sensitive channels by increased acidic store luminal Ca^{2+} [270,271]. This bidirectional shuttling of Ca^{2+} between ER and lysosome may play an important role in NAADP-mediated Ca^{2+} oscillations. Importantly, such physiological Ca^{2+}-dependent cross-talk was unaffected by the slow Ca^{2+} buffer EGTA, indicative of local, Ca^{2+} microdomain-driven phenomena between lysosome and ER [269]. Recently in mammalian cell lines, lysosomal Ca^{2+} uptake can modulate the amplitude of ER Ca^{2+} release [272], analogous to the buffering action of the mitochondria.

In mammalian cells, a hallmark of NAADP-induced Ca^{2+} release, as in intact sea urchin eggs, is that it often appears to be a two-component response, consisting of the release from two pools of Ca^{2+} sequestering compartments. The initial component is often thought of as a small lysosomal Ca^{2+} release, which is only marginally reported by high affinity cytosolic Ca^{2+} dyes and is sensitive to a variety of inhibitors that lower lysosomal Ca^{2+} content [273–276]. The second component is often more pronounced, subsequent to the initial lysosomal Ca^{2+} release, which can be observed as a global Ca^{2+} transient that is readily reported by cytosolic Ca^{2+} dyes and shows sensitivity to a variety of inhibitors that prevent Ca^{2+} release from the ER [273–275]. Based on these observations, the prevailing view is that a small, potentially local lysosomal Ca^{2+} release is subsequently amplified by the action of CICR channels on the ER, which leads to a large cytoplasmic Ca^{2+} transient. This has been referred to as the 'trigger hypothesis' [237,238,277], a hypothesis that implicates an obligate cross-talk between the lysosomes and the ER. Lysosomes have been shown to closely associate with RyRs [273,275] and these are the sites of Ca^{2+} signal initiation in response to both endothelin-1 and NAADP.

As is the case for many Ca^{2+} transport mechanisms in organelles, as outlined above, it will come as no surprise that the molecular nature of NAADP-gated Ca^{2+} channels is controversial. The first candidate to emerge was TRPML1 or mucoplin1 [278,279], a member of the TRP family of ion channels, which is exclusively expressed in endolysosomes. Mutations in this channel are associated with the lysosomal storage disease mucolipidosis IV [280]. However, the candidature of this channel as an NAADP-gated channel has been ruled out by others [281] and this channel may instead be regulated by phosphatidylinositol 3,5,bisphosphate (PtdIns3,5,P_2) [282]. Recently, much steam has gathered behind a novel class of endolysosomal ion channel, the three member family of two-pore channel proteins (TPCs) as the target for NAADP [283–285]. Over the last three years, compelling evidence has been presented by a number of different laboratories using a variety of approaches, to support this view [286], with particular emphasis on TPC2 since it is the major form expressed in lysosomes. These include reconstitution of NAADP-gated Ca^{2+} channels in lipid bilayers by immunopurified TPCs [270], mutation of TPC2 leading to a change in ion selectivity of NAADP-gated currents in patch-clamped lysosomes [287], and redirection of TPC2 to the plasma membrane yielding an NAADP-gated current at the plasma membrane dissociated from ER coupling [288]. However, subsequent work showed that NAADP probably does not directly bind TPCs directly [289], but instead its effects are mediated by an NAADP-binding protein which was shown to associate with TPCs in sea urchin egg preparations [290,291]. This may in part explain the failure of some groups to see NAADP modulation of TPCs in patch clamp studies of vacuolin-enlarged lysosomes [292,293], with other regulators including PtdIns3,5,P_2 [292], and indirect action of ATP via mTOR proposed [293], apparently regulating a TPC-mediated sodium current. If this later assertion proves to be correct, then it is possible that TPCs may act to provide a pathway for counterion movement to facilitate Ca^{2+} release from lysosomes by a yet-to-be identified NAADP-gated Ca^{2+} channel, rather like the proposed role of TRIC channels in facilitating Ca^{2+} release from the SR (see Section 4.3).

8.3. Molecular aspects of lysosomal–ER junctions

An important model for understanding ER–lysosomal interactions has come from parallel studies in yeast examining the interactions of the vacuole (the yeast equivalent of a lysosome) with the membrane of the nucleus, which as discussed above, is contiguous with the ER. Lysosome–ER contact sites have been observed in electron micrographs, forming junctions of ~20 nm separation [294,295]. Like other membrane contact sites, lysosomes and the ER are held juxtaposed via protein contacts. Lysosomal movements are determined by bidirectional cytoskeletal motor proteins (kinesins and dyneins) under the control of small GTPases such as those of the Rab and Arf families [241], with members of the kinesin family promoting positive end movement, and dyneins favoring minus-end movement. Tethering and movement are controlled by a single molecular unit assembled on the late endosomal/lysosomal GTPase Rab7 protein complex. GTP-bound Rab7 forms a tripartite complex with its two effectors RILP and the cholesterol-sensing ORP1L [296]. RILP regulates minus-end movement, which is connected to the molecular motor dynein through its interaction with the adaptor protein $p150^{glued}$ [296,297]. ORP1L, under permissive conditions, is recognized by the ER transmembrane protein VAP-A through its FFAT motif, allowing the juxtaposition of lysosomes and the ER [294,298]. Switching between tethering and movement is regulated by the ORP1L ligand cholesterol and the conformational state of ORP1L. Through elegant FRET and biochemical experiments, it has been shown that cholesterol

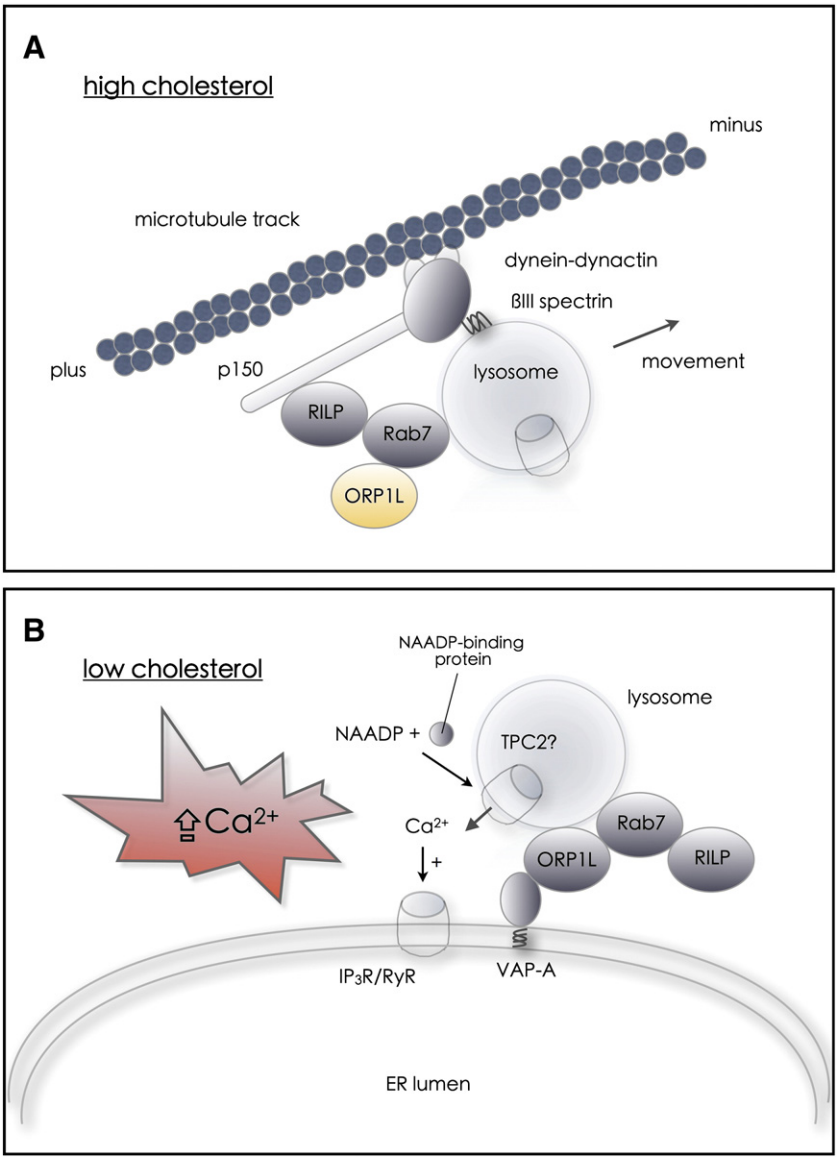

Fig. 4. Dynamic regulation of ER–lysosome junction. A. An endo-lysosomal organelle undergoing minus-end movement under high cholesterol conditions. Minus-end movement of lysosomes is mediated by the dynein/dynactin complex. Cholesterol induces a closed conformation of ORP1L, allowing the interaction between $p150^{Glued}$ and RILP thereby forming a complete physical linkage between motor proteins and the organelle. Dynactin binding to the organellar motor receptor beta-III spectrin depends on the function of cholesterol-bound ORP1L. B. ER–endolysosomal junctions are formed under low cholesterol conditions. Endolysosomal tethering to the ER is mediated by the ER transmembrane protein VAP-A, which recognizes the FFAT motif of ORP1L exposed when ORP1L is in a cholesterol-free open conformation. This displaces $p150^{glued}$ from the Rab–RILP–ORP1L complex and thus minus-end movement and tethering are counteractive. The current model hypothesizes that NAADP together with the putative NAADP-binding protein(s) [289,291] may activate lysosomal two-pore channels (TPC2) [283], allowing CICR between the lysosome and the ER within ER–lysosome junctions and subsequent global increase in Ca^{2+} [269,275].

binds to ORP1L and induces a conformational change of ORP1L, thereby allowing graded alternation between the ORP1L/VAP-A interaction and the RILP/$p150^{glued}$ interaction, and thus switching between ER tethering and minus-end movement [294]. Indeed, lysosomes tethered to the ER are more peripherally located, which also leads to the redistribution of the ER VAP-A into peripheral clusters that overlap with late endosomal/lysosomal markers [294,299]. In addition, lysosomes also undergo plus-end movements. This has recently been shown to be regulated by a novel lysosomal GTPase Arl8b and its effector SKIP [300]. SKIP interacts directly with the plus-end motor kinesin-1 through its WD domain thereby activating plus-end movement [300]. As this complex can exist in parallel with the Rab7/ORP1L/VAP-A complex that tethers lysosomes to the ER, plus-end movement of ER–juxtaposed lysosomes may occur and hence ER–tethered lysosomes may not be static and may promote protein translocation to the ER membrane. Interestingly, movement of lysosomes along ER tubules has recently been observed [272].

To date, it is not known at the molecular level how Ca^{2+} signaling between lysosomes and the ER is related to their juxtaposition. Although mammalian lysosome-associated ER membrane proteins have not been identified, a MAM-like domain is envisaged. There, NAADP-regulated channels and ER Ca^{2+}-sensitive Ca^{2+} release channels, for example the RyR/IP_3 receptor may be found, thereby forming a local Ca^{2+} amplification unit that may underlie the 'trigger hypothesis'. Further, the unprecedented link between cellular cholesterol level and lysosome–ER tethering implies the potential role of cholesterol in regulating lysosome–ER signaling. This may be of particular interest given that cellular cholesterol level is dysregulated under

certain pathological conditions, such as those seen in familial hypercholesterolemia, atherosclerosis [301,302] and congenital diseases such as Niemann–Pick type C (NPC) disease [248,303].

9. Conclusions

The ER is now established as the major Ca^{2+} storage organelle in cells, and pivotal to Ca^{2+} signaling and homeostasis. However, it generally does not work in isolation, and by forming junctions with the plasma membrane and the membranes of other organelles, it acts to coordinate intracellular Ca^{2+} signaling not only throughout the cytoplasm but also by regulating and recruiting additional Ca^{2+} pools. Studies of the structural and molecular basis of organellar interactions with the ER and visualization of local Ca^{2+} signaling at inter-organellar junctions, are suggestive of a key role for organelle architecture in the control and spatial aspects of Ca^{2+} signaling, crucial for the specific regulation of Ca^{2+}-sensitive processes that abound in all cell types. Clearly the mechanisms for the formation of organellar junctions, the identification and localization of Ca^{2+} signaling proteins at these interfaces, and the regulation of their stability and dynamics will prove to be an emerging major theme in Ca^{2+} signaling in the future.

References

[1] M.J. Berridge, P. Lipp, M.D. Bootman, The versatility and universality of calcium signalling, Nat. Rev. Mol. Cell Biol. 1 (2000) 11–21.
[2] D.E. Clapham, Calcium signaling, Cell 80 (1995) 259–268.
[3] D. Burdakov, O.H. Petersen, A. Verkhratsky, Intraluminal calcium as a primary regulator of endoplasmic reticulum function, Cell Calcium 38 (2005) 303–310.
[4] E.F. Corbett, M. Michalak, Calcium, a signaling molecule in the endoplasmic reticulum? Trends Biochem. Sci. 25 (2000) 307–311.
[5] A. Sakuntabhai, V. Ruiz-Perez, S. Carter, N. Jacobsen, S. Burge, S. Monk, M. Smith, C.S. Munro, M. O'Donovan, N. Craddock, R. Kucherlapati, J.L. Rees, M. Owen, G.M. Lathrop, A.P. Monaco, T. Strachan, A. Hovnanian, Mutations in ATP2A2, encoding a Ca^{2+} pump, cause Darier disease, Nat. Genet. 21 (1999) 271–277.
[6] S. Ebashi, Historical overview. Calcium ion and contractile proteins, Ann. N. Y. Acad. Sci. 522 (1988) 51–59.
[7] D.H. MacLennan, C.J. Brandl, B. Korczak, N.M. Green, Amino-acid sequence of a Ca^{2+} Mg^{2+}-dependent ATPase from rabbit muscle sarcoplasmic reticulum, deduced from its complementary DNA sequence, Nature 316 (1985) 696–700.
[8] T.R. Jackson, S.I. Patterson, O. Thastrup, M.R. Hanley, A novel tumour promoter, thapsigargin, transiently increases cytoplasmic free Ca^{2+} without generation of inositol phosphates in NG115-401L neuronal cells, Biochem. J. 253 (1988) 81–86.
[9] M. Endo, M. Tanaka, Y. Ogawa, Calcium induced release of calcium from the sarcoplasmic reticulum of skinned skeletal muscle fibres, Nature 228 (1970) 34–36.
[10] L.E. Ford, R.J. Podolsky, Regenerative calcium release within muscle cells, Science 167 (1970) 58–59.
[11] S.P. Nielsen, O.H. Petersen, Transport of calcium in the perfused submandibular gland of the cat, J. Physiol. 223 (1972) 685–697.
[12] H. Streb, R.F. Irvine, M.J. Berridge, I. Schulz, Release of Ca^{2+} from a nonmitochondrial intracellular store in pancreatic acinar cells by inositol-1,4,5-trisphosphate, Nature 306 (1983) 67–69.
[13] T. Furuichi, S. Yoshikawa, A. Miyawaki, K. Wada, N. Maeda, K. Mikoshiba, Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P400, Nature 342 (1989) 32–38.
[14] I. Bezprozvanny, J. Watras, B.E. Ehrlich, Bell-shaped calcium-response curves of Ins(1,4,5)P3- and calcium-gated channels from endoplasmic reticulum of cerebellum, Nature 351 (1991) 751–754.
[15] A. Galione, H.C. Lee, W.B. Busa, Ca^{2+}-induced Ca^{2+} release in sea urchin egg homogenates: modulation by cyclic ADP-ribose, Science 253 (1991) 1143–1146.
[16] M.J. Berridge, Neuronal calcium signaling, Neuron 21 (1998) 13–26.
[17] M.H. Wussling, K. Krannich, V. Drygalla, H. Podhaisky, Calcium waves in agarose gel with cell organelles: implications of the velocity curvature relationship, Biophys. J. 80 (2001) 2658–2666.
[18] G. Csordas, A.P. Thomas, G. Hajnoczky, Quasi-synaptic calcium signal transmission between endoplasmic reticulum and mitochondria, EMBO J. 18 (1999) 96–108.
[19] G.E. Palade, Keith Roberts Porter and the development of contemporary cell biology, J. Cell Biol. 75 (1977) D3–D19.
[20] L.L. Costantin, C. Franzini-Armstrong, R.J. Podolsky, Localization of calcium-accumulating structures in striated muscle fibers, Science 147 (1965) 158–160.
[21] M. Terasaki, N.T. Slater, A. Fein, A. Schmidek, T.S. Reese, Continuous network of endoplasmic reticulum in cerebellar Purkinje neurons, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 7510–7514.
[22] K. Subramanian, T. Meyer, Calcium-induced restructuring of nuclear envelope and endoplasmic reticulum calcium stores, Cell 89 (1997) 963–971.
[23] H. Mogami, K. Nakano, A.V. Tepikin, O.H. Petersen, Ca^{2+} flow via tunnels in polarized cells: recharging of apical Ca^{2+} stores by focal Ca^{2+} entry through basal membrane patch, Cell 88 (1997) 49–55.
[24] V.A. Golovina, M.P. Blaustein, Spatially and functionally distinct Ca^{2+} stores in sarcoplasmic and endoplasmic reticulum, Science 275 (1997) 1643–1648.
[25] M.T. Nelson, H. Cheng, M. Rubart, L.F. Santana, A.D. Bonev, H.J. Knot, W.J. Lederer, Relaxation of arterial smooth muscle by calcium sparks, Science 270 (1995) 633–637.
[26] C. Franzini-Armstrong, F. Protasi, Ryanodine receptors of striated muscles: a complex channel capable of multiple interactions, Physiol. Rev. 77 (1997) 699–729.
[27] S. Doroudgar, C.C. Glembotski, New concepts of endoplasmic reticulum function in the heart: programmed to conserve, J. Mol. Cell. Cardiol. 55 (2013) 85–91.
[28] I.F. Smith, S.M. Wiltgen, J. Shuai, I. Parker, Ca^{2+} puffs originate from preestablished stable clusters of inositol trisphosphate receptors, Sci. Signal. 2 (2009) ra77.
[29] H. Vais, J.K. Foskett, D.O. Mak, $InsP_3R$ channel gating altered by clustering? Nature 478 (2011) E1–E2, (discussion E2-3.).
[30] Y. Tateishi, M. Hattori, T. Nakayama, M. Iwai, H. Bannai, T. Nakamura, T. Michikawa, T. Inoue, K. Mikoshiba, Cluster formation of inositol 1,4,5-trisphosphate receptor requires its transition to open state, J. Biol. Chem. 280 (2005) 6816–6822.
[31] R. Taufiq Ur, A. Skupin, M. Falcke, C.W. Taylor, Clustering of InsP3 receptors by InsP3 retunes their regulation by $InsP_3$ and Ca^{2+}, Nature 458 (2009) 655–659.
[32] R. Rizzuto, T. Pozzan, Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences, Physiol. Rev. 86 (2006) 369–408.
[33] S. Papp, E. Dziak, M. Michalak, M. Opas, Is all of the endoplasmic reticulum created equal? The effects of the heterogeneous distribution of endoplasmic reticulum Ca^{2+}-handling proteins, J. Cell Biol. 160 (2003) 475–479.
[34] G. Gatti, S. Trifari, N. Mesaeli, J.M. Parker, M. Michalak, J. Meldolesi, Head-to-tail oligomerization of calsequestrin: a novel mechanism for heterogeneous distribution of endoplasmic reticulum luminal proteins, J. Cell Biol. 154 (2001) 525–534.
[35] T.R. Shannon, T. Guo, D.M. Bers, Ca^{2+} scraps: local depletions of free Ca^{2+} in cardiac sarcoplasmic reticulum during contractions leave substantial Ca^{2+} reserve, Circ. Res. 93 (2003) 40–45.
[36] C.M. Ribeiro, R.R. McKay, E. Hosoki, G.S. Bird, J.W. Putney Jr., Effects of elevated cytoplasmic calcium and protein kinase C on endoplasmic reticulum structure and function in HEK293 cells, Cell Calcium 27 (2000) 175–185.
[37] D.C. Renard-Rooney, G. Hajnoczky, M.B. Seitz, T.G. Schneider, A.P. Thomas, Imaging of inositol 1,4,5-trisphosphate-induced Ca^{2+} fluxes in single permeabilized hepatocytes. Demonstration of both quantal and nonquantal patterns of Ca^{2+} release, J. Biol. Chem. 268 (1993) 23601–23610.
[38] M. Terasaki, L.A. Jaffe, Organization of the sea urchin egg endoplasmic reticulum and its reorganization at fertilization, J. Cell Biol. 114 (1991) 929–940.
[39] I. Benedeczky, E. Molnar, P. Somogyi, The cisternal organelle as a Ca^{2+}-storing compartment associated with GABAergic synapses in the axon initial segment of hippocampal pyramidal neurones, Exp. Brain Res. 101 (1994) 216–230.
[40] T. Deller, M. Korte, S. Chabanis, A. Drakew, H. Schwegler, G.G. Stefani, A. Zuniga, K. Schwarz, T. Bonhoeffer, R. Zeller, M. Frotscher, P. Mundel, Synaptopodin-deficient mice lack a spine apparatus and show deficits in synaptic plasticity, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 10494–10499.
[41] A. Vlachos, Synaptopodin and the spine apparatus organelle-regulators of different forms of synaptic plasticity? Ann. Anat. 194 (2012) 317–320.
[42] M.J. Boulware, J.S. Marchant, IP_3 receptor activity is differentially regulated in endoplasmic reticulum subdomains during oocyte maturation, Curr. Biol. 15 (2005) 765–770.
[43] M.J. Boulware, J.S. Marchant, Nuclear pore disassembly from endoplasmic reticulum membranes promotes Ca^{2+} signalling competency, J. Physiol. 586 (2008) 2873–2888.
[44] L. Stehno-Bittel, C. Perez-Terzic, D.E. Clapham, Diffusion across the nuclear envelope inhibited by depletion of the nuclear Ca^{2+} store, Science 270 (1995) 1835–1838.
[45] D.R. Higazi, C.J. Fearnley, F.M. Drawnel, A. Talasila, E.M. Corps, O. Ritter, F. McDonald, K. Mikoshiba, M.D. Bootman, H.L. Roderick, Endothelin-1-stimulated $InsP_3$-induced Ca^{2+} release is a nexus for hypertrophic signaling in cardiac myocytes, Mol. Cell 33 (2009) 472–482.
[46] A. Malhas, C. Goulbourne, D.J. Vaux, The nucleoplasmic reticulum: form and function, Trends Cell Biol. 21 (2011) 362–373.
[47] W. Echevarria, M.F. Leite, M.T. Guerra, W.R. Zipfel, M.H. Nathanson, Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum, Nat. Cell Biol. 5 (2003) 440–446.
[48] P. Marius, M.T. Guerra, M.H. Nathanson, B.E. Ehrlich, M.F. Leite, Calcium release from ryanodine receptors in the nucleoplasmic reticulum, Cell Calcium 39 (2006) 65–73.
[49] F.W. Tse, A. Tse, B. Hille, Cyclic Ca^{2+} changes in intracellular stores of gonadotropes during gonadotropin-releasing hormone-stimulated Ca^{2+} oscillations, Proc. Natl. Acad. Sci. U. S. A. 91 (1994) 9750–9754.
[50] A.E. Palmer, C. Jin, J.C. Reed, R.Y. Tsien, Bcl-2-mediated alterations in endoplasmic reticulum Ca^{2+} analyzed with an improved genetically encoded fluorescent sensor, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17404–17409.
[51] N. Solovyova, A. Verkhratsky, Monitoring of free calcium in the neuronal endoplasmic reticulum: an overview of modern approaches, J. Neurosci. Methods 122 (2002) 1–12.
[52] A. Miyawaki, J. Llopis, R. Heim, J.M. McCaffery, J.A. Adams, M. Ikura, R.Y. Tsien, Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin, Nature 388 (1997) 882–887.

[53] J. Nakai, M. Ohkura, K. Imoto, A high signal-to-noise Ca^{2+} probe composed of a single green fluorescent protein, Nat. Biotechnol. 19 (2001) 137–141.
[54] A.E. Palmer, R.Y. Tsien, Measuring calcium signaling using genetically targetable fluorescent indicators, Nat. Protoc. 1 (2006) 1057–1065.
[55] Y. Zhao, S. Araki, J. Wu, T. Teramoto, Y.F. Chang, M. Nakano, A.S. Abdelfattah, M. Fujiwara, T. Ishihara, T. Nagai, R.E. Campbell, An expanded palette of genetically encoded Ca(2)(+) indicators, Science 333 (2011) 1888–1891.
[56] K. Ishii, K. Hirose, M. Iino, Ca^{2+} shuttling between endoplasmic reticulum and mitochondria underlying Ca^{2+} oscillations, EMBO Rep. 7 (2006) 390–396.
[57] M. Canato, M. Scorzeto, M. Giacomello, F. Protasi, C. Reggiani, G.J. Stienen, Massive alterations of sarcoplasmic reticulum free calcium in skeletal muscle fibers lacking calsequestrin revealed by a genetically encoded probe, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 22326–22331.
[58] S. Tang, H.C. Wong, Z.M. Wang, Y. Huang, J. Zou, Y. Zhuo, A. Pennati, G. Gadda, O. Delbono, J.J. Yang, Design and application of a class of sensors to monitor Ca^{2+} dynamics in high Ca^{2+} concentration cellular compartments, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 16265–16270.
[59] R.Y. Tsien, New calcium indicators and buffers with high selectivity against magnesium and protons: design, synthesis, and properties of prototype structures, Biochemistry 19 (1980) 2396–2404.
[60] G. Grynkiewicz, M. Poenie, R.Y. Tsien, A new generation of Ca^{2+} indicators with greatly improved fluorescence properties, J. Biol. Chem. 260 (1985) 3440–3450.
[61] R.Y. Tsien, A non-disruptive technique for loading calcium buffers and indicators into cells, Nature 290 (1981) 527–528.
[62] D. Thomas, S.C. Tovey, T.J. Collins, M.D. Bootman, M.J. Berridge, P. Lipp, A comparison of fluorescent Ca^{2+} indicator properties and their use in measuring elementary and global Ca^{2+} signals, Cell Calcium 28 (2000) 213–223.
[63] A.P. Ziman, C.W. Ward, G.G. Rodney, W.J. Lederer, R.J. Bloch, Quantitative measurement of Ca^{2+} in the sarcoplasmic reticulum lumen of mammalian skeletal muscle, Biophys. J. 99 (2010) 2705–2714.
[64] A.M. Hofer, T.E. Machen, Technique for in situ measurement of calcium in intracellular inositol 1,4,5-trisphosphate-sensitive stores using the fluorescent indicator mag-fura-2, Proc. Natl. Acad. Sci. U. S. A. 90 (1993) 2598–2602.
[65] G. Hajnoczky, A.P. Thomas, Minimal requirements for calcium oscillations driven by the IP_3 receptor, EMBO J. 16 (1997) 3533–3543.
[66] M.K. Park, O.H. Petersen, A.V. Tepikin, The endoplasmic reticulum as one continuous Ca^{2+} pool: visualization of rapid Ca(2+) movements and equilibration, EMBO J. 19 (2000) 5729–5739.
[67] N. Solovyova, N. Veselovsky, E.C. Toescu, A. Verkhratsky, Ca^{2+} dynamics in the lumen of the endoplasmic reticulum in sensory neurons: direct visualization of Ca^{2+}-induced Ca^{2+}release triggered by physiological Ca(2+) entry, EMBO J. 21 (2002) 622–630.
[68] M. Rehberg, A. Lepier, B. Solchenberger, P. Osten, R. Blum, A new non-disruptive strategy to target calcium indicator dyes to the endoplasmic reticulum, Cell Calcium 44 (2008) 386–399.
[69] J.K. Foskett, C. White, K.H. Cheung, D.O. Mak, Inositol trisphosphate receptor Ca^{2+} release channels, Physiol. Rev. 87 (2007) 593–658.
[70] M. Fill, J.A. Copello, Ryanodine receptor calcium release channels, Physiol. Rev. 82 (2002) 893–922.
[71] E. Sammels, B. Devogelaere, D. Mekahli, G. Bultynck, L. Missiaen, J.B. Parys, Y. Cai, S. Somlo, H. De Smedt, Polycystin-2 activation by inositol 1,4,5-trisphosphate-induced Ca^{2+} release requires its direct association with the inositol 1,4,5-trisphosphate receptor in a signaling microdomain, J. Biol. Chem. 285 (2010) 18794–18805.
[72] E.T. Petri, A. Celic, S.D. Kennedy, B.E. Ehrlich, T.J. Boggon, M.E. Hodsdon, Structure of the EF-hand domain of polycystin-2 suggests a mechanism for Ca^{2+}-dependent regulation of polycystin-2 channel activity, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 9176–9181.
[73] A. Galione, A. McDougall, W.B. Busa, N. Willmott, I. Gillot, M. Whitaker, Redundant mechanisms of calcium-induced calcium release underlying calcium waves during fertilization of sea urchin eggs, Science 261 (1993) 348–352.
[74] S. Supattapone, P.F. Worley, J.M. Baraban, S.H. Snyder, Solubilization, purification, and characterization of an inositol trisphosphate receptor, J. Biol. Chem. 263 (1988) 1530–1534.
[75] A. Miyawaki, T. Furuichi, N. Maeda, K. Mikoshiba, Expressed cerebellar-type inositol 1,4,5-trisphosphate receptor, P400, has calcium release activity in a fibroblast L cell line, Neuron 5 (1990) 11–18.
[76] D.O. Mak, S. McBride, V. Raghuram, Y. Yue, S.K. Joseph, J.K. Foskett, Single-channel properties in endoplasmic reticulum membrane of recombinant type 3 inositol trisphosphate receptor, J. Gen. Physiol. 115 (2000) 241–256.
[77] H. Vais, J.K. Foskett, D.O. Mak, Unitary Ca^{2+} current through recombinant type 3 $InsP_3$ receptor channels under physiological ionic conditions, J. Gen. Physiol. 136 (2010) 687–700.
[78] D.O. Mak, J.K. Foskett, Effects of divalent cations on single-channel conduction properties of *Xenopus* IP_3 receptor, Am. J. Physiol. 275 (1998) C179–C188.
[79] D. Gillespie, M. Fill, Intracellular calcium release channels mediate their own countercurrent: the ryanodine receptor case study, Biophys. J. 95 (2008) 3706–3714.
[80] C.W. Taylor, S.C. Tovey, IP(3) receptors: toward understanding their activation, Cold Spring Harb. Perspect. Biol. 2 (2010) a004010.
[81] D.O. Mak, S. McBride, J.K. Foskett, Inositol 1,4,5-trisphosphate activation of inositol trisphosphate receptor Ca^{2+} channel by ligand tuning of Ca^{2+} inhibition, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 15821–15825.
[82] M.D. Bootman, M.J. Berridge, P. Lipp, Cooking with calcium: the recipes for composing global signals from elementary events, Cell 91 (1997) 367–373.
[83] G. Dupont, L. Combettes, G.S. Bird, J.W. Putney, Calcium oscillations, Cold Spring Harb. Perspect. Biol. 3 (2011).
[84] L. Missiaen, H. De Smedt, G. Droogmans, R. Casteels, Ca^{2+} release induced by inositol 1,4,5-trisphosphate is a steady-state phenomenon controlled by luminal Ca^{2+} in permeabilized cells, Nature 357 (1992) 599–602.
[85] D.L. Nunn, C.W. Taylor, Luminal Ca^{2+} increases the sensitivity of Ca^{2+} stores to inositol 1,4,5-trisphosphate, Mol. Pharmacol. 41 (1992) 115–119.
[86] I. Bezprozvanny, B.E. Ehrlich, Inositol (1,4,5)-trisphosphate ($InsP_3$)-gated Ca channels from cerebellum: conduction properties for divalent cations and regulation by intraluminal calcium, J. Gen. Physiol. 104 (1994) 821–856.
[87] H. Vais, J.K. Foskett, G. Ullah, J.E. Pearson, D.O. Daniel Mak, Permeant calcium ion feed-through regulation of single inositol 1,4,5-trisphosphate receptor channel gating, J. Gen. Physiol. 140 (2012) 697–716.
[88] K. Mikoshiba, IP_3 receptor/Ca^{2+} channel: from discovery to new signaling concepts, J. Neurochem. 102 (2007) 1426–1446.
[89] M.L. Mayer, Glutamate receptors at atomic resolution, Nature 440 (2006) 456–462.
[90] I. Bosanac, H. Yamazaki, T. Matsu-Ura, T. Michikawa, K. Mikoshiba, M. Ikura, Crystal structure of the ligand binding suppressor domain of type 1 inositol 1,4,5-trisphosphate receptor, Mol. Cell 17 (2005) 193–203.
[91] H. Ando, A. Mizutani, T. Matsu-ura, K. Mikoshiba, IRBIT, a novel inositol 1,4,5-trisphosphate (IP3) receptor-binding protein, is released from the IP_3 receptor upon IP_3 binding to the receptor, J. Biol. Chem. 278 (2003) 10602–10612.
[92] H. Ando, A. Mizutani, H. Kiefer, D. Tsuzurugi, T. Michikawa, K. Mikoshiba, IRBIT suppresses IP_3 receptor activity by competing with IP_3 for the common binding site on the IP3 receptor, Mol. Cell 22 (2006) 795–806.
[93] N. DeSouza, S. Reiken, K. Ondrias, Y.M. Yang, S. Matkovich, A.R. Marks, Protein kinase A and two phosphatases are components of the inositol 1,4,5-trisphosphate receptor macromolecular signaling complex, J. Biol. Chem. 277 (2002) 39397–39400.
[94] C. Giorgi, K. Ito, H.K. Lin, C. Santangelo, M.R. Wieckowski, M. Lebiedzinska, A. Bononi, M. Bonora, J. Duszynski, R. Bernardi, R. Rizzuto, C. Tacchetti, P. Pinton, P.P. Pandolfi, PML regulates apoptosis at endoplasmic reticulum by modulating calcium release, Science 330 (2010) 1247–1251.
[95] C.D. Ferris, R.L. Huganir, D.S. Bredt, A.M. Cameron, S.H. Snyder, Inositol trisphosphate receptor: phosphorylation by protein kinase C and calcium calmodulin-dependent protein kinases in reconstituted lipid vesicles, Proc. Natl. Acad. Sci. U. S. A. 88 (1991) 2232–2235.
[96] P. Komalavilas, T.M. Lincoln, Phosphorylation of the inositol 1,4,5-trisphosphate receptor by cyclic GMP-dependent protein kinase, J. Biol. Chem. 269 (1994) 8701–8707.
[97] J. Schlossmann, A. Ammendola, K. Ashman, X. Zong, A. Huber, G. Neubauer, G.X. Wang, H.D. Allescher, M. Korth, M. Wilm, F. Hofmann, P. Ruth, Regulation of intracellular calcium by a signalling complex of IRAG, IP3 receptor and cGMP kinase Ibeta, Nature 404 (2000) 197–201.
[98] K. Yokoyama, I.H.Su. Ih, T. Tezuka, T. Yasuda, K. Mikoshiba, A. Tarakhovsky, T. Yamamoto, BANK regulates BCR-induced calcium mobilization by promoting tyrosine phosphorylation of IP(3) receptor, EMBO J. 21 (2002) 83–92.
[99] T. Jayaraman, K. Ondrias, E. Ondriasova, A.R. Marks, Regulation of the inositol 1,4,5-trisphosphate receptor by tyrosine phosphorylation, Science 272 (1996) 1492–1494.
[100] T.S. Tang, H. Tu, Z. Wang, I. Bezprozvanny, Modulation of type 1 inositol (1,4,5)-trisphosphate receptor function by protein kinase a and protein phosphatase 1alpha, J. Neurosci. 23 (2003) 403–415.
[101] F.A. Lai, H.P. Erickson, E. Rousseau, Q.Y. Liu, G. Meissner, Purification and reconstitution of the calcium release channel from skeletal muscle, Nature 331 (1988) 315–319.
[102] M. Inui, A. Saito, S. Fleischer, Purification of the ryanodine receptor and identity with feet structures of junctional terminal cisternae of sarcoplasmic reticulum from fast skeletal muscle, J. Biol. Chem. 262 (1987) 1740–1747.
[103] J.T. Lanner, D.K. Georgiou, A.D. Joshi, S.L. Hamilton, Ryanodine receptors: structure, expression, molecular details, and function in calcium release, Cold Spring Harb. Perspect. Biol. 2 (2010) a003996.
[104] A.R. Lindsay, S.D. Manning, A.J. Williams, Monovalent cation conductance in the ryanodine receptor-channel of sheep cardiac muscle sarcoplasmic reticulum, J. Physiol. 439 (1991) 463–480.
[105] J.S. Smith, T. Imagawa, J. Ma, M. Fill, K.P. Campbell, R. Coronado, Purified ryanodine receptor from rabbit skeletal muscle is the calcium-release channel of sarcoplasmic reticulum, J. Gen. Physiol. 92 (1988) 1–26.
[106] A. Tinker, A.J. Williams, Divalent cation conduction in the ryanodine receptor channel of sheep cardiac muscle sarcoplasmic reticulum, J. Gen. Physiol. 100 (1992) 479–493.
[107] A. Tinker, A.R. Lindsay, A.J. Williams, Cation conduction in the calcium release channel of the cardiac sarcoplasmic reticulum under physiological and pathophysiological conditions, Cardiovasc. Res. 27 (1993) 1820–1825.
[108] R. Mejia-Alvarez, C. Kettlun, E. Rios, M. Stern, M. Fill, Unitary Ca^{2+} current through cardiac ryanodine receptor channels under quasi-physiological ionic conditions, J. Gen. Physiol. 113 (1999) 177–186.
[109] S.M. Baylor, W.K. Chandler, M.W. Marshall, Sarcoplasmic reticulum calcium release in frog skeletal muscle fibres estimated from Arsenazo III calcium transients, J. Physiol. 344 (1983) 625–666.
[110] M.D. Stern, Buffering of calcium in the vicinity of a channel pore, Cell Calcium 13 (1992) 183–192.
[111] G.D. Smith, Analytical steady-state solution to the rapid buffering approximation near an open Ca^{2+} channel, Biophys. J. 71 (1996) 3064–3072.
[112] E. Neher, Vesicle pools and Ca^{2+} microdomains: new tools for understanding their roles in neurotransmitter release, Neuron 20 (1998) 389–399.
[113] E. Neher, Usefulness and limitations of linear approximations to the understanding of Ca++ signals, Cell Calcium 24 (1998) 345–357.

[114] D.R. Laver, T.M. Baynes, A.F. Dulhunty, Magnesium inhibition of ryanodine-receptor calcium channels: evidence for two independent mechanisms, J. Membr. Biol. 156 (1997) 213–229.
[115] L. Xu, G. Mann, G. Meissner, Regulation of cardiac Ca^{2+} release channel (ryanodine receptor) by Ca^{2+}, H^+, Mg^{2+}, and adenine nucleotides under normal and simulated ischemic conditions, Circ. Res. 79 (1996) 1100–1109.
[116] J.S. Smith, R. Coronado, G. Meissner, Sarcoplasmic reticulum contains adenine nucleotide-activated calcium channels, Nature 316 (1985) 446–449.
[117] L.G. Meszaros, J. Bak, A. Chu, Cyclic ADP-ribose as an endogenous regulator of the non-skeletal type ryanodine receptor channel, Nature 364 (1993) 76–79.
[118] R. Sitsapesan, A.J. Williams, Cyclic ADP-ribose and related compounds activate sheep skeletal sarcoplasmic reticulum Ca^{2+} release channel, Am. J. Physiol. 268 (1995) C1235–C1240.
[119] I. Gyorke, S. Gyorke, Regulation of the cardiac ryanodine receptor channel by luminal Ca^{2+} involves luminal Ca^{2+} sensing sites, Biophys. J. 75 (1998) 2801–2810.
[120] R. Sitsapesan, A.J. Williams, The gating of the sheep skeletal sarcoplasmic reticulum Ca^{2+}-release channel is regulated by luminal Ca^{2+}, J. Membr. Biol. 146 (1995) 133–144.
[121] H. Kong, R. Wang, W. Chen, L. Zhang, K. Chen, Y. Shimoni, H.J. Duff, S.R. Chen, Skeletal and cardiac ryanodine receptors exhibit different responses to Ca^{2+} overload and luminal Ca^{2+}, Biophys. J. 92 (2007) 2757–2770.
[122] L.L. Ching, A.J. Williams, R. Sitsapesan, Evidence for Ca^{2+} activation and inactivation sites on the luminal side of the cardiac ryanodine receptor complex, Circ. Res. 87 (2000) 201–206.
[123] J.H. Hwang, F. Zorzato, N.F. Clarke, S. Treves, Mapping domains and mutations on the skeletal muscle ryanodine receptor channel, Trends Mol. Med. 18 (2012) 644–657.
[124] L.M. Blayney, F.A. Lai, Ryanodine receptor-mediated arrhythmias and sudden cardiac death, Pharmacol. Ther. 123 (2009) 151–177.
[125] A.B. Brillantes, K. Ondrias, A. Scott, E. Kobrinsky, E. Ondriasova, M.C. Moschella, T. Jayaraman, M. Landers, B.E. Ehrlich, A.R. Marks, Stabilization of calcium release channel (ryanodine receptor) function by FK506-binding protein, Cell 77 (1994) 513–523.
[126] S.O. Marx, S. Reiken, Y. Hisamatsu, T. Jayaraman, D. Burkhoff, N. Rosemblit, A.R. Marks, PKA phosphorylation dissociates FKBP12.6 from the calcium release channel (ryanodine receptor): defective regulation in failing hearts, Cell 101 (2000) 365–376.
[127] D.M. Bers, Cardiac excitation–contraction coupling, Nature 415 (2002) 198–205.
[128] A.M. Garcia, C. Miller, Channel-mediated monovalent cation fluxes in isolated sarcoplasmic reticulum vesicles, J. Gen. Physiol. 83 (1984) 819–839.
[129] A.V. Somlyo, H.G. Gonzalez-Serratos, H. Shuman, G. McClellan, A.P. Somlyo, Calcium release and ionic changes in the sarcoplasmic reticulum of tetanized muscle: an electron-probe study, J. Cell Biol. 90 (1981) 577–594.
[130] C. Miller, Voltage-gated cation conductance channel from fragmented sarcoplasmic reticulum: steady-state electrical properties, J. Membr. Biol. 40 (1978) 1–23.
[131] R. Coronado, R.L. Rosenberg, C. Miller, Ionic selectivity, saturation, and block in a K^+-selective channel from sarcoplasmic reticulum, J. Gen. Physiol. 76 (1980) 425–446.
[132] C. Miller, Bis-quaternary ammonium blockers as structural probes of the sarcoplasmic reticulum K^+ channel, J. Gen. Physiol. 79 (1982) 869–891.
[133] G. Meissner, D. McKinley, Permeability of canine cardiac sarcoplasmic reticulum vesicles to K^+, Na^+, H^+, and Cl, J. Biol. Chem. 257 (1982) 7704–7711.
[134] C.W. Abramcheck, P.M. Best, Physiological role and selectivity of the in situ potassium channel of the sarcoplasmic reticulum in skinned frog skeletal muscle fibers, J. Gen. Physiol. 93 (1989) 1–21.
[135] M. Yazawa, C. Ferrante, J. Feng, K. Mio, T. Ogura, M. Zhang, P.H. Lin, Z. Pan, S. Komazaki, K. Kato, M. Nishi, X. Zhao, N. Weisleder, C. Sato, J. Ma, H. Takeshima, TRIC channels are essential for Ca^{2+} handling in intracellular stores, Nature 448 (2007) 78–82.
[136] S.J. Pitt, K.H. Park, M. Nishi, T. Urashima, S. Aoki, D. Yamazaki, J. Ma, H. Takeshima, R. Sitsapesan, Charade of the SR K^+-channel: two ion-channels, TRIC-A and TRIC-B, masquerade as a single K^+-channel, Biophys. J. 99 (2010) 417–426.
[137] D. Yamazaki, Y. Tabara, S. Kita, H. Hanada, S. Komazaki, D. Naitou, A. Mishima, M. Nishi, H. Yamamura, S. Yamamoto, S. Kakizawa, H. Miyachi, S. Yamamoto, T. Miyata, Y. Kawano, K. Kamide, T. Ogihara, A. Hata, S. Umemura, M. Soma, N. Takahashi, Y. Imaizumi, T. Miki, T. Iwamoto, H. Takeshima, TRIC-A channels in vascular smooth muscle contribute to blood pressure maintenance, Cell Metab. 14 (2011) 231–241.
[138] D. Yamazaki, S. Komazaki, H. Nakanishi, A. Mishima, M. Nishi, M. Yazawa, T. Yamazaki, R. Taguchi, H. Takeshima, Essential role of the TRIC-B channel in Ca^{2+} handling of alveolar epithelial cells and in perinatal lung maturation, Development 136 (2009) 2355–2361.
[139] C. Camello, R. Lomax, O.H. Petersen, A.V. Tepikin, Calcium leak from intracellular stores—the enigma of calcium signalling, Cell Calcium 32 (2002) 355–361.
[140] R.B. Lomax, C. Camello, F. Coppenolle Fv, O.H. Petersen, A.V. Tepikin, Basal and physiological Ca^{2+} leak from the endoplasmic reticulum of pancreatic acinar cells: second messenger—activated channels and translocons, J. Biol. Chem. 277 (2002) 26479–26485.
[141] F. Vanden Abeele, G. Bidaux, D. Gordienko, B. Beck, Y.V. Panchin, A.V. Baranova, D.V. Ivanov, R. Skryma, N. Prevarskaya, Functional implications of calcium permeability of the channel formed by pannexin 1, J. Cell Biol. 174 (2006) 535–546.
[142] H. Tu, O. Nelson, A. Bezprozvanny, Z. Wang, S.F. Lee, Y.H. Hao, L. Serneels, B. De Strooper, G. Yu, I. Bezprozvanny, Presenilins form ER Ca^{2+} leak channels, a function disrupted by familial Alzheimer's disease-linked mutations, Cell 126 (2006) 981–993.
[143] C. Li, X. Wang, H. Vais, C.B. Thompson, J.K. Foskett, C. White, Apoptosis regulation by Bcl-x(L) modulation of mammalian inositol 1,4,5-trisphosphate receptor channel isoform gating, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 12565–12570.
[144] M.A. Leissring, I. Parker, F.M. LaFerla, Presenilin-2 mutations modulate amplitude and kinetics of inositol 1, 4,5-trisphosphate-mediated calcium signals, J. Biol. Chem. 274 (1999) 32535–32538.
[145] K.H. Cheung, L. Mei, D.O. Mak, I. Hayashi, T. Iwatsubo, D.E. Kang, J.K. Foskett, Gain-of-function enhancement of IP3 receptor modal gating by familial Alzheimer's disease-linked presenilin mutants in human cells and mouse neurons, Sci. Signal. 3 (2010) ra22.
[146] Y. Elbaz, M. Schuldiner, Staying in touch: the molecular era of organelle contact sites, Trends Biochem. Sci. 36 (2011) 616–623.
[147] S.C. Helle, G. Kanfer, K. Kolar, A. Lang, A.H. Michel, B. Kornmann, Organization and function of membrane contact sites, Biochim. Biophys. Acta (2013), (pii: S0167-4889(13)00043-8).
[148] G.S. Bird, S.Y. Hwang, J.T. Smyth, M. Fukushima, R.R. Boyles, J.W. Putney Jr., STIM1 is a calcium sensor specialized for digital signaling, Curr. Biol. 19 (2009) 1724–1729.
[149] J. Soboloff, B.S. Rothberg, M. Madesh, D.L. Gill, STIM proteins: dynamic calcium signal transducers, Nat. Rev. Mol. Cell Biol. 13 (2012) 549–565.
[150] P. Varnai, L. Hunyady, T. Balla, STIM and Orai: the long-awaited constituents of store-operated calcium entry, Trends Pharmacol. Sci. 30 (2009) 118–128.
[151] G.C. Wellman, M.T. Nelson, Signaling between SR and plasmalemma in smooth muscle: sparks and the activation of Ca^{2+}-sensitive ion channels, Cell Calcium 34 (2003) 211–229.
[152] A.G. Manford, C.J. Stefan, H.L. Yuan, J.A. Macgurn, S.D. Emr, ER-to-plasma membrane tethering proteins regulate cell signaling and ER morphology, Dev. Cell 23 (2012) 1129–1140.
[153] H. Takeshima, M. Iino, H. Takekura, M. Nishi, J. Kuno, O. Minowa, H. Takano, T. Noda, Excitation–contraction uncoupling and muscular degeneration in mice lacking functional skeletal muscle ryanodine-receptor gene, Nature 369 (1994) 556–559.
[154] R.S. Lewis, The molecular choreography of a store-operated calcium channel, Nature 446 (2007) 284–287.
[155] M. Endo, Calcium-induced calcium release in skeletal muscle, Physiol. Rev. 89 (2009) 1153–1176.
[156] D.X. Brochet, D. Yang, A. Di Maio, W.J. Lederer, C. Franzini-Armstrong, H. Cheng, Ca^{2+} blinks: rapid nanoscopic store calcium signaling, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 3099–3104.
[157] Y. Takagishi, K. Yasui, N.J. Severs, Y. Murata, Species-specific difference in distribution of voltage-gated L-type Ca^{2+} channels of cardiac myocytes, Am. J. Physiol. Cell Physiol. 279 (2000) C1963–C1969.
[158] C. Franzini-Armstrong, F. Protasi, V. Ramesh, Comparative ultrastructure of Ca^{2+} release units in skeletal and cardiac muscle, Ann. N. Y. Acad. Sci. 853 (1998) 20–30.
[159] S.L. Carl, K. Felix, A.H. Caswell, N.R. Brandt, W.J. Ball Jr., P.L. Vaghy, G. Meissner, D.G. Ferguson, Immunolocalization of sarcolemmal dihydropyridine receptor and sarcoplasmic reticular triadin and ryanodine receptor in rabbit ventricle and atrium, J. Cell Biol. 129 (1995) 673–682.
[160] D.R. Scriven, P. Dan, E.D. Moore, Distribution of proteins implicated in excitation–contraction coupling in rat ventricular myocytes, Biophys. J. 79 (2000) 2682–2691.
[161] M.D. Stern, G. Pizarro, E. Rios, Local control model of excitation–contraction coupling in skeletal muscle, J. Gen. Physiol. 110 (1997) 415–440.
[162] C. Franzini-Armstrong, F. Protasi, V. Ramesh, Shape, size, and distribution of Ca^{2+} release units and couplons in skeletal and cardiac muscles, Biophys. J. 77 (1999) 1528–1539.
[163] P.S. Haddock, W.A. Coetzee, E. Cho, L. Porter, H. Katoh, D.M. Bers, M.S. Jafri, M. Artman, Subcellular $[Ca^{2+}]i$ gradients during excitation–contraction coupling in newborn rabbit ventricular myocytes, Circ. Res. 85 (1999) 415–427.
[164] J.W. Putney Jr., A model for receptor-regulated calcium entry, Cell Calcium 7 (1986) 1–12.
[165] J. Liou, M.L. Kim, W.D. Heo, J.T. Jones, J.W. Myers, J.E. Ferrell Jr., T. Meyer, STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx, Curr. Biol. 15 (2005) 1235–1241.
[166] J. Roos, P.J. DiGregorio, A.V. Yeromin, K. Ohlsen, M. Lioudyno, S. Zhang, O. Safrina, J.A. Kozak, S.L. Wagner, M.D. Cahalan, G. Velicelebi, K.A. Stauderman, STIM1, an essential and conserved component of store-operated Ca^{2+} channel function, J. Cell Biol. 169 (2005) 435–445.
[167] S.L. Zhang, Y. Yu, J. Roos, J.A. Kozak, T.J. Deerinck, M.H. Ellisman, K.A. Stauderman, M.D. Cahalan, STIM1 is a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane, Nature 437 (2005) 902–905.
[168] B. Minke, B. Cook, TRP channel proteins and signal transduction, Physiol. Rev. 82 (2002) 429–472.
[169] M. Vig, C. Peinelt, A. Beck, D.L. Koomoa, D. Rabah, M. Koblan-Huberson, S. Kraft, H. Turner, A. Fleig, R. Penner, J.P. Kinet, CRACM1 is a plasma membrane protein essential for store-operated Ca^{2+} entry, Science 312 (2006) 1220–1223.
[170] S.L. Zhang, A.V. Yeromin, X.H. Zhang, Y. Yu, O. Safrina, A. Penna, J. Roos, K.A. Stauderman, M.D. Cahalan, Genome-wide RNAi screen of Ca^{2+} influx identifies genes that regulate Ca^{2+} release-activated Ca^{2+} channel activity, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 9357–9362.
[171] S. Feske, Y. Gwack, M. Prakriya, S. Srikanth, S.H. Puppel, B. Tanasa, P.G. Hogan, R.S. Lewis, M. Daly, A. Rao, A mutation in Orai1 causes immune deficiency by abrogating CRAC channel function, Nature 441 (2006) 179–185.
[172] R.M. Luik, B. Wang, M. Prakriya, M.M. Wu, R.S. Lewis, Oligomerization of STIM1 couples ER calcium depletion to CRAC channel activation, Nature 454 (2008) 538–542.

[173] M.M. Wu, J. Buchanan, R.M. Luik, R.S. Lewis, Ca^{2+} store depletion causes STIM1 to accumulate in ER regions closely associated with the plasma membrane, J. Cell Biol. 174 (2006) 803–813.

[174] R.M. Luik, M.M. Wu, J. Buchanan, R.S. Lewis, The elementary unit of store-operated Ca^{2+} entry: local activation of CRAC channels by STIM1 at ER–plasma membrane junctions, J. Cell Biol. 174 (2006) 815–825.

[175] M. Muik, M. Fahrner, R. Schindl, P. Stathopulos, I. Frischauf, I. Derler, P. Plenk, B. Lackner, K. Groschner, M. Ikura, C. Romanin, STIM1 couples to ORAI1 via an intramolecular transition into an extended conformation, EMBO J. 30 (2011) 1678–1689.

[176] N. Calloway, M. Vig, J.P. Kinet, D. Holowka, B. Baird, Molecular clustering of STIM1 with Orai1/CRACM1 at the plasma membrane depends dynamically on depletion of Ca^{2+} stores and on electrostatic interactions, Mol. Biol. Cell 20 (2009) 389–399.

[177] C.Y. Park, P.J. Hoover, F.M. Mullins, P. Bachhawat, E.D. Covington, S. Raunser, T. Walz, K.C. Garcia, R.E. Dolmetsch, R.S. Lewis, STIM1 clusters and activates CRAC channels via direct binding of a cytosolic domain to Orai1, Cell 136 (2009) 876–890.

[178] J.P. Yuan, W. Zeng, M.R. Dorwart, Y.J. Choi, P.F. Worley, S. Muallem, SOAR and the polybasic STIM1 domains gate and regulate Orai channels, Nat. Cell Biol. 11 (2009) 337–343.

[179] S. Srikanth, M. Jew, K.D. Kim, M.K. Yee, J. Abramson, Y. Gwack, Junctate is a Ca^{2+}-sensing structural component of Orai1 and stromal interaction molecule 1 (STIM1), Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 8682–8687.

[180] S. Srikanth, H.J. Jung, K.D. Kim, P. Souda, J. Whitelegge, Y. Gwack, A novel EF-hand protein, CRACR2A, is a cytosolic Ca^{2+} sensor that stabilizes CRAC channels in T cells, Nat. Cell Biol. 12 (2010) 436–446.

[181] R. Palty, A. Raveh, I. Kaminsky, R. Meller, E. Reuveny, SARAF inactivates the store operated calcium entry machinery to prevent excess calcium refilling, Cell 149 (2012) 425–438.

[182] W.D. Heo, T. Inoue, W.S. Park, M.L. Kim, B.O. Park, T.J. Wandless, T. Meyer, $PI(3,4,5)P_3$ and PI(4,5)P2 lipids target proteins with polybasic clusters to the plasma membrane, Science 314 (2006) 1458–1461.

[183] M.K. Korzeniowski, M.A. Popovic, Z. Szentpetery, P. Varnai, S.S. Stojilkovic, T. Balla, Dependence of STIM1/Orai1-mediated calcium entry on plasma membrane phosphoinositides, J. Biol. Chem. 284 (2009) 21027–21035.

[184] D. Bakowski, C. Nelson, A.B. Parekh, Endoplasmic reticulum–mitochondria coupling: local Ca(2)(+) signalling with functional consequences, Pflugers Arch. 464 (2012) 27–32.

[185] D.G. Nicholls, Mitochondria and calcium signaling, Cell Calcium 38 (2005) 311–317.

[186] P. Pacher, P. Csordas, T. Schneider, G. Hajnoczky, Quantification of calcium signal transmission from sarco-endoplasmic reticulum to the mitochondria, J. Physiol. 529 (2000) 553–564.

[187] H.F. Deluca, G.W. Engstrom, Calcium uptake by rat kidney mitochondria, Proc. Natl. Acad. Sci. U. S. A. 47 (1961) 1744–1750.

[188] G. Hajnoczky, L.D. Robb-Gaspers, M.B. Seitz, A.P. Thomas, Decoding of cytosolic calcium oscillations in the mitochondria, Cell 82 (1995) 415–424.

[189] P. Pizzo, I. Drago, R. Filadi, T. Pozzan, Mitochondrial Ca^{2+} homeostasis: mechanism, role, and tissue specificities, Pflugers Arch. 464 (2012) 3–17.

[190] J. Santo-Domingo, L. Vay, E. Hernandez-Sanmiguel, C.D. Lobaton, A. Moreno, M. Montero, J. Alvarez, The plasma membrane Na^+/Ca^{2+}exchange inhibitor KB-R7943 is also a potent inhibitor of the mitochondrial Ca^{2+} uniporter, Br. J. Pharmacol. 151 (2007) 647–654.

[191] L. Vay, E. Hernandez-Sanmiguel, J. Santo-Domingo, C.D. Lobaton, A. Moreno, M. Montero, J. Alvarez, Modulation of Ca^{2+} release and Ca^{2+} oscillations in HeLa cells and fibroblasts by mitochondrial Ca^{2+} uniporter stimulation, J. Physiol. 580 (2007) 39–49.

[192] R. Rizzuto, A.W. Simpson, M. Brini, T. Pozzan, Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin, Nature 358 (1992) 325–327.

[193] R. Rizzuto, M. Brini, M. Murgia, T. Pozzan, Microdomains with high Ca^{2+} close to IP3-sensitive channels that are sensed by neighboring mitochondria, Science 262 (1993) 744–747.

[194] M. Montero, M.T. Alonso, E. Carnicero, I. Cuchillo-Ibanez, A. Albillos, A.G. Garcia, J. Garcia-Sancho, J. Alvarez, Chromaffin-cell stimulation triggers fast millimolar mitochondrial Ca^{2+} transients that modulate secretion, Nat. Cell Biol. 2 (2000) 57–61.

[195] F. Ichas, L.S. Jouaville, J.P. Mazat, Mitochondria are excitable organelles capable of generating and conveying electrical and calcium signals, Cell 89 (1997) 1145–1153.

[196] D. Prins, M. Michalak, Organellar calcium buffers, Cold Spring Harb. Perspect. Biol. 3 (2011).

[197] E. Carafoli, Mitochondrial uptake of calcium ions and the regulation of cell function, Biochem. Soc. Symp. (1974) 89–109.

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

[199] G. Csordas, C. Renken, P. Varnai, L. Walter, D. Weaver, K.F. Buttle, T. Balla, C.A. Mannella, G. Hajnoczky, Structural and functional features and significance of the physical linkage between ER and mitochondria, J. Cell Biol. 174 (2006) 915–921.

[200] O.M. de Brito, L. Scorrano, Mitofusin 2 tethers endoplasmic reticulum to mitochondria, Nature 456 (2008) 605–610.

[201] J.R. Friedman, L.L. Lackner, M. West, J.R. DiBenedetto, J. Nunnari, G.K. Voeltz, ER tubules mark sites of mitochondrial division, Science 334 (2011) 358–362.

[202] P. Bernardi, V. Paradisi, T. Pozzan, G.F. Azzone, Pathway for uncoupler-induced calcium efflux in rat liver mitochondria: inhibition by ruthenium red, Biochemistry 23 (1984) 1645–1651.

[203] M. Trenker, R. Malli, I. Fertschai, S. Levak-Frank, W.F. Graier, Uncoupling proteins 2 and 3 are fundamental for mitochondrial Ca^{2+} uniport, Nat. Cell Biol. 9 (2007) 445–452.

[204] P.S. Brookes, N. Parker, J.A. Buckingham, A. Vidal-Puig, A.P. Halestrap, T.E. Gunter, D.G. Nicholls, P. Bernardi, J.J. Lemasters, M.D. Brand, UCPs—unlikely calcium porters, Nat. Cell Biol. 10 (2008) 1235–1237, (author reply 1237–1240.).

[205] D. De Stefani, A. Raffaello, E. Teardo, I. Szabo, R. Rizzuto, A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter, Nature 476 (2011) 336–340.

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

[207] F. Perocchi, V.M. Gohil, H.S. Girgis, X.R. Bao, J.E. McCombs, A.E. Palmer, V.K. Mootha, MICU1 encodes a mitochondrial EF hand protein required for Ca^{2+} uptake, Nature 467 (2010) 291–296.

[208] K. Mallilankaraman, P. Doonan, C. Cardenas, H.C. Chandramoorthy, M. Muller, R. Miller, N.E. Hoffman, R.K. Gandhirajan, J. Molgo, M.J. Birnbaum, B.S. Rothberg, D.O. Mak, J.K. Foskett, M. Madesh, MICU1 is an essential gatekeeper for MCU-mediated mitochondrial Ca^{2+} uptake that regulates cell survival, Cell 151 (2012) 630–644.

[209] K. Mallilankaraman, C. Cardenas, P.J. Doonan, H.C. Chandramoorthy, K.M. Irrinki, T. Golenar, G. Csordas, P. Madireddi, J. Yang, M. Muller, R. Miller, J.E. Kolesar, J. Molgo, B. Kaufman, G. Hajnoczky, J.K. Foskett, M. Madesh, MCUR1 is an essential component of mitochondrial Ca(2+) uptake that regulates cellular metabolism, Nat. Cell Biol. 15 (2012) 123.

[210] C. Brenner, M. Moulin, Physiological roles of the permeability transition pore, Circ. Res. 111 (2012) 1237–1247.

[211] A. Rasola, P. Bernardi, Mitochondrial permeability transition in Ca^{2+}-dependent apoptosis and necrosis, Cell Calcium 50 (2011) 222–233.

[212] R. Palty, W.F. Silverman, M. Hershfinkel, T. Caporale, S.L. Sensi, J. Parnis, C. Nolte, D. Fishman, V. Shoshan-Barmatz, S. Herrmann, D. Khananshvili, I. Sekler, NCLX is an essential component of mitochondrial Na^+/Ca^{2+} exchange, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 436–441.

[213] D. Jiang, L. Zhao, D.E. Clapham, Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca^{2+}/H^+ antiporter, Science 326 (2009) 144–147.

[214] K. Nowikovsky, T. Pozzan, R. Rizzuto, L. Scorrano, P. Bernardi, Perspectives on: SGP symposium on mitochondrial physiology and medicine: the pathophysiology of LETM1, J. Gen. Physiol. 139 (2012) 445–454.

[215] J. Santo-Domingo, N. Demaurex, Calcium uptake mechanisms of mitochondria, Biochim. Biophys. Acta 1797 (2010) 907–912.

[216] R. Rizzuto, D. De Stefani, A. Raffaello, C. Mammucari, Mitochondria as sensors and regulators of calcium signalling, Nat. Rev. Mol. Cell Biol. 13 (2012) 566–578.

[217] J.G. McCormack, A.P. Halestrap, R.M. Denton, Role of calcium ions in regulation of mammalian intramitochondrial metabolism, Physiol. Rev. 70 (1990) 391–425.

[218] R.G. Hansford, Physiological role of mitochondrial Ca^{2+} transport, J. Bioenerg. Biomembr. 26 (1994) 495–508.

[219] J.G. McCormack, R.M. Denton, The effects of calcium ions and adenine nucleotides on the activity of pig heart 2-oxoglutarate dehydrogenase complex, Biochem. J. 180 (1979) 533–544.

[220] P. Pacher, A.P. Thomas, G. Hajnoczky, Ca^{2+} marks: miniature calcium signals in single mitochondria driven by ryanodine receptors, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 2380–2385.

[221] C. Cardenas, R.A. Miller, I. Smith, T. Bui, J. Molgo, M. Muller, H. Vais, K.H. Cheung, J. Yang, I. Parker, C.B. Thompson, M.J. Birnbaum, K.R. Hallows, J.K. Foskett, Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca^{2+} transfer to mitochondria, Cell 142 (2010) 270–283.

[222] J.J. Lemasters, T.P. Theruvath, Z. Zhong, A.L. Nieminen, Mitochondrial calcium and the permeability transition in cell death, Biochim. Biophys. Acta 1787 (2009) 1395–1401.

[223] M.C. Bassik, L. Scorrano, S.A. Oakes, T. Pozzan, S.J. Korsmeyer, Phosphorylation of BCL-2 regulates ER Ca^{2+} homeostasis and apoptosis, EMBO J. 23 (2004) 1207–1216.

[224] P. Pinton, C. Giorgi, R. Siviero, E. Zecchini, R. Rizzuto, Calcium and apoptosis: ER–mitochondria Ca^{2+} transfer in the control of apoptosis, Oncogene 27 (2008) 6407–6418.

[225] C.B. Thompson, Apoptosis in the pathogenesis and treatment of disease, Science 267 (1995) 1456–1462.

[226] G. Perry, A. Nunomura, P. Lucassen, H. Lassmann, M.A. Smith, Apoptosis and Alzheimer's disease, Science 282 (1998) 1268–1269.

[227] F. Celsi, P. Pizzo, M. Brini, S. Leo, C. Fotino, P. Pinton, R. Rizzuto, Mitochondria, calcium and cell death: a deadly triad in neurodegeneration, Biochim. Biophys. Acta 1787 (2009) 335–344.

[228] H. Du, L. Guo, F. Fang, D. Chen, A.A. Sosunov, G.M. McKhann, Y. Yan, C. Wang, H. Zhang, J.D. Molkentin, F.J. Gunn-Moore, J.P. Vonsattel, O. Arancio, J.X. Chen, S.D. Yan, Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease, Nat. Med. 14 (2008) 1097–1105.

[229] H. Chen, S.A. Detmer, A.J. Ewald, E.E. Griffin, S.E. Fraser, D.C. Chan, Mitofusins Mfn1 and Mfn2 coordinately regulate mitochondrial fusion and are essential for embryonic development, J. Cell Biol. 160 (2003) 189–200.

[230] C. Cerqua, V. Anesti, A. Pyakurel, D. Liu, D. Naon, G. Wiche, R. Baffa, K.S. Dimmer, L. Scorrano, Trichoplein/mitostatin regulates endoplasmic reticulum–mitochondria juxtaposition, EMBO Rep. 11 (2010) 854–860.

[231] J.E. Vance, Phospholipid synthesis in a membrane fraction associated with mitochondria, J. Biol. Chem. 265 (1990) 7248–7256.

[232] G. Szabadkai, K. Bianchi, P. Varnai, D. De Stefani, M.R. Wieckowski, D. Cavagna, A.I. Nagy, T. Balla, R. Rizzuto, Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels, J. Cell Biol. 175 (2006) 901–911.

[233] T. Hayashi, T.P. Su, Sigma-1 receptor chaperones at the ER–mitochondrion interface regulate Ca(2+) signaling and cell survival, Cell 131 (2007) 596–610.

[234] M. Giacomello, I. Drago, M. Bortolozzi, M. Scorzeto, A. Gianelle, P. Pizzo, T. Pozzan, Ca^{2+} hot spots on the mitochondrial surface are generated by Ca^{2+} mobilization from stores, but not by activation of store-operated Ca^{2+} channels, Mol. Cell 38 (2010) 280–290.

[235] G. Csordas, P. Varnai, T. Golenar, S. Roy, G. Purkins, T.G. Schneider, T. Balla, G. Hajnoczky, Imaging interorganelle contacts and local calcium dynamics at the ER–mitochondrial interface, Mol. Cell 39 (2010) 121–132.

[236] P. Saftig, J. Klumperman, Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function, Nat. Rev. Mol. Cell Biol. 10 (2009) 623–635.

[237] A.J. Morgan, F.M. Platt, E. Lloyd-Evans, A. Galione, Molecular mechanisms of endolysosomal Ca^{2+} signalling in health and disease, Biochem. J. 439 (2011) 349–374.

[238] A. Galione, J. Parrington, T. Funnell, Physiological roles of NAADP-mediated Ca^{2+} signaling, Sci. China Life Sci. 54 (2011) 725–732.

[239] D. Shen, X. Wang, X. Li, X. Zhang, Z. Yao, S. Dibble, X.P. Dong, T. Yu, A.P. Lieberman, H.D. Showalter, H. Xu, Lipid storage disorders block lysosomal trafficking by inhibiting a TRP channel and lysosomal calcium release, Nat. Commun. 3 (2012) 731.

[240] H.V. McCue, J.D. Wardyn, R.D. Burgoyne, L.P. Haynes, Generation and characterization of a lysosomally targeted, genetically encoded Ca^{2+}-sensor, Biochem. J. 449 (2013) 449–457.

[241] V.I. Korolchuk, S. Saiki, M. Lichtenberg, F.H. Siddiqi, E.A. Roberts, S. Imarisio, L. Jahreiss, S. Sarkar, M. Futter, F.M. Menzies, C.J. O'Kane, V. Deretic, D.C. Rubinsztein, Lysosomal positioning coordinates cellular nutrient responses, Nat. Cell Biol. 13 (2011) 453–460.

[242] C. Pous, P. Codogno, Lysosome positioning coordinates mTORC1 activity and autophagy, Nat. Cell Biol. 13 (2011) 342–344.

[243] M. Laplante, D.M. Sabatini, mTOR signaling in growth control and disease, Cell 149 (2012) 274–293.

[244] P.R. Pryor, B.M. Mullock, N.A. Bright, S.R. Gray, J.P. Luzio, The role of intraorganellar Ca^{2+} in late endosome–lysosome heterotypic fusion and in the reformation of lysosomes from hybrid organelles, J. Cell Biol. 149 (2000) 1053–1062.

[245] J.C. Hay, Calcium: a fundamental regulator of intracellular membrane fusion? EMBO Rep. 8 (2007) 236–240.

[246] F.M. Platt, B. Boland, A.C. van der Spoel, The cell biology of disease: lysosomal storage disorders: the cellular impact of lysosomal dysfunction, J. Cell Biol. 199 (2012) 723–734.

[247] K.A. Christensen, J.T. Myers, J.A. Swanson, pH-dependent regulation of lysosomal calcium in macrophages, J. Cell Sci. 115 (2002) 599–607.

[248] E. Lloyd-Evans, A.J. Morgan, X. He, D.A. Smith, E. Elliot-Smith, D.J. Sillence, G.C. Churchill, E.H. Schuchman, A. Galione, F.M. Platt, Niemann–Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium, Nat. Med. 14 (2008) 1247–1255.

[249] T.J. Jentsch, Chloride and the endosomal–lysosomal pathway: emerging roles of CLC chloride transporters, J. Physiol. 578 (2007) 633–640.

[250] B.E. Steinberg, K.K. Huynh, A. Brodovitch, S. Jabs, T. Stauber, T.J. Jentsch, S. Grinstein, A cation counterflux supports lysosomal acidification, J. Cell Biol. 189 (2010) 1171–1186.

[251] M. Koivusalo, B.E. Steinberg, D. Mason, S. Grinstein, In situ measurement of the electrical potential across the lysosomal membrane using FRET, Traffic 12 (2011) 972–982.

[252] C. Grimm, S. Hassan, C. Wahl-Schott, M. Biel, Role of TRPML and two-pore channels in endolysosomal cation homeostasis, J. Pharmacol. Exp. Ther. 342 (2012) 236–244.

[253] A.H. Guse, Linking NAADP to ion channel activity: a unifying hypothesis, Sci. Signal. 5 (2012) pe18.

[254] S. Patel, R. Docampo, Acidic calcium stores open for business: expanding the potential for intracellular Ca^{2+} signaling, Trends Cell Biol. 20 (2010) 277–286.

[255] H.C. Lee, Cyclic ADP-ribose and nicotinic acid adenine dinucleotide phosphate (NAADP) as messengers for calcium mobilization, J. Biol. Chem. 287 (2012) 31633–31640.

[256] J.M. Cancela, G.C. Churchill, A. Galione, Coordination of agonist-induced Ca^{2+}-signalling patterns by NAADP in pancreatic acinar cells, Nature 398 (1999) 74–76.

[257] R. Masgrau, G.C. Churchill, A.J. Morgan, S.J. Ashcroft, A. Galione, NAADP: a new second messenger for glucose-induced Ca^{2+} responses in clonal pancreatic beta cells, Curr. Biol. 13 (2003) 247–251.

[258] M. Yamasaki, R. Masgrau, A.J. Morgan, G.C. Churchill, S. Patel, S.J. Ashcroft, A. Galione, Organelle selection determines agonist-specific Ca^{2+} signals in pancreatic acinar and beta cells, J. Biol. Chem. 279 (2004) 7234–7240.

[259] V. Pandey, C.C. Chuang, A.M. Lewis, P.K. Aley, E. Brailoiu, N.J. Dun, G.C. Churchill, S. Patel, Recruitment of NAADP-sensitive acidic Ca^{2+} stores by glutamate, Biochem. J. 422 (2009) 503–512.

[260] F.X. Boittin, A. Galione, A.M. Evans, Nicotinic acid adenine dinucleotide phosphate mediates Ca^{2+} signals and contraction in arterial smooth muscle via a two-pool mechanism, Circ. Res. 91 (2002) 1168–1175.

[261] S. Patel, E. Brailoiu, Triggering of Ca^{2+} signals by NAADP-gated two-pore channels: a role for membrane contact sites? Biochem. Soc. Trans. 40 (2012) 153–157.

[262] D.L. Clapper, T.F. Walseth, P.J. Dargie, H.C. Lee, Pyridine nucleotide metabolites stimulate calcium release from sea urchin egg microsomes desensitized to inositol trisphosphate, J. Biol. Chem. 262 (1987) 9561–9568.

[263] H.C. Lee, R. Aarhus, A derivative of NADP mobilizes calcium stores insensitive to inositol trisphosphate and cyclic ADP-ribose, J. Biol. Chem. 270 (1995) 2152–2157.

[264] G.C. Churchill, Y. Okada, J.M. Thomas, A.A. Genazzani, S. Patel, A. Galione, NAADP mobilizes Ca(2+) from reserve granules, lysosome-related organelles, in sea urchin eggs, Cell 111 (2002) 703–708.

[265] G.C. Churchill, A. Galione, Spatial control of Ca^{2+} signaling by nicotinic acid adenine dinucleotide phosphate diffusion and gradients, J. Biol. Chem. 275 (2000) 38687–38692.

[266] D. Lim, K. Kyozuka, G. Gragnaniello, E. Carafoli, L. Santella, $NAADP^{+}$ initiates the Ca^{2+} response during fertilization of starfish oocytes, FASEB J. 15 (2001) 2257–2267.

[267] G.C. Churchill, A. Galione, Prolonged inactivation of nicotinic acid adenine dinucleotide phosphate-induced Ca^{2+} release mediates a spatiotemporal Ca^{2+} memory, J. Biol. Chem. 276 (2001) 11223–11225.

[268] A.J. Morgan, A. Galione, Fertilization and nicotinic acid adenine dinucleotide phosphate induce pH changes in acidic Ca^{2+} stores in sea urchin eggs, J. Biol. Chem. 282 (2007) 37730–37737.

[269] A.J. Morgan, L.C. Davis, S.K.T.Y. Wagner, A.M. Lewis, J. Parrington, G.C. Churchill, A. Galione, Bidirectional Ca^{2+} signalling occurs between the endoplasmic reticulum and acidic organelles, J. Cell Biol. 200 (2013) 789–805.

[270] S.J. Pitt, T.M. Funnell, M. Sitsapesan, E. Venturi, K. Rietdorf, M. Ruas, A. Ganesan, R. Gosain, G.C. Churchill, M.X. Zhu, J. Parrington, A. Galione, R. Sitsapesan, TPC2 is a novel NAADP-sensitive Ca^{2+} release channel, operating as a dual sensor of luminal pH and Ca^{2+}, J. Biol. Chem. 285 (2010) 35039–35046.

[271] A.J. Morgan, J. Parrington, A. Galione, The luminal Ca^{2+} chelator, TPEN, inhibits NAADP-induced Ca^{2+} release, Cell Calcium 52 (2012) 481–487.

[272] C.I. Lopez-Sanjurjo, S.C. Tovey, D.L. Prole, C.W. Taylor, Lysosomes shape IP_3--evoked Ca^{2+} signals by selectively sequestering Ca^{2+} released from the endoplasmic reticulum, J. Cell Sci. 126 (2013) 289–300.

[273] N.P. Kinnear, F.X. Boittin, J.M. Thomas, A. Galione, A.M. Evans, Lysosome-sarcoplasmic reticulum junctions. A trigger zone for calcium signaling by nicotinic acid adenine dinucleotide phosphate and endothelin-1, J. Biol. Chem. 279 (2004) 54319–54326.

[274] G. Gambara, R.A. Billington, M. Debidda, A. D'Alessio, F. Palombi, E. Ziparo, A.A. Genazzani, A. Filippini, NAADP-induced Ca^{2+} signaling in response to endothelin is via the receptor subtype B and requires the integrity of lipid rafts/caveolae, J. Cell. Physiol. 216 (2008) 396–404.

[275] N.P. Kinnear, C.N. Wyatt, J.H. Clark, P.J. Calcraft, S. Fleischer, L.H. Jeyakumar, G.F. Nixon, A.M. Evans, Lysosomes co-localize with ryanodine receptor subtype 3 to form a trigger zone for calcium signalling by NAADP in rat pulmonary arterial smooth muscle, Cell Calcium 44 (2008) 190–201.

[276] L.C. Davis, A.J. Morgan, J.L. Chen, C.M. Snead, D. Bloor-Young, E. Shenderov, M.N. Stanton-Humphreys, S.J. Conway, G.C. Churchill, J. Parrington, V. Cerundolo, A. Galione, NAADP activates two-pore channels on T cell cytolytic granules to stimulate exocytosis and killing, Curr. Biol. 22 (2012) 2331–2337.

[277] A. Galione, NAADP, a new intracellular messenger that mobilizes Ca^{2+} from acidic stores, Biochem. Soc. Trans. 34 (2006) 922–926.

[278] F. Zhang, P.L. Li, Reconstitution and characterization of a nicotinic acid adenine dinucleotide phosphate (NAADP)-sensitive Ca^{2+} release channel from liver lysosomes of rats, J. Biol. Chem. 282 (2007) 25259–25269.

[279] F. Zhang, S. Jin, F. Yi, P.L. Li, TRP-ML1 functions as a lysosomal NAADP-sensitive Ca^{2+} release channel in coronary arterial myocytes, J. Cell. Mol. Med. 13 (2009) 3174–3185.

[280] G. Bach, Mucolipin 1: endocytosis and cation channel—a review, Pflugers Arch. 451 (2005) 313–317.

[281] V. Rybalchenko, M. Ahuja, J. Coblentz, D. Churamani, S. Patel, K. Kiselyov, S. Muallem, Membrane potential regulates nicotinic acid adenine dinucleotide phosphate (NAADP) dependence of the pH- and Ca^{2+}-sensitive organellar two-pore channel TPC1, J. Biol. Chem. 287 (2012) 20407–20416.

[282] X.P. Dong, D. Shen, X. Wang, T. Dawson, X. Li, Q. Zhang, X. Cheng, Y. Zhang, L.S. Weisman, M. Delling, H. Xu, $PI(3,5)P_2$ controls membrane trafficking by direct activation of mucolipin Ca^{2+} release channels in the endolysosome, Nat. Commun. 1 (2010) 38.

[283] P.J. Calcraft, M. Ruas, Z. Pan, X. Cheng, A. Arredouani, X. Hao, J. Tang, K. Rietdorf, L. Teboul, K.T. Chuang, P. Lin, R. Xiao, C. Wang, Y. Zhu, Y. Lin, C.N. Wyatt, J. Parrington, J. Ma, A.M. Evans, A. Galione, M.X. Zhu, NAADP mobilizes calcium from acidic organelles through two-pore channels, Nature 459 (2009) 596–600.

[284] X. Zong, M. Schieder, H. Cuny, S. Fenske, C. Gruner, K. Rotzer, O. Griesbeck, H. Harz, M. Biel, C. Wahl-Schott, The two-pore channel TPCN2 mediates NAADP-dependent Ca^{2+}-release from lysosomal stores, Pflugers Arch. 458 (2009) 891–899.

[285] E. Brailoiu, D. Churamani, X. Cai, M.G. Schrlau, G.C. Brailoiu, X. Gao, R. Hooper, M.J. Boulware, N.J. Dun, J.S. Marchant, S. Patel, Essential requirement for two-pore channel 1 in NAADP-mediated calcium signaling, J. Cell Biol. 186 (2009) 201–209.

[286] A. Galione, NAADP receptors, Cold Spring Harb. Perspect. Biol. 3 (2011) a004036.

[287] M. Schieder, K. Rotzer, A. Bruggemann, M. Biel, C.A. Wahl-Schott, Characterization of two-pore channel 2 (TPCN2)-mediated Ca^{2+} currents in isolated lysosomes, J. Biol. Chem. 285 (2010) 21219–21222.

[288] E. Brailoiu, T. Rahman, D. Churamani, D.L. Prole, G.C. Brailoiu, R. Hooper, C.W. Taylor, S. Patel, An NAADP-gated two-pore channel targeted to the plasma membrane uncouples triggering from amplifying Ca^{2+} signals, J. Biol. Chem. 285 (2010) 38511–38516.

[289] Y. Lin-Moshier, T.F. Walseth, D. Churamani, S.M. Davidson, J.T. Slama, R. Hooper, E. Brailoiu, S. Patel, J.S. Marchant, Photoaffinity labeling of nicotinic acid adenine dinucleotide phosphate (NAADP) targets in mammalian cells, J. Biol. Chem. 287 (2012) 2296–2307.

[290] M. Ruas, K. Rietdorf, A. Arredouani, L.C. Davis, E. Lloyd-Evans, H. Koegel, T.M. Funnell, A.J. Morgan, J.A. Ward, K. Watanabe, X. Cheng, G.C. Churchill, M.X. Zhu, F.M. Platt, G.M. Wessel, J. Parrington, A. Galione, Purified TPC isoforms form NAADP receptors with distinct roles for Ca^{2+} signaling and endolysosomal trafficking, Curr. Biol. 20 (2010) 703–709.
[291] T.F. Walseth, Y. Lin-Moshier, P. Jain, M. Ruas, J. Parrington, A. Galione, J.S. Marchant, J.T. Slama, Photoaffinity labeling of high affinity nicotinic acid adenine dinucleotide phosphate (NAADP)-binding proteins in sea urchin egg, J. Biol. Chem. 287 (2012) 2308–2315.
[292] X. Wang, X. Zhang, X.P. Dong, M. Samie, X. Li, X. Cheng, A. Goschka, D. Shen, Y. Zhou, J. Harlow, M.X. Zhu, D.E. Clapham, D. Ren, H. Xu, TPC proteins are phosphoinositide-activated sodium-selective ion channels in endosomes and lysosomes, Cell 151 (2012) 372–383.
[293] C. Cang, Y. Zhou, B. Navarro, Y.-J. Seo, K. Aranda, L. Shi, S. Battaglia-Hsu, I. Nissim, D.E. Clapham, D. Ren, mTOR regulates lysosomal ATP-sensitive two-pore Na^{+} channels to adapt to metabolic state, Cell 152 (2013) 778–790.
[294] N. Rocha, C. Kuijl, R. van der Kant, L. Janssen, D. Houben, H. Janssen, W. Zwart, J. Neefjes, Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP-p150 Glued and late endosome positioning, J. Cell Biol. 185 (2009) 1209–1225.
[295] B.S. Kilpatrick, E.R. Eden, A.H. Schapira, C.E. Futter, S. Patel, Direct mobilisation of lysosomal Ca^{2+} triggers complex Ca^{2+} signals, J. Cell Sci. 26 (2012) 60–66.
[296] M. Johansson, N. Rocha, W. Zwart, I. Jordens, L. Janssen, C. Kuijl, V.M. Olkkonen, J. Neefjes, Activation of endosomal dynein motors by stepwise assembly of Rab7-RILP-p150Glued, ORP1L, and the receptor betalll spectrin, J. Cell Biol. 176 (2007) 459–471.
[297] I. Jordens, M. Fernandez-Borja, M. Marsman, S. Dusseljee, L. Janssen, J. Calafat, H. Janssen, R. Wubbolts, J. Neefjes, The Rab7 effector protein RILP controls lysosomal transport by inducing the recruitment of dynein–dynactin motors, Curr. Biol. 11 (2001) 1680–1685.
[298] S.E. Kaiser, J.H. Brickner, A.R. Reilein, T.D. Fenn, P. Walter, A.T. Brunger, Structural basis of FFAT motif-mediated ER targeting, Structure 13 (2005) 1035–1045.
[299] T. Vihervaara, R.L. Uronen, G. Wohlfahrt, I. Bjorkhem, E. Ikonen, V.M. Olkkonen, Sterol binding by OSBP-related protein 1L regulates late endosome motility and function, Cell. Mol. Life Sci. 68 (2011) 537–551.
[300] C. Rosa-Ferreira, S. Munro, Arl8 and SKIP act together to link lysosomes to kinesin-1, Dev. Cell 21 (2011) 1171–1178.
[301] P. Libby, Inflammation in atherosclerosis, Nature 420 (2002) 868–874.
[302] C. Weber, H. Noels, Atherosclerosis: current pathogenesis and therapeutic options, Nat. Med. 17 (2011) 1410–1422.
[303] S. Mukherjee, F.R. Maxfield, Lipid and cholesterol trafficking in NPC, Biochim. Biophys. Acta 1685 (2004) 28–37.
[304] M.K. Korzeniowski, I.M. Manjarres, P. Varnai, T. Balla, Activation of STIM1–Orai1 involves an intramolecular switching mechanism, Sci. Signal. 3 (2010) ra82.