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Review

Calcium signaling at the endoplasmic reticulum: fine-tuning stress responses

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ABSTRACT

Endoplasmic reticulum (ER) calcium signaling is implicated in a myriad of coordinated cellular processes. The ER calcium content is tightly regulated as it allows a favorable environment for protein folding, in addition to operate as a major reservoir for fast and specific release of calcium. Altered ER homeostasis impacts protein folding, activating the unfolded protein response (UPR) as a rescue mechanism to restore proteostasis. ER calcium release impacts mitochondrial metabolism and also fine-tunes the threshold to undergo apoptosis under chronic stress. The global coordination between UPR signaling and energetic demands takes place at mitochondrial associated membranes (MAMs), specialized subdomains mediating interorganelle communication. Here we discuss current models explaining the functional relationship between ER homeostasis and various cellular responses to coordinate proteostasis and metabolic maintenance.

1. Introduction

Calcium is a highly versatile molecule, functioning as a key secondary messenger participating in the regulation of a wide variety of processes such as fertilization, metabolism, secretion, muscle contraction, neuronal activity and cell death, among many others [1]. Calcium signaling is fast and efficient due to the establishment of a steep calcium gradient concentration –as large as 10^5-fold –between the extracellular and intracellular spaces. Indeed, this gradient is also conserved between different intracellular organelles and the cytosol, facilitating a variety of specific calcium-driven signaling events. For instance, the endoplasmic reticulum (ER), the largest intracellular calcium store, has a free luminal concentration of about 100–800 μM. Calcium increases in the cytosol may result in the engagement of distinct cellular processes, such as the activation of the NFAT pathway in the cytosol, increase oxidative phosphorylation or even cell death through the canonical mitochondrial apoptosis pathway [1]. The maintenance of the calcium gradient has an energetic cost which is achieved through the activity of several pumps and transporters that use ATP to work against electrochemical gradients [2]. Importantly, disturbances on calcium gradients, and specifically on luminal calcium levels have been related to multiple diseases including diabetes, neurological and vascular disorders, viral infections and cancer [3].

The ER is responsible for the folding and maturation of around 30% of the total proteome, a process mediated by luminal resident chaperones and foldases. Many of these folding factors are characterized by a high capacity to bind calcium with low affinity, operating as a cofactor for their optimal chaperone activity. Various conditions can alter the protein folding process at the ER including ER calcium depletion, physiological demands such as high secretory activity, or the expression of mutant proteins of the secretory pathway, resulting in a condition termed ER stress [4,5]. ER stress engages the unfolded protein response (UPR), a signaling network that enforces adaptive programs to restore ER homeostasis. The UPR triggers an initial signaling phase that promotes cell survival through the activation of transcriptional responses aiming to alleviate the intracellular misfolded protein burden [6]. However, if ER homeostasis cannot be re-established, the UPR switches its signaling toward the activation of cell death by apoptosis [7,8].

As part of the adaptive phase of the UPR, crosstalk between the ER and the mitochondria has been reported to modulate energy consumption, boosting ATP production [9,10]. This increase in the generation of ATP is thought to be mediated by the release of calcium from the ER followed by its entry into the mitochondria through specialized structures named mitochondria associated membranes (MAMs). Importantly, MAMs have acquired great interest as they function as a signaling node, controlling different aspects of cellular biology, including lipid biosynthesis, ER-to-mitochondria calcium transfer, cell death and macroautophagy [11]. Importantly, many components of the

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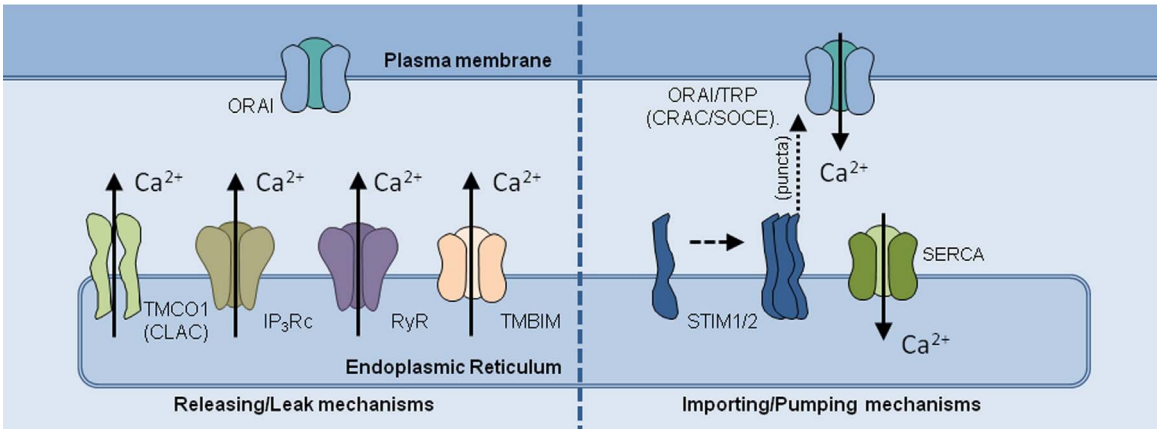

Fig. 1. Calcium homeostasis mechanisms in the ER. Left panel: Calcium leak mechanisms imply (among others) TMBIMs, which are speculated to pH-dependent calcium leak channels and TMCO1 that opens upon high ER calcium levels. In addition IP3Rc and RyRs are involved in calcium signaling in the cells and tightly controlled by different agonists form the plasma membrane to amplify calcium signaling. Right panel: calcium importing or pumping mechanisms are carried by a large family of proteins know as SERCA. SERCA pumps import calcium into the cell against the electrochemical gradient, thus expending ATP. SERCA activity is coupled to SOCE mechanisms that sense ER calcium depletion and communicate CRAC channels in the plasma membrane to open and induce a calcium entry to the cytosol in regions known as puncta, coupled to SERCA, to allow store refilling.

UPR pathway, as well as the calcium-handling machinery, are localized to the MAMs. In this review, we discuss the evidence supporting a functional relevance of ER calcium homeostasis in sustaining ER proteostasis, cellular bioenergetics and the threshold to undergo cell death.

2. Calcium handling mechanisms in the ER

Maintaining a high calcium concentration in the ER is essential to create a differential gradient with the cytosol, a phenomenon controlled by several mechanisms. Three main molecular systems generate and preserve this calcium gradient: i) molecular chaperones that bind and buffer calcium; ii) calcium importing mechanisms that increase ER calcium content; and iii) channels and pores that leak or release calcium from the ER to the cytosol. The crosstalk between these systems determines the maintenance of a steady-state calcium level within the ER [12].

The concentration of *free* luminal calcium in the ER is maintained at the micromolar level, but *total* calcium levels are in the order of 1–3 mM. Thus, the vast majority of the molecules of calcium within the ER are trapped in the surfaces of ER proteins [13]. As mentioned, many ER-localized chaperones can buffer many calcium molecules because either the number of binding sites are high, or the calcium association/dissociation ratio (Koff/Kon = Kd) is low. These chaperones include for example the lectin-like protein calnexin (CNX) and calreticulin (CRT), 78-kDa glucose-regulated protein/immunoglobulin heavy chain binding protein (GRP78/BiP), GRP94 and the protein disulfide isomerase PDI [14,15]. In addition to directly bind calcium and catalyze protein folding, some of these proteins physically associate with calcium pumps and channels, regulating their activities [16,17] (see below). Thus, ER chaperones and other ER-localized enzymes play a dual role, contributing to the maintenance of a high intraluminal free calcium environment in addition to assist protein folding and maturation.

ER calcium importing mechanisms are mainly driven by sarco/endoplasmic-reticulum Ca^{2+} ATPase (SERCA) proteins. SERCA proteins pump calcium to the luminal space of the ER against its electrochemical gradient, consuming ATP in the process (reviewed in Ref. [18]). SERCA pumps represent a large family of proteins whose expression and calcium affinities differ in a tissue and cell-specific manner [19]. Different mechanisms regulate SERCA activity, including posttranslational modifications by certain ER luminal chaperones (see above), membrane lipid composition, and the levels of co-factors (e.g. sarcolipin/phospholamban) [17,20–23]. Other mechanisms to refill intracellular stores are also relevant to sustain ER calcium homeostasis. For example, store operated calcium entry (SOCE) impacts ER calcium content through the activity of calcium-released activated channels (CRAC). CRAC channels are mainly composed by ORAI proteins, which are activated by the ER calcium sensors stromal interacting proteins or STIMs [24,25] (Fig. 1).

ER-calcium release is mediated by well-known channels and pores on its membrane including the ryanodine receptors (RyR) and inositol 1,4,5-triphosphate (IP_3)-receptors (IP_3R). Both families of proteins are composed by three members and form high-order homo- and hetero-tetramers. Like SERCA proteins, RyRs and IP_3Rs have different calcium affinities and are differentially expressed in distinct tissues. For instance RyR receptors are highly expressed in muscle and neurons and are activated by calcium to auto-amplify its signaling in the cytosol (reviewed in Ref. [26]). IP_3Rs are widely expressed in most cell types and their calcium conductance and affinity to agonists are regulated through their homo- and hetero-oligomerization, phosphorylation, chaperone binding, co-factors, and their suborganellar distribution within the ER (reviewed in Ref. [27]). RyRs and IP_3Rs are well-known to regulate a myriad of calcium-signaling related functions, reviewed elsewhere [1,28,29].

Calcium is also released from the ER in a passive manner. A constant leakage of calcium from the ER is normally observed, evidenced by the fact that the inhibition of the SERCA pump with thapsigargin results in the complete depletion of the ER calcium pool within minutes, triggering ER stress and cell death. The molecular identity of the calcium leak channel has remained elusive for many years and different proteins have been proposed to mediate this activity [27,30]. Some members of the Transmembrane BAX inhibitor motif-containing (TMBIM) family of proteins have been proposed as calcium leak channels. TMBIM proteins include six family members and are implicated in cell death control [31]. Structural and functional analysis of Bax inhibitor 1 (BI-1/TMBIM6) and its bacterial ortholog suggest that these proteins are pH-dependent calcium leak channels [32,33]. Some of these family members have been spotted in the ER where their expression reduce ER calcium levels [34]. Another well described leak channel is the translocon-ribosome complex where Sec61alpha plays a key role [35–37]. In addition, the anti-apoptotic protein BCL-2 also locates at the ER and has been suggested to have a calcium leakage function (reviewed in Ref. [38]) as well as other ER proteins including, presenilins [39], or a truncated version of SERCA pump named SERCA1T [27,40]. However, the role of some of these proteins as ER calcium leak channels is still debated [41]. Recently, an additional mechanism to prevent ER calcium overload has been proposed, mediated by the calcium load-activated calcium (CLAC) channel, involving the transmembrane and coiled-coil domains 1 (TMCO1)