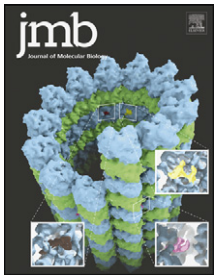
Review



Regulation of Calcium Homeostasis by ER Redox: A Close-Up of the ER/Mitochondria Connection

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Abstract

Calcium signaling plays an important role in cell survival by influencing mitochondria-related processes such as energy production and apoptosis. The endoplasmic reticulum (ER) is the main storage compartment for cell calcium (Ca^{2+}; ~60–500 μM), and the Ca^{2+} released by the ER has a prompt effect on the homeostasis of the juxtaposed mitochondria. Recent findings have highlighted a close connection between ER redox and Ca^{2+} signaling that is mediated by Ca^{2+}-handling proteins. This paper describes the redox-regulated mediators and mechanisms that orchestrate Ca^{2+} signals from the ER to mitochondria.

Introduction

Calcium (Ca^{2+}) is an important signal transducer that acts by association with proteins. Since the affinity of different proteins for Ca^{2+} varies greatly (from nM to mM), Ca^{2+} binding results either in buffering or in triggering specific functions that alter cellular metabolism [1]. Cell Ca^{2+} is highly concentrated in the endoplasmic reticulum (ER), which is not simply a place of storage but a dynamic reservoir that responds to electrical and chemical cell stimulation by releasing or taking up Ca^{2+}, thus favoring rapid physiological Ca^{2+}-mediated signaling [2,3].

The ER and the juxtaposed mitochondria share sites of close apposition known as mitochondria-associated membranes (MAMs), where the Ca^{2+} released by the ER accumulates in microdomains and can be easily taken up by mitochondria [4]. This finding has been reinforced by electron microscopic tomographic studies showing that the ER and mitochondria are joined by proteinaceous tethers (10/25 nm) whose shortening induces mitochondrial Ca^{2+} overload, observed both in an artificial tethering model and under apoptosis-inducing conditions [5]. One of the main functions of the interactions between the ER and mitochondria is to control Ca^{2+} signaling: the related Ca^{2+} ions regulate ER chaperone-assisted protein folding and, in mitochondria, regulate the dehydrogenases involved in ATP-producing citric acid cycle reactions (and the related oxidative phosphorylation) and the activation of the Ca^{2+}-dependent enzymes involved in programmed cell death [6].

Mitochondria have a pivotal function in shaping the Ca^{2+} signal released by the ER through inositol 1,4,5-trisphosphate receptors (IP3Rs) and ryanodine receptors (RyRs), as they rapidly sequester Ca^{2+} via voltage-dependent anion channel (VDAC) and mitochondrial Ca^{2+} uniporter (MCU) and then return it to the ER via sarcoplasmic/ER Ca^{2+} ATPase (SERCA). The Ca^{2+} signal is tuned by the activity of Ca^{2+} pumps (SERCAs) and channels (RyRs and IP3Rs) on the ER side and of VDAC and MCU channels on the mitochondria side (Fig. 1). These mediators of Ca^{2+} signaling orchestrate the Ca^{2+} flux between the ER and mitochondria by means of finely tuned redox regulation (Fig. 2) mediated by chaperones [calnexin (CNX) (CNX) and calreticulin (CRT)] and redox-active proteins [ERp57, ERp44, ER oxidoreductin 1α (ERO1α), NADPH oxidase 4 (NOX4), selenoprotein N1 (SEPN1), thioredoxin-related transmembrane protein 1 (TMX1), and ERdj5]. While controversial findings are reported on the enrichment of the Ca^{2+} channels/pumps in the MAMs, many of their regulatory proteins have been detected in this

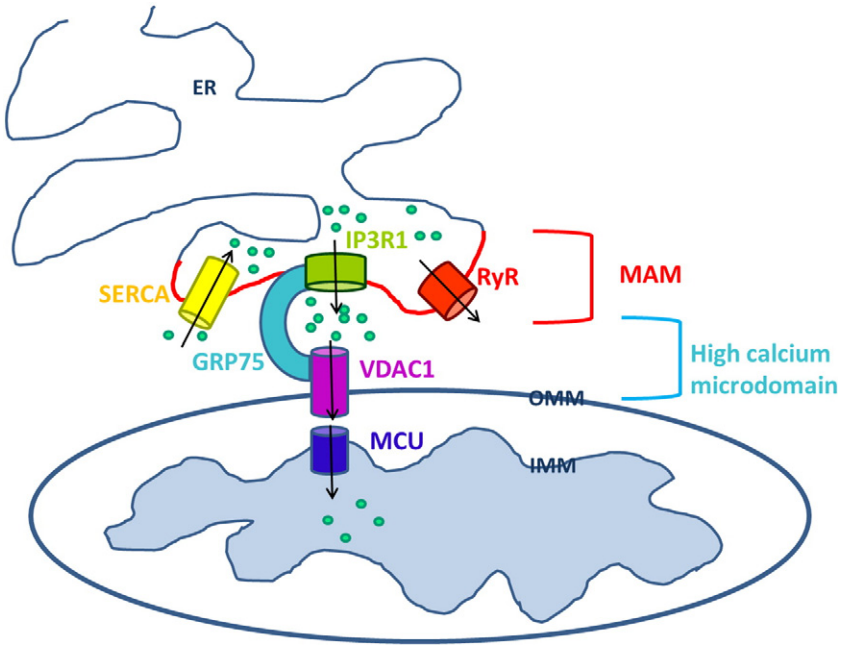

Fig. 1. General scheme of the ER/mitochondria contact site showing the localization of key Ca^{2+}-handling proteins in the MAM and mitochondrial membranes.

region, suggesting their role in controlling local Ca^{2+} signaling [7]. The upstream signaling pathways may also regulate MAM Ca^{2+} signaling. Transforming growth factor β (TGF-β) and CCAAT/enhancer-binding protein homologous protein (CHOP) pathways are considered the main examples, as they act directly on key regulators of the ER redox poise and control cell survival/apoptosis, as will be further discussed (Fig. 3).

The altered Ca^{2+} flow between the ER and mitochondria characterized by mitochondrial Ca^{2+} overload can affect mitochondrial metabolism and trigger apoptosis, particularly during prolonged ER stress, which stimulates Ca^{2+} release from the ER and consequently increases Ca^{2+} in the mitochondrial matrix [8].

The possible tissue-specific activity of Ca^{2+} regulators and handling proteins, together with differences in the stability and pattern of the ER/mitochondria junction, may influence Ca^{2+} transfer between the ER and mitochondria and determine downstream signal transduction [9].

Mechanistic Insights into Ca^{2+} Pumps/Channels: Structure and Regulation

SERCAs

SERCAs are the main Ca^{2+} pumps in the ER/sarcoplasmic reticulum (SR) and transport two Ca^{2+} ions per single hydrolyzed ATP molecule from the cytoplasm to the ER/SR lumen. Given the importance of SERCA pumps in Ca^{2+} metabolism, it is not surprising that their activity is tightly regulated at many levels, including gene transcription, protein expression, interactions with endogenous proteins, and various post-translational modifications [10].

The three main paralogs (SERCA1–3) are expressed at different levels in different cell types, and their protein diversity is further enhanced by alternative splicing that leads to 10 different SERCA isoforms. Structurally, SERCA1a is the most widely studied isoform: the multiple three-dimensional structures available from the Protein Data Bank mainly refer to a protein from rabbit muscle that was first resolved by Toyoshima *et al.* [11]. The proposed general model of the enzyme has 3 cytoplasmic domains joined to a set of 10 transmembrane helices by a narrow extramembrane pentahelical stalk [12]. The cytoplasmic portion of SERCA1a appears split with three clearly separated domains (designated A, N, and P). Domain A is the main effector of Ca^{2+} binding and release, while domain N contains the nucleotide-binding site. Domain P contains the Asp351 phosphorylation site, whose modification is important for cytoplasmic domain rearrangement during the reaction cycle [13,14]. The transmembrane region (M) consists of 10 alpha-helices (M1 to M10). The luminal loops between these helices are short except for the loop connecting M7 and M8, which comprises ~35 residues. In the bovine version of SERCA1a, this loop has a slightly different structure, which may explain why it can pump less Ca^{2+} than the rabbit enzyme [14].