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REVIEW

Calcium and bioenergetics: from endoplasmic reticulum to mitochondria

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Controlling metabolism throughout life is a necessity for living creatures, and perturbation of energy balance elicits disorders such as type-2 diabetes mellitus and cardiovascular disease. Ca^{2+} plays a key role in regulating energy generation. Ca^{2+} homeostasis of the endoplasmic reticulum (ER) lumen is maintained through the action of Ca^{2+} channels and the Ca^{2+} ATPase pump. Once released from the ER, Ca^{2+} is taken up by mitochondria where it facilitates energy metabolism. Mitochondrial Ca^{2+} serves as a key metabolic regulator and determinant of cell fate, necrosis, and/or apoptosis. Here, we focus on Ca^{2+} transport from the ER to mitochondria, and Ca^{2+}-dependent regulation of mitochondrial energy metabolism.

Keywords: Ca^{2+} homeostasis; receptor; endoplasmic reticulum; mitochondria; energy metabolism

Introduction

The regulation and balance of cellular energy metabolism are fundamental requirements for all living organisms. Adenosine-5′-triphosphate (ATP) is generated by the catabolism of nutrients and consumption of oxygen in the mitochondria. When ATP is hydrolyzed, free energy is released to support the metabolic needs of the cell and maintain homeostatic processes.

The endoplasmic reticulum (ER), found in all eukaryotic cells, plays a fundamental role in protein synthesis, maturation, and sorting to their final destination. Additionally, the ER lumen is a major reservoir of intracellular Ca^{2+}, and fluctuations in ER Ca^{2+} results in impaired movement of Ca^{2+} between the ER and Golgi (Ashby and Tepikin 2001), and impeded transport of small molecules across the nuclear pore (Greber and Gerace 1995). ER Ca^{2+} homeostasis and Ca^{2+} signaling are maintained by controlling Ca^{2+} release from the ER by the inositol 1,4,5-triphosphate receptor ($InsP_3R$) and ryanodine receptor (RyR). The sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump is responsible for returning Ca^{2+} to the ER lumen. The ER and mitochondria form close contacts, and Ca^{2+} released from the ER *via* the $InsP_3R$ is efficiently transported into the mitochondria where it supports ATP synthesis (Cardenas et al. 2010).

Mitochondrial Ca^{2+} homeostasis is an emerging field of research that includes study of the regulation of basic mitochondrial functions and cell fate (life and/or death). In this review, we have focused on the mechanisms responsible for transfer of Ca^{2+} from the $InsP_3$-sensitive ER to the mitochondria matrix and the effect of Ca^{2+} on mitochondrial metabolism.

Ca^{2+} homeostasis in the ER

The $InsP_3R$ is one of two major Ca^{2+} channels that generates cell signaling-derived Ca^{2+} release from the ER lumen to the cytosol (Yule et al. 2010). This receptor is activated by $InsP_3$, a second messenger, as well as Ca^{2+}, which is an allosteric modulator and plays an important role in shaping the $InsP_3R$-evoked Ca^{2+} response. Interestingly, Ca^{2+} enhances the probability that the $InsP_3R$ will be open but never allows independent opening of the receptor in the absence of $InsP_3$ ligand. The RyR is the second ER Ca^{2+} channel, which is ubiquitously expressed in cells and shows high-conductance relative to other nonspecific cation channels. The mammalian genome includes three RyR isoforms. RyR1 is prominent in skeletal muscle, where it functions in excitation–contraction (EC) coupling and muscle contraction. RyR2 is predominantly expressed in cardiac muscle and is also the major form in brain tissue. Lastly, RyR3 exhibits a low level widespread expression pattern and is found in striated, smooth, and cardiac muscle, as well as in T lymphocytes and in the brain-specific regions for learning and memory (Hertle and Yeckel 2007). Furthermore, Ca^{2+} is the principal activator of all three RyR isoforms, however, each isoform displays different sensitivities to cytosolic Ca^{2+} (RyR1 > RyR2 > RyR3) (Fill and Copello 2002).

Uptake of cytosolic Ca^{2+} into the ER lumen is mediated by the SERCA pump *via* ATP hydrolysis. The SERCA2a isoform is expressed both in cardiac and slow skeletal muscle, whereas SERCA2b is ubiquitously expressed in non-muscle tissues, particularly in the brain (Carafoli and Brini 2000). In the lumen of the ER Ca^{2+} is stored bound to ER luminal Ca^{2+} buffering chaperones and folding enzymes (Michalak

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et al. 2009). Ca^{2+} also binds to the EF hand domain of stromal interacting molecule (STIM), a regulator of store-operated Ca^{2+} entry (SOCE) (Roos et al. 2005). Upon depletion of ER Ca^{2+} stores and disassociation of Ca^{2+} from STIM1, the protein undergoes oligomerization at sites immediately adjacent to the plasma membrane, and binds Orai, a SOCE channel (Hewavitharana et al. 2007). Thus, the STIM1–Orai complex SOCE refills ER stores with Ca^{2+} funneled from the extracellular space directly into the ER lumen (Lewis 2007; Prins et al. 2011). A large portion of the luminal ER Ca^{2+} is free (50–800 μM) (Bygrave and Benedetti 1996), and this allows for rapid diffusion of Ca^{2+} throughout the ER lumen. High-capacity Ca^{2+} buffering proteins such as calreticulin play a critical role in maintaining ER Ca^{2+} homeostasis (Michalak et al. 2009). Calreticulin is one of the Ca^{2+}-buffering proteins in the ER lumen. The protein utilizes a carboxyl terminal acidic region as the high-capacity Ca^{2+}-binding site to bind 25 mol of Ca^{2+} per mol of protein with low affinity ($K_d = 2$ mM) (Baksh and Michalak 1991). Over 50% of Ca^{2+} stored in the ER lumen is bound to calreticulin (Nakamura et al. 2001), and up-regulation of calreticulin leads to increased amounts of Ca^{2+} in ER intracellular stores (Arnaudeau et al. 2002), whereas calreticulin-deficient cells have reduced Ca^{2+}-storage capacity in the ER and delayed agonist-induced Ca^{2+} release (Guo et al. 2001; Michalak et al. 2009). Besides calreticulin, binding immunoglobulin protein (BiP), glucose-regulated protein 94 (GRP94), and protein disulfide isomerase (PDI) also take part in the regulation of ER Ca^{2+} homeostasis. BiP binds Ca^{2+} with a relatively low capacity (1–2 mol of Ca^{2+} per mol of protein), however, is responsible for as much as 25% of the total Ca^{2+} binding capacity of the ER (Lievremont et al. 1997). GRP94 is one of the most abundant Ca^{2+}-buffering proteins of the ER with both low-affinity and high-capacity for Ca^{2+}. GRP94 has 15 moderate-affinity sites ($K_d = \sim 2$ μM) with low capacity (1 mol Ca^{2+} per mol of protein), and 11 low-affinity sites ($K_d = \sim 600$ μM) with high capacity (10 mol of Ca^{2+} per mol of protein) (Argon and Simen 1999). As a Ca^{2+}-buffering oxidoreductase in the ER lumen, PDI binds Ca^{2+} with a high capacity (19 mol Ca^{2+} per mol of protein) and weak affinity ($K_d = 2$–5 mM) (Lebeche et al. 1994).

Mitochondrial Ca^{2+} uptake

Ca^{2+} uptake can be monitored using imaging techniques such as the Ca^{2+}-sensitive photoprotein, aequorin (Rizzuto et al. 1992). This protein allows the selective measurement and monitoring of mitochondrial Ca^{2+} concentration and uptake. Using engineered Cameleon probes (Dcpv), Ca^{2+} hotspots have been directly visualized in intact cells, demonstrating the close apposition of mitochondria to ER Ca^{2+} channels and some types of plasma membrane Ca^{2+} channels (Giacomello et al. 2010). The mitochondrial outer membrane is highly permeable to ions and solutes compare with the mitochondrial inner membrane, which is ion impermeable (Kirichok et al. 2004). The voltage-dependent anion channel (VDAC) is clustered at ER/mitochondrial contact sites and it influences Ca^{2+} uptake by mitochondria (Rapizzi et al. 2002). High-speed single cell imaging has shown that up-regulation of VDAC triggers a significant rise in the peak Ca^{2+} concentration and reduces the delay between the cytosolic and mitochondrial upstroke (Rapizzi et al. 2002). Therefore, VDAC, located in the outer mitochondrial membrane, plays a critical role in the rapid transfer of the high Ca^{2+} microdomain from the outside of the mitochondria to internal mitochondrial space. Interestingly, VDAC shuttles between open and closed states (Tan and Colombini 2007). Ca^{2+} may also play a role in control of the conductance of VDAC (Tan and Colombini 2007), thus suggesting that mitochondrial Ca^{2+} uptake may be facilitated by a reduced barrier of the mitochondrial outer membrane during intracellular Ca^{2+} signaling.

In mitochondria-associated ER membrane (MAM), proteins within the ER are associated directly with proteins and lipids of the mitochondrial outer membrane. Isolation of MAM fractions has allowed the identification of proteins that might be important in ER/mitochondrial Ca^{2+} communication. For example, chaperones and proteins controlling the fusion and fission of mitochondria have been isolated from MAM fractions (Szabadkai et al. 2006). Glucose-regulated protein 75 (GRP75), a chaperone which assists with the refolding of newly imported proteins in the mitochondria matrix, was identified as a VDAC binding partner in a yeast two-hybrid screening. GRP75 mediated the interaction of VDAC1 with the $InsP_3R1$ at MAM, allowing the $InsP_3R$ to facilitate mitochondria Ca^{2+} uptake (Szabadkai et al. 2006). The sigma-1 receptor is a Ca^{2+}-sensitive ER chaperone that stabilizes $InsP_3R$ when the Ca^{2+} concentration within the ER drops, and is another component of MAM that contributes to the maintenance of ER–mitochondria communications (Szabadkai et al. 2006).

Since most of the mitochondrial Ca^{2+} effectors are localized in the mitochondrial matrix, Ca^{2+} transport from the outside environment to the matrix through the highly permeable outer membrane and ion impermeable inner membrane is a key limiting factor. Major efforts have been made to elucidate the inner membrane Ca^{2+} transporters. Mitochondria Ca^{2+} uniporter (MCU) was identified, which is an electrophoretic system that has the properties of a highly selective ion

channel (Kirichok et al. 2004), and allows Ca^{2+} to be accumulated down the electrical gradient established by the respiratory chain (Carafoli 2003). Mitochondrial Ca^{2+} uptake 1 (MICU1) was identified by screening for the expected properties of MCU. MICU1 is associated with the inner mitochondrial membrane and possesses 2 EF-hand Ca^{2+}-binding sites as well as a single transmembrane stretch (Perocchi et al. 2010). In intact and permeabilized cells, silencing of MICU1 abolishes mitochondrial Ca^{2+} uptake without interfering with mitochondrial respiration or membrane potential (Perocchi et al. 2010). Indeed MICU1 is required for the metabolic coupling between cytosolic Ca^{2+} transients and activation of matrix dehydrogenase.

Cellular metabolism by mitochondria Ca^{2+}

In the mitochondrial matrix, the physiological function of pyruvate-, α-ketoglutarate- and isocitrate dehydrogenases, and F_0F_1 ATPase are regulated by Ca^{2+}. Consequently, rising Ca^{2+} in the mitochondrial matrix of stimulated cells could serve to stimulate Ca^{2+}-sensitive dehydrogenases of the tricarboxylic acid (TCA) cycle, resulting in increased ATP synthesis as a result of the needs of a stimulated cell (McCormack et al. 1990). An increase in mitochondrial and cytosolic ATP, which depends on an increase in the mitochondrial Ca^{2+} concentration, was confirmed by direct measurement of ATP levels using a targeted luciferase probe (mtLUC) (Jouaville et al. 1999). Recent reports have indicated that inhibition of $InsP_3R$-dependent Ca^{2+} transfer from the ER to the mitochondria results in reduced ATP production, enhanced activation of AMP-activated protein kinase, and autophagy (Cardenas et al. 2010). Cells lacking all three $InsP_3R$ isoforms are protected from nutrient deprivation and recover much faster than wild-type cells when nutrient supply is restored (Cardenas et al. 2010). Thus, by activating pro-survival autophagy, suppression of $InsP_3R$-mediated Ca^{2+} signaling represents a response mechanism to bioenergetic stress even in the presence of nutrients. Conversely, a constitutive low level of $InsP_3R$-mediated Ca^{2+} transfer to mitochondria under normal condition is required for autophagy suppression in the absence of specific agonist stimulation, and maintains optimal mitochondria bioenergetics by supporting oxidative phosphorylation (Cardenas et al. 2010).

The mitochondria is also considered a checkpoint in the intrinsic pathway of apoptosis, and Ca^{2+} plays a crucial sensitizing signal in the pro-apoptotic transition of the organelle, by establishing that the release of caspase cofactors, such as cytochrome *c* and a second mitochondria-derived activator of caspase/diablo, is the signal causing the assembly of the apoptosome and the commitment of the cell to apoptotic death (Szydlowska and Tymianski 2010). As to mechanisms of release, a key role is played by organelle fragmentation and swelling triggered by the opening of a large-conductance channel, the permeability transition pore (PTP) (Bernardi et al. 2006). Ca^{2+} is the most important trigger for PTP opening, acting in living cells in conjunction with a variety of pathological challenges. Otherwise, mitochondrial Ca^{2+} overload has been known to be a critical in the bioenergetics crisis related to cell death by necrosis (Szydlowska and Tymianski 2010). It is conceivable, that mitochondrial Ca^{2+} loading may play an important role, allowing a variety of toxic challenges to cause the release of caspase cofactors from the mitochondria resulting in the induction of cell death. Consequently, alteration of mitochondrial cellular response plays a role in the pathogenesis of human disorders.

Conclusions

Ca^{2+} loading from the ER and/or outside of the cell to the mitochondria controls pivotal metabolic processes in the mitochondria (Figure 1), and alterations in this movement of Ca^{2+} plays an important role in the pathologies of diverse human diseases. A decrease in cellular metabolism elicits various pathologies such as type-2 diabetes mellitus, insulin resistance, Alzheimer's disease, and cardiovascular disease. Alterations in cellular Ca^{2+} homeostasis, signs of ER stress, and decreases in mitochondrial membrane potential as well as reduced ATP levels are recurrent molecular events associated with these pathologies (Kelley et al. 2002). Additionally, modification of the contacts between the ER and mitochondria are also critical events in the regulation of cellular metabolism, and interruption of communication between the ER and mitochondria could underlie mitochondrial dysfunction and metabolic imbalance (Decuypere et al. 2011). There has been considerable progression in our understanding of the impact of mitochondrial Ca^{2+} signaling in the control of fundamental cell functions in terms of aerobic metabolism and the cell death pathway. The next challenges will be the identification of addition key transporters responsible for the transport of selective Ca^{2+} or other cations across mitochondrial membrane, of the relationship between transporter structure and function, and generation of transgenic animal models to allow understanding of the roles these transporters in the pathogenesis of human diseases.

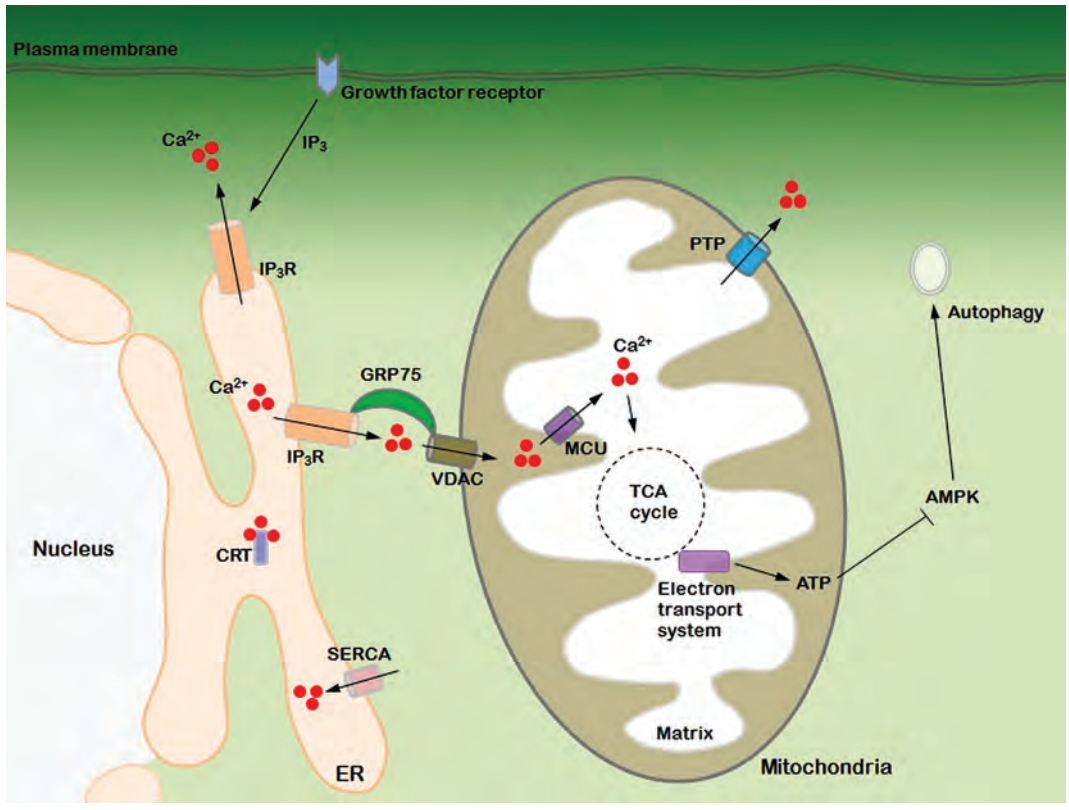

Figure 1. Metabolic communication between the endoplasmic reticulum (ER) and mitochondria *via* Ca^{2+}. Ligand binds to growth factor receptor on the plasma membrane. The receptor generates an $InsP_3$ signaling molecule, which binds to the $InsP_3R$ in the membrane of the ER. Ca^{2+} in the ER is released through the $InsP_3R$ to form high [Ca^{2+}] microdomains located between the ER and mitochondria. Ca^{2+} from the microdomains is translocated by VDAC in the outer membrane of the mitochondria. Subsequently, Ca^{2+} is translocated by the MCU located in the inner membrane of mitochondria. Ca^{2+} found in the matrix promotes the TCA cycle and ATP is generated by the electron transport cascade. Reduction of ATP production due to limited Ca^{2+} efflux from the ER activates AMP-activated protein kinase (AMPK), which in turn stimulates autophagy by the cell. Thus, Ca^{2+} in the mitochondria originates from the ER and regulates cellular metabolism.

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References

Argon Y, Simen BB. 1999. GRP94, an ER chaperone with protein and peptide binding properties. Semin Cell Dev Biol. 10:495–505.

Arnaudeau S, Frieden M, Nakamura K, Castelbou C, Michalak M, Demaurex N. 2002. Calreticulin differentially modulates calcium uptake and release in the endoplasmic reticulum and mitochondria. J Biol Chem. 277:46696–46705.

Ashby MC, Tepikin AV. 2001. ER calcium and the functions of intracellular organelles. Semin Cell Dev Biol. 12:11–17.

Baksh S, Michalak M. 1991. Expression of calreticulin in *Escherichia coli* and identification of its Ca^{2+} binding domains. J Biol Chem. 266:21458–21465.

Bernardi P, Krauskopf A, Basso E, Petronilli V, Blachly-Dyson E, Di Lisa F, Forte MA. 2006. The mitochondrial permeability transition from in vitro artifact to disease target. FEBS J. 273:2077–2099.

Bygrave FL, Benedetti A. 1996. What is the concentration of calcium ions in the endoplasmic reticulum? Cell Calcium. 19:547–551.

Carafoli E. 2003. Historical review: mitochondria and calcium: ups and downs of an unusual relationship. Trends Biochem Sci. 28:175–181.

Carafoli E, Brini M. 2000. Calcium pumps: structural basis for and mechanism of calcium transmembrane transport. Curr Opin Chem Biol. 4:152–161.

Cardenas C, Miller RA, Smith I, Bui T, Molgo J, Muller M, Vais H, Cheung KH, Yang J, Parker I, et al. 2010. Essential regulation of cell bioenergetics by constitutive InsP3 receptor Ca^{2+} transfer to mitochondria. Cell. 142:270–283.

Decuypere JP, Monaco G, Bultynck G, Missiaen L, De Smedt H, Parys JB. 2011. The IP3 receptor-mitochondria connection in apoptosis and autophagy. Biochim Biophys Acta. 1813:1003–1013.

Fill M, Copello JA. 2002. Ryanodine receptor calcium release channels. Physiol Rev. 82:893–922.

Giacomello M, Drago I, Bortolozzi M, Scorzeto M, Gianelle A, Pizzo P, Pozzan T. 2010. 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. 38:280–290.

Greber UF, Gerace L. 1995. Depletion of calcium from the lumen of endoplasmic reticulum reversibly inhibits passive diffusion and signal-mediated transport into the nucleus. J Cell Biol. 128:5–14.

Guo L, Lynch J, Nakamura K, Fliegel L, Kasahara H, Izumo S, Komuro I, Agellon LB, Michalak M. 2001. COUP-TF1 antagonizes Nkx2.5-mediated activation of the calreticulin gene during cardiac development. J Biol Chem. 276:2797–2801.

Hertle DN, Yeckel MF. 2007. Distribution of inositol-1,4,5-trisphosphate receptor isotypes and ryanodine receptor isotypes during maturation of the rat hippocampus. Neuroscience. 150:625–638.

Hewavitharana T, Deng X, Soboloff J, Gill DL. 2007. Role of STIM and Orai proteins in the store-operated calcium signaling pathway. Cell Calcium. 42:173–182.

Jouaville LS, Pinton P, Bastianutto C, Rutter GA, Rizzuto R. 1999. Regulation of mitochondrial ATP synthesis by calcium: evidence for a long-term metabolic priming. Proc Natl Acad Sci USA. 96:13807–13812.

Kelley DE, He J, Menshikova EV, Ritov VB. 2002. Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. Diabetes. 51:2944–2950.

Kirichok Y, Krapivinsky G, Clapham DE. 2004. The mitochondrial calcium uniporter is a highly selective ion channel. Nature. 427:360–364.

Lebeche D, Lucero HA, Kaminer B. 1994. Calcium binding properties of rabbit liver protein disulfide isomerase. Biochem Biophys Res Commun. 202:556–561.

Lewis RS. 2007. The molecular choreography of a store-operated calcium channel. Nature. 446:284–287.

Lievremont JP, Rizzuto R, Hendershot L, Meldolesi J. 1997. BiP, a major chaperone protein of the endoplasmic reticulum lumen, plays a direct and important role in the storage of the rapidly exchanging pool of Ca^{2+}. J Biol Chem. 272:30873–30879.

McCormack JG, Halestrap AP, Denton RM. 1990. Role of calcium ions in regulation of mammalian intramitochondrial metabolism. Physiol Rev. 70:391–425.

Michalak M, Groenendyk J, Szabo E, Gold LI, Opas M. 2009. Calreticulin, a multi-process calcium-buffering chaperone of the endoplasmic reticulum. Biochem J. 417:651–666.

Nakamura K, Zuppini A, Arnaudeau S, Lynch J, Ahsan I, Krause R, Papp S, De Smedt H, Parys JB, Muller-Esterl W, et al. 2001. Functional specialization of calreticulin domains. J Cell Biol. 154:961–972.

Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK. 2010. MICU1 encodes a mitochondrial EF hand protein required for Ca^{2+} uptake. Nature. 467:291–296.

Prins D, Groenendyk J, Touret N, Michalak M. 2011. Modulation of STIM1 and capacitative Ca^{2+} entry by the endoplasmic reticulum luminal oxidoreductase ERp57. EMBO Rep. 12:1182–1188.

Rapizzi E, Pinton P, Szabadkai G, Wieckowski MR, Vandecasteele G, Baird G, Tuft RA, Fogarty KE, Rizzuto R. 2002. Recombinant expression of the voltage-dependent anion channel enhances the transfer of Ca^{2+} microdomains to mitochondria. J Cell Biol. 159:613–624.

Rizzuto R, Simpson AW, Brini M, Pozzan T. 1992. Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin. Nature. 358:325–327.

Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, et al. 2005. STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. J Cell Biol. 169:435–445.

Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, Cavagna D, Nagy AI, Balla T, Rizzuto R. 2006. Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca^{2+} channels. J Cell Biol. 175:901–911.

Szydlowska K, Tymianski M. 2010. Calcium, ischemia and excitotoxicity. Cell Calcium. 47:122–129.

Tan W, Colombini M. 2007. VDAC closure increases calcium ion flux. Biochim Biophys Acta. 1768:2510–2515.

Yule DI, Betzenhauser MJ, Joseph SK. 2010. Linking structure to function: recent lessons from inositol 1,4,5-trisphosphate receptor mutagenesis. Cell Calcium. 47:469–479.