

Mitochondrial Ca^{2+} levels lower down rate of metabolic diseases and cardiomyopathies

Abstract

Present review article explains role of mitochondria in regulation of calcium metabolism. Besides, combustion of fuel and ATP generation for all physiological and metabolic activities, it regulates Ca^{2+} uptake, that is essentially required for intracellular Ca^{2+} signaling, cell metabolism cell proliferation and survival. However, buffering of cytosolic Ca^{2+} levels regulate mitochondrial effectors. Mitochondria work as a Ca^{2+} sink that is formed by electrochemical gradient generated during oxidative phosphorylation, which makes tunneling of the cation an exergonic process. However, excessive calcium influx increases ROS generation and induces mitochondrial depolarization that results in metabolic diseases and evokes cardiomyopathies. In the present article calcium regulated mitochondrial functions have been explained with their wide concern to metabolic defects and cardiac muscle irregularities.

Keywords: Mitochondria, Cytosolic Ca^{2+} levels, transporters, metabolic diseases and cardiomyopathy

Volume 4 Issue 3 - 2018

Ravi Kant Upadhyay

Department of Zoology, DDU Gorakhpur University, India

Correspondence: Ravi Kant Upadhyay, Department of Zoology, DDU Gorakhpur University, Gorakhpur, 273009, U.P. India, Email rkupadhyay@yahoo.com

Received: June 06, 2018 | **Published:** August 17, 2018

Introduction

Mitochondria is an important cell organelle, generates ATP that is used as sole energy molecule for all physiological and metabolic activities. It supplies cellular energy and assist in signaling, cell metabolism, cellular differentiation, cell survival and other cell-specific functions. Calcium uptake takes place through mitochondrial outer membrane by voltage-dependent anion channels. After formation of electrochemical gradient and equilibrium on both sides' mitochondrial functions become normal. It is maintained during oxidative phosphorylation. Thus buffering of cytosolic Ca^{2+} levels regulate mitochondrial effector functions. Ca^{2+} transported into mitochondria regulates its metabolism and causes transient depolarisation of mitochondrial membrane. Imbalance in Ca^{2+} levels cause cardiac myocyte injury that is increased with the decline in pH.¹ Dysregulated mitochondrial Ca_{2+} level and its imbalances generate ischemia neurodegenerative diseases, neuropsychiatric disorders and cancer.² Accumulation of extra calcium in mitochondria also increases production and modulation of reactive oxygen species. Therefore, balanced Ca^{2+} buffering is required for normal mitochondrial functions, cell survival and longevity. Mitochondria also involve in control of cell cycle and cell growth. Hence, excessive calcium influx increases ROS generation, induces mitochondrial depolarization and triggers sever pathogenesis. Contrary to this low calcium level affects homoeostasis and redox signaling.³ It also gives rise stress particularly, nitrosative or oxidative stress. More often, excessive calcium uptake of calcium and accumulation of it in cardiac muscle cells result in mitochondrial dysfunctions that impose heart disease. Mitochondria play a central role in cell life and cell death. Availability of Ca^{2+} in cell from endoplasmic reticulum plays a pivotal role in cell proliferation.

Cardiomyopathy is group of diseases that is characterized by loss of function of cardiac muscles because they become enlarged, thick or rigid. It results in shortness of breath, and swelling of the legs. In young adults mainly athletes cardiac muscles become unable to relax and due to overload they become non flexible and go under sudden arrest due to dysregulation of calcium uptake. An irregular heart

beat results in fainting. Contrary to this older adults face restrictive cardiomyopathy in which heart becomes rigid because abnormal tissue (e.g. scar tissue). The muscle function depends on calcium influx and its transportation. Once intracellular Ca^{2+} flux disturbs it and results in morbidity. Normally, mild calcium influx from cytosol into the mitochondrial matrix causes transient depolarization which is further corrected by pumping out protons. In contrast, excessive calcium influx results in generation of more ROS that induces mitochondrial depolarization. It is also associated with opening of the permeability transition pore (PTP).³

Loss of cardiac muscles by programmed cell death contributes progression of ischemic heart diseases.¹ This death pathway is activated by hypoxia-acidosis that is driven by a combination of calcium-activated calpains and pro-death factors (DNases) secreted by the mitochondria. But accumulation of cytochrome c in the cytoplasm during hypoxia-acidosis induces programmed cell death.¹ Moreover, combined hypoxia with acidosis, is marker of ischemia that promotes cardiac myocyte injury. Its severity increases with pH decline.¹ Calpain inhibitors provide vigorous protection against hypoxia-acidosis-induced programmed cell death beside this, metabolite waste buildup during hypoxia that initiates caspase dependent and independent cell death pathways.¹

Ca^{2+} transporters play important role in metabolic functions of mitochondria. Diffusion of calcium ions takes place through mitochondrial outer membrane by the activity of voltage-dependent anion channels (VDAC). Both VDAC channel and mPTP permeability is maintained by calcium ion influx. But calcium overload causes pore opening (Figure 1).⁴ Besides this, calcium release units (CRUs) and mitochondria control myoplasmic $[\text{Ca}^{2+}]$ levels and ATP production in muscle cells. ATP production takes place under active role of ATP synthase found in the inner membrane. Both organelles are structurally connected by tethers and assist in Ca^{2+} signaling.⁵ This density of CRUs and mitochondria is decreased in muscle fibers with an increase in percentage of mitochondria damages.¹ Further, a reduced association between CRUs and mitochondria with aging

contributes impaired relations between the two organelles. It results in a significant reduction of efficiency in activity-dependent ATP production. This is the main reason that in older person age-dependent decline of skeletal muscle performance occurs.

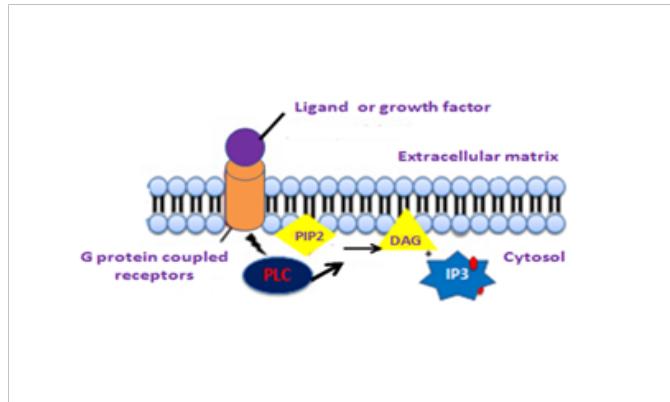


Figure 1 Phospholipase C cleaving PIP2 into IP3 and DAG at inner membrane and cytosol.

Accumulation of calcium into mitochondria trigger pathology. Low or very high calcium uptake causes nitrosative or oxidative stress. Oxidative stress in cardiomyocytes, indicates mitochondrial calcium overload. In case of oxidative stress generates cerebral ischemia-reperfusion injury which involves multiple independently fatal terminal pathways in the mitochondria. These pathways include the reactive oxygen species (ROS) generation caused by changes in mitochondrial membrane potential due to calcium overload. It results in apoptosis via cytochrome c (Cyt c) release.⁶ Mitochondrial permeability of calcium occurs through transition pore (PTP) but sudden increase in calcium cause ischemia-reperfusion-induced cell death. Among few regulators matrix protein cyclophilin D (CypD) is the best known regulator of PTP opening.⁷ Besides, calcium overload amino acid glutamate generates neurotoxicity after activation of *N*-methyl-D-aspartate (NMDA) receptors. NMDA receptors and neuronal nitric oxide synthase generates nitric oxide that leads to the collapse of mitochondrial membrane potential followed by cell death. Impaired mitochondrial energy supply coupled to increased H_2O_2 emission under energy/redox stress leads to myocardial dysfunction and also cause Type I diabetes.⁸ Caffeine stimulates transfer of calcium from sarcoplasmic reticulum (SR) to mitochondria. CypD prevents PTP opening.⁷

Calcium buffering, cell survival and longevity

Calcium transport and signaling is essential role for cell survival. It assists in production of energy, calcium buffering, all oxygen based physiological functions, and in regulation of Ca^{2+} and exocytotic signals in mature cells (Figure 1).⁹ More often, Ca^{2+} signals, integrate extracellular and intracellular fluxes, and play important role in synaptic plasticity and memory. These are also required for, neurotransmitter release, and neuronal excitability. It also plays role in regulating apoptosis and gene transcription in mitochondria. All mitochondrial dysfunctions cause impairment of the mitochondrial respiratory chain, generate excessive of reactive oxygen species, and induce excitotoxicity.⁹ This disturbance in calcium uptake and release impose pathogenesis results in certain neurodegenerative diseases, neuropsychiatric disorders, and cancer.² Calcium release in neurons causes an instant increase in cytosolic and

mitochondrial synchronization of electrical activity that start energy metabolism. Further, for the activation of isocitrate dehydrogenase calcium levels in mitochondrial matrix should reach the tens of micromolar levels. This is one of the key regulatory enzymes of the Krebs cycle.^{10,11} Besides ATP driven functions mitochondria actively assist in other non-ATP-related functions that are intimately involved with most of the major metabolic pathways used by a cell to build, break down, and recycle its molecular building blocks. Mitochondria detoxify ammonia generated in liver cells during the urea cycle. It also play important role in cholesterol metabolism, estrogen and testosterone synthesis, neurotransmitter metabolism, and free radical production and detoxification.

Endoplasmic reticulum stress is also related to Ca^{2+} signaling. It also mediates unfolded protein response, that induce ER associate degradation (ERAD) and autophagy.¹² Hence, for normal health calcium buffering should be balanced because it is essentially required for cell survival and longevity. Ca^{2+} mediated events are performed when the released Ca^{2+} binds to and activates the regulatory protein calmodulin. Calmodulin activate calcium-calmodulin-dependent protein kinase and act directly on other effector proteins.¹³ Besides calmodulin, there are many other Ca^{2+} -binding proteins that mediate the biological effects of Ca^{2+} . But do not show behavior like calmodulin. Ca^{2+} permeation and/or binding to the skeletal muscle L-type Ca^{2+} channel (CaV1.1) facilitates activation of Ca^{2+} /calmodulin kinase type II (CaMKII) and Ca^{2+} store refilling. It reduces muscle fatigue and atrophy.¹⁴ CaV1.1-mediated CaMKII activation impacts muscle energy expenditure. Calcium ions also function as second messenger and involve in intra- and extracellular signaling cascades and plays an essential role in cell life and death decisions. The Ca^{2+} signaling network regulate cellular processes through calcium buffering that helps to operate pumps and exchangers on the plasma membrane. It send extra calcium into internal stores. Calcium signaling pathways interact with other cellular signaling systems such as reactive oxygen species (ROS). Hence, a fine tuning of cellular signaling networks is essential for cellular health, once failed it leads to dysfunctions and impose harmful effects which might contribute to the pathogenesis of various disorders.¹⁵

Cell proliferation

Cell proliferation is also operated through diverse proteins related to calcium Ca^{2+} signaling inside the cell. There is an interrelationship in calcium stores to the nucleus and signaling peptides synthesized in response to calcium level. However, for performing cellular functions plasma membrane cellular Ca^{2+} influx occurs, which is followed by absorption of Ca^{2+} ions by mitochondria and endoplasmic reticulum. This fluctuation of Ca^{2+} from the endoplasmic reticulum plays important physiological role for cell proliferation. However, Ca^{2+} depletion in the endoplasmatic reticulum triggers Ca^{2+} influx across the plasma membrane. It results in store-operated calcium entries (SOCEs).¹⁶ Further, for maintaining calcium level mitochondrial Ca^{2+} uniporter plays important roles. It is a pore-forming mitochondrial Ca^{2+} uniporter protein (MCU), whose scaffolding is essential for MCU regulator (EMRE), and mitochondrial calcium uptake by MICU1/2. It forms a Ca^{2+} -selective protein complex that negatively regulate mitochondrial Ca^{2+} uptake. UCP2 assists in mitochondrial Ca^{2+} uptake because it is as a selective modulator of just one distinct MCU/EMRE-dependent mitochondrial Ca^{2+} inward movement.¹⁷ Sirtuin 3 inhibits cardiomyocyte apoptosis by reducing cytochrome C release in myocardial H9c2 cells with calcium overload.¹⁸

For normal functioning of cells and its metabolism calcium buffering is highly important because it is required for maintaining cell signaling.⁹ Moreover, Cav1.1→CaMKII→NOS occurs in skeletal muscles regulate the intracellular distribution of the fatty acid lead by a transport protein CD36. It alters fatty acid metabolism. Blocking of this pathway results in decreased mitochondrial β -oxidation and decreased energy expenditure. Mainly CaV1.1-mediated pathway regulates energy utilization in skeletal muscles.¹⁹ Thus Ca^{2+} permeation and/or binding to the skeletal muscle L-type Ca^{2+} channel (CaV1.1) facilitates activation of Ca^{2+} /calmodulin kinase type II (CaMKII) and Ca^{2+} store refilling. Both processes reduce muscle fatigue and atrophy (Figure 2).¹⁴ Ca^{2+} level in mitochondria and exocytotic signals are required for catecholamine secretory response.⁹ Mitochondria both encode and decode Ca^{2+} signals which largely put impact on cell signaling and metabolism. More specifically, a mitochondrial protein Fus1 a tumor suppressor effectively controls immune response and tumor growth via maintenance of mitochondrial homeostasis and Ca^{2+} accumulation. It assists in Ca^{2+} signaling, mitochondrial Ca^{2+} transport and ROS production in the activation of NFAT and NF- κ B transcription factors.²⁰ However, proliferating cancer cells and lymphocytes need energy for maintaining signaling and calcium flux and buffering.

Excitation-contraction coupling

Mitochondria assists in cardiac contractility functions of heart as ATP generated by it is utilized for such functions. This is excitation-contraction (E-C) coupling is closely interconnected with the SR, and Ca^{2+} uptake. It mainly depends on calcium (Ca^{2+}) released from the sarcoplasmic reticulum (SR). But excess of Ca^{2+} impairs mitochondrial function, decrease in ATP production and an increase in release of reactive oxygen species (ROS). Oxidative stress generates after high calcium accumulation in heart muscles also important cause of heart failure.²¹ Mitochondria maintain uptake, storage and release of Ca^{2+} within the intact cell by steady-state cycling of Ca^{2+} across the inner membrane. Naturally, due to a regulated influx and out flux of calcium is established by independent uptake and efflux pathways operated by distinctive kinetics of the uniporter. Uniporter maintains a level between external free Ca^{2+} concentration and the efflux calcium. These disallow excess of calcium and keep out it by calcium phosphate complex and mitochondria reversibly sequester transient elevations in cytoplasmic Ca^{2+} . CO induces a two-component metabolic response: uncoupling of mitochondrial respiration dependent on the activation of mitoBKCa channels and inhibition of glycolysis independent of mitoBKCa channels.²² Further, under non-stimulated conditions, the same transport regulates matrix Ca^{2+} concentrations and citric acid cycle activity.²³

Calcium influx regulation and cell signaling

In resting state concentration of Ca^{2+} in the cytoplasm remains around 100nM. It is that is 20,000 to 100,000-fold lower than typical extracellular concentration.^{24,25} However, for maintaining low concentration, Ca^{2+} is actively pumped from the cytosol to the extracellular space or into the endoplasmic reticulum (ER), and sometimes into the mitochondria (Figure 2). Calmodulin found in cytoplasm bind Ca^{2+} ions and maintain buffer state. Signaling starts when cell gets stimulation to release calcium ions (Ca^{2+}) from intracellular space. Moreover, affinity of Ca^{2+} channels which found on outer mitochondrial membrane responds to changes in intracellular Ca^{2+} flux.²⁶ Further, Ca^{2+} micro-domains found between

MAM and mitochondria situation associate to form contact points through which efficient Ca^{2+} transmission from the ER to the mitochondria occurs.²⁶ It proceeds in response to Ca^{2+} puffs produced by spontaneous clustering and activation of IP3R, an ER membrane Ca^{2+} channel.^{26,27} As soon as MAM receives Ca^{2+} exposure, it starts working as a firewall that essentially buffers Ca^{2+} puffs by acting as a sink into which free ions released into the cytosol which are funneled (Figure 2).^{26,28,29} Thus Ca^{2+} tunneling occurs through the low-affinity Ca^{2+} receptor VDAC1, physically tethered to the IP3R clusters on the ER membrane and enriched at the MAM.^{26,28,30} Regulating ER release of Ca^{2+} at the MAM is highly important Ca^{2+} uptake sustains the mitochondria. Consequently it maintains homeostasis (Figure 2) and does fine regulation of Ca^{2+} signaling. Once it fails results in several neurodegenerative diseases.³⁰

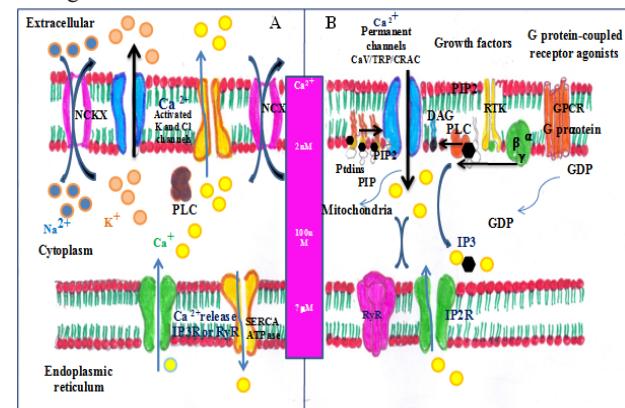


Figure 2 Maintaining and using Ca^{2+} gradients for signaling.

Permeability of calcium ions maintains cell signaling. Specific signals are generated after sudden increase in the cytoplasmic Ca^{2+} level up to 500–1,000nM due to opening of transport channels located in the endoplasmic reticulum or the plasma membrane. As this signal enters into cytosol it exerts allosteric regulatory effects on many enzymes and proteins. Calcium ions activate ion channels through signal transduction mechanism and behave as second messenger and bind to G-protein-coupled receptors. The most common signaling pathway that increases cytoplasmic calcium concentration is the phospholipase C pathway. G coupled and tyrosine kinase receptors located on cell surface receptors, activate the phospholipase C enzyme. This hydrolyses the membrane phospholipid PIP2 to form IP3 and diacylglycerol. The IP3 receptor serves as a Ca^{2+} channel, and releases Ca^{2+} from the endoplasmic reticulum. The Ca^{2+} ions also bind to PKC, and activate it.³¹

Ca^{2+} oscillations are observed after Ca^{2+} influx occurs across the plasma membrane. Ca^{2+} buffering of mitochondria also requires operating Ca^{2+} shuttling pathways in primary mesothelial cells. As during Ca^{2+} oscillations Ca^{2+} is shuttled between the ER and mitochondria, from ER and the extracellular space or the ER and cytoplasmic Ca^{2+} buffers.³² Further, spatio-temporal dynamics of intracellular calcium, $[\text{Ca}^{2+}]$, regulate the contractile function of cardiac muscle cells.³³ Depletion of calcium from the endoplasmic reticulum results in Ca^{2+} entry from outside the cell by activation of store-operated channels.³⁴ This inflowing calcium current depends on release of calcium from stored reserves that generates Ca^{2+} -release-activated Ca^{2+} current (Figure 2). Thus movement of calcium ions from the extracellular compartment to the intracellular compartment alters membrane potential. It happens in cardiac muscle cells of heart,

during the plateau phase of ventricular contraction. Here, calcium ions itself maintain depolarization of the heart.

Calcium signaling through ion channels is also important in neuronal synaptic transmission. However, ATP is required for normal electrical activities of neurons and synaptic transmission. Additionally, calcium signaling is also required for neurotransmitter synthesis, calcium homoeostasis, redox signaling, production and modulation of reactive oxygen species, and neuronal death.³⁵ For maintaining calcium signaling in mitochondria ER plays important role. Thus over all changes occur in intracellular Ca^{2+} flux due to disturbance in calcium channels either by inhibition of channel ports by an inhibitor or low affinity of Ca^{2+} channels localized on the outer mitochondrial membrane.²¹ MAM dynamics determines the propagation of Ca^{2+} waves throughout the cell in an integrated manner.²⁸ More specifically, transmission of Ca^{2+} is not unidirectional; it is a two-way path.²¹ For operation of Ca^{2+} pump SERCA and the channel IP3R found on the ER membrane do feedback regulation coordinated by MAM function. More specifically, clearance of Ca^{2+} by the MAM decides pattern of Ca^{2+} signaling as Ca^{2+} alters IP3R activity in a biphasic cellular manner.²⁶ Further, SERCA is affected by mitochondrial feedback, and uptake of Ca^{2+} by the MAM stimulates ATP production. Thus energy production assists SERCA to reload the ER with Ca^{2+} for continued Ca^{2+} efflux at the MAM.^{28,30} Thus, the MAM modulate Ca^{2+} signaling through feedback loops that affect ER dynamics. Functions of mitochondria vary according to the cell

type, highly excitable cells possess highly active mitochondria. The most important function of mitochondria is production of energy by utilizing metabolites such as fats, carbohydrates and proteins. After glycolysis and decarboxylation processes these are converted into acetyl Co A that easily enters the mitochondrial membrane. Mainly with in mitochondria acetyl Co A processed to produce charged molecules by combining with oxygen and produce ATP molecules in the process of oxidative phosphorylation. It is important to maintain proper concentration of calcium ions with in various compartments of cell.

Mitochondrial sustainability depends on Ca^{2+} uptake which is regulated by ER, through release of Ca^{2+} at the MAM. For activating dehydrogenase enzymes before citric acid cycle sufficient intraorganelle Ca^{2+} signaling is required.³⁶ Only when Ca^{2+} flux crossed a certain threshold, signal is passed on to mitochondria to stimulate the intrinsic pathway of apoptosis. It collapse the mitochondrial membrane potential which is required for CAC metabolism.²⁶ For metabolic performance anti-apoptotic factor Bcl_2 interact with IP3Rs to reduce Ca^{2+} filling of the ER. It leads to reduce efflux at the MAM that preventing collapse of the mitochondrial membrane potential by generating post-apoptotic stimuli.²⁶ If any how this, fine regulation of Ca^{2+} signaling is not maintained, it dysregulates mitochondrial Ca^{2+} that results in several neurodegenerative diseases and apoptosis (Figure 3).³⁰

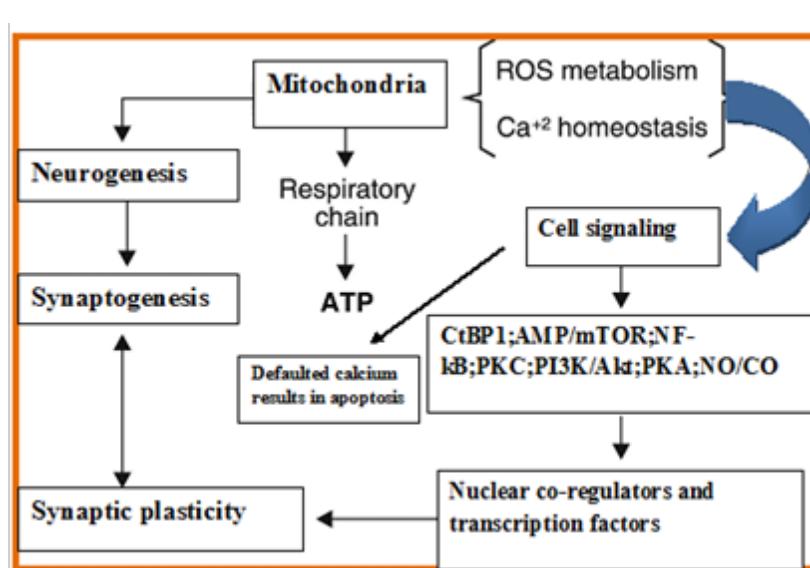


Figure 3 Regulation of various mitochondrial functions by calcium signaling mechanism.

Conclusion

Mitochondria are an important cell organelle that generates ATP that is utilized in various cellular activities. Ca^{2+} uptake is highly important mechanism as it controls intracellular Ca^{2+} signaling, cell metabolism, cell survival and other cell-type specific functions. For over all metabolic functions calcium ionic equilibrium and cytosolic buffering is important for prevention of programmed cell death. Cytosolic Ca^{2+} level regulate mitochondrial effectors. In maintaining Ca^{2+} level mitochondrial transporters play important role. The major process involved for calcium influx is opening of the mitochondrial permeability transition pore. It functions like a gateway for conduction of ions. Once it disturbs it leads to collapse of mitochondrial

membrane potential, ATP depletion and necrotic cell death, and apoptosis. Calcium ions play important functions in biochemistry and physiology of cell as it perform signal transduction, assists in release of neurotransmitters neurons, assists in contraction of muscle cells. Alteration in mitochondrial calcium give rise biochemical changes and its ultimate consequence is cell death. Calcium deficit cells face traumatic death due to acute cellular injury or necrosis. Defect in transient depolarization of mitochondrial membrane potential shows loss of vitality in cells. For all normal activities cell needs un-interrupted supply of adenosine triphosphate (ATP) that is utilized in various cellular activities such as signaling, cellular differentiation, progression of cell cycle, cell growth and cell death. Mitochondria detoxify ammonia in the urea cycle, involve in free radical production.

It also works for cholesterol metabolism, estrogen and synthesis, and neurotransmitter metabolism. Mitochondria oxidize fat, protein, and carbohydrates. In blood clotting many enzymes require Ca^{2+} as a co-factor. In excitable cells extracellular calcium maintain the potential difference across excitable cell membranes. Calcium has important role in organization of bone tissue and fertilization. Mitochondria promptly respond to Ca^{2+} -mediated cell stimulations with a rapid accumulation of the cation into the matrix. Defaulted permeability causes Ca^{2+} ion imbalance, failure of cell signaling, and largely effect energy production and homeostasis. Failure of Ca^{2+} homeostasis results in chronic mitochondrial diseases loss of muscle coordination, heart, strokes, seizures, muscle fatigue, gastrointestinal problems, liver problems, diabetes and obesity. Conclusively calcium overload is a major component of the programmed cell death. This eventually leads in failure of the organ system. It can even prove to be fatal in some cases.

Acknowledgements

Thanks for Prof. R.N. K. Bamezai, former dean, JNU New Delhi for important discussion.

Conflict of interest

Author declares that there is none of the conflicts.

References

1. Graham RM, Thompson JW, Webster KA. BNIP3 promotes calcium and calpain-dependent cell death. *Life Sci.* 2015;142:26–35.
2. de Oliveira MR, Elangovan N, Ljubkovic M, et al. The roads to mitochondrial dysfunction. *Biomed Res Int.* 2015;235370.
3. Karnkowska Anna, Vacek Vojtěch, Zubáčová Zuzana, et al. A Eukaryote without a Mitochondrial Organelle. *Current Biology.* 2016;26(10):1274–1284.
4. Azarashvili T, Krestinina O, Baburina Y, et al. Combined effect of G3139 and TSPO ligands on Ca^{2+} -induced permeability transition in rat brain mitochondria. *Arch Biochem Biophys.* 2015;587:70–77.
5. Pietrangelo L, Dincecco A, Ainbinder A, et al. Age-dependent uncoupling of mitochondria from Ca^{2+} release units in skeletal muscle. *Oncotarget.* 2015;6(34):35358–35371.
6. Hu Y, Deng H, Xu S, et al. MicroRNAs Regulate Mitochondrial Function in Cerebral Ischemia–Reperfusion Injury. *Int J Mol Sci.* 2015;16(10):24895–24917.
7. Teixeira G, Chiari P, Fauconnier J, et al. Involvement of Cyclophilin D and Calcium in Isoflurane-induced Preconditioning. *Anesthesiology.* 2015;123(6):1374–1384.
8. Tocchetti CG, Stanley BA, Sivakumaran V, et al. Impaired mitochondrial energy supply coupled to increased H_2O_2 emission under energy/redox stress leads to myocardial dysfunction during Type I diabetes. *Clin Sci (Lond).* 2015;129(7):561–574.
9. Vestring S, Fernández–Morales JC, Méndez–López I, et al. Tight mitochondrial control of calcium and exocytotic signals in chromaffin cells at embryonic life. *Pflügers Arch.* 2015;467(12):2589–2601.
10. Ivannikov M, Macleod GT. Mitochondrial Free Ca^{2+} Levels and Their Effects on Energy Metabolism in Drosophila Motor Nerve Terminals. *Biophys J.* 2013;104(11):2353–2361.
11. Ivannikov M, Sugimori M, Llinás RR. Synaptic vesicle exocytosis in hippocampal synaptosomes correlates directly with total mitochondrial volume. *J Mol Neurosci.* 2013;49(1):223–230.
12. Berridge M. Neuronal calcium signaling. *Neuron.* 1998;21(1):13–26.
13. Berg Jeremy, Tymoczko John L, Gatto Gregory J, et al. *Biochemistry.* 8th ed. In: WH Freeman editor. New York; 2015. 407 p.
14. Lee C, Zeng J, Drew BG, et al. The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance. *Cell Metab.* 2015;21(3):443–54.
15. Görlich A, Dimova EY, Petry A, et al. Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved? *Redox Biol.* 2015;6:372–85.
16. Pinto MC, Kihara AH, Goulart VA, et al. Calcium signaling and cell proliferation. *Cell Signal.* 2015;27(11):2139–2149.
17. Deak AT, Jean–Quartier C, Bondarenko AI, et al. Assessment of mitochondrial Ca^{2+} uptake. *Methods Mol Biol.* 2015;1264:421–439.
18. Ye L, Yang S. Sirtuin 3 inhibits cardiomyocyte apoptosis by reducing cytochrome C release in myocardial H9c2 cells with calcium overload. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi.* 2015;31(8):1031–1035.
19. Georgiou DK, Dagnino–Acosta A, Lee CS, et al. Ca^{2+} Binding/Permeation via Calcium Channel, CaV1.1, Regulates the Intracellular Distribution of the Fatty Acid Transport Protein, CD36, and Fatty Acid Metabolism. *J Biol Chem.* 2015;290(39):23751–23765.
20. Uzhachenko R, Shanker A, Yarbrough WG, et al. Mitochondria, calcium, and tumor suppressor Fus1: At the crossroad of cancer, inflammation, and autoimmunity. *Oncotarget.* 2015;6(25):20754–20772.
21. Santulli G, Xie W, Reiken SR, et al. Mitochondrial calcium overload is a key determinant in heart failure. *Proc Natl Acad Sci USA.* 2015;112(36):11389–11394.
22. Kaczara P, Motterlini R, Rosen GM, et al. Carbon monoxide released by CORM–401 uncouples mitochondrial respiration and inhibits glycolysis in endothelial cells: A role for mitoBKCa channels. *Biochim Biophys Acta.* 2015;1847(10):1297–1309.
23. Nicholls DG. Mitochondria and calcium signaling. *Cell Calcium.* 2005;38(3–4):311–317.
24. Clapham DE. Calcium Signaling. *Cell.* 2007;131(6):1047–1058.
25. Demaurex N, Nunes P. The role of STIM and ORAI proteins in phagocytic immune cells. *American Journal of Physiology. Cell Physiology.* 2016;310(7):C496–C508.
26. Rizzuto R, Marchi Saverio, Bonora Massimo, et al. Ca^{2+} transfer from the ER to mitochondria: when, how and why. *Biochim Biophys Acta.* 2009;1787(11):1342–1351.
27. Santulli Gaetano, Marks Andrew. Essential Roles of Intracellular Calcium Release Channels in Muscle, Brain, Metabolism, and Aging. *Current Molecular Pharmacology.* 2015;8(2):206–222.
28. Kopach O, Kruglikov Illya, Pivneva Tatyana, et al. Functional coupling between ryanodine receptors, mitochondria and Ca^{2+} ATPases in rat submandibular acinar cells. *Cell Calcium.* 2008;43(5):469–481.
29. Csordas G, Hajnóczky G. Sorting of calcium signals at the junctions of endoplasmic reticulum and mitochondria. *Cell Calcium.* 2001;29(4):249–262.
30. Decuyper JP, Monaco Giovanni, Bultynck Geert, et al. The IP3 receptor–mitochondria connection in apoptosis and autophagy. *Biochim Biophys Acta.* 2011;1813(5):1003–1013.
31. Liberts, Bray, Hopkin, et al. *Essential Cell Biology.* 4th ed. New York: Garland Science; 2014. p. 548–549.
32. Pecze L, Blum W, Schwaller B. Routes of Ca^{2+} shuttling during Ca^{2+} oscillations: Focus on role of mitochondrial Ca^{2+} handling and cytosolic Ca^{2+} buffers. *J Biol Chem.* 2015;290(47):28214–28230.

33. Rajagopal V, Bass G, Walker CG, et al. Examination of the Effects of Heterogeneous Organization of RyR Clusters, Myofibrils and Mitochondria on Ca^{2+} Release Patterns in Cardiomyocytes. *PLoS Comput Biol.* 2015;11(9):e1004417.

34. Putney James W, Tomita Takuro. Phospholipase C Signaling and Calcium Influx. *Advances in biological regulation.* 2012;52(1):152–164.

35. Zsurka G, Kunz WS. Mitochondrial dysfunction and seizures: the neuronal energy crisis. *Lancet Neurol.* 2015;14(9):956–966.

36. Hajnóczky G, Csordás G, Yi M. Old players in a new role: mitochondria-associated membranes, VDAC, and ryanodine receptors as contributors to calcium signal propagation from endoplasmic reticulum to the mitochondria. *Cell Calcium.* 2011;32(5–6):363–377.