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Different approaches to modeling analysis of mitochondrial swelling

Sabzali Javadov^{1,*}, Xavier Chapa-Dubocq¹, and Vladimir Makarov²

¹Department of Physiology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico, USA

²Department of Physics, Rio Piedras Campus, University of Puerto Rico, San Juan, Puerto Rico, USA

Abstract

Mitochondria are critical players involved in both cell life and death through multiple pathways. Structural integrity, metabolism and function of mitochondria are regulated by matrix volume due to physiological changes of ion homeostasis in cellular cytoplasm and mitochondria. Ca^{2+} and K^{+} presumably play a critical role in physiological and pathological swelling of mitochondria when increased uptake (influx)/decreased release (efflux) of these ions enhances osmotic pressure accompanied by high water accumulation in the matrix. Changes in the matrix volume in the physiological range have a stimulatory effect on electron transfer chain and oxidative phosphorylation to satisfy metabolic requirements of the cell. However, excessive matrix swelling associated with the sustained opening of mitochondrial permeability transition pores (PTP) and other PTP-independent mechanisms compromises mitochondrial function and integrity leading to cell death. The mechanisms of transition from reversible (physiological) to irreversible (pathological) swelling of mitochondria remain unknown. Mitochondrial swelling is involved in the pathogenesis of many human diseases such as neurodegenerative and cardiovascular diseases. Therefore, modeling analysis of the swelling process is important for understanding the mechanisms of cell dysfunction. This review attempts to describe the role of mitochondrial swelling in cell life and death and the main mechanisms involved in the maintenance of ion homeostasis and swelling. The review also summarizes and discusses different kinetic models and approaches that can be useful for the development of new models for better simulation and prediction of *in vivo* mitochondrial swelling.

*To whom correspondence should be addressed: Sabzali Javadov, MD, PhD, Department of Physiology, University of Puerto Rico School of Medicine San Juan, PR 00936-5067 USA, Tel: 787-758-2525; Fax: 787-753-0120, sabzali.javadov@upr.edu.

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1. Introduction

Mitochondria are subcellular organelles that originated from primitive bacterial invasion of primordial eukaryotic cells (Sagan, 1967). Mitochondria are the powerhouse of the cell; ~90% of oxygen consumed by mammalian cells are used by mitochondria to produce ATP through oxidative phosphorylation (Rolfe and Brown, 1997). In addition to ATP production, mitochondria maintain a myriad of metabolic pathways such as ion homeostasis, cell growth, lipid oxidation and synthesis, and redox signaling. Mitochondria play a central role not only in cell life but also cell death (Balaban et al., 2005; O'Rourke et al., 1994; Tan et al., 1998). Due to their ability to store Ca^{2+} and respond to cytosolic Ca^{2+} signals, mitochondria together with endo(sarco)plasmic reticulum, participate in the regulation of intracellular Ca^{2+} homeostasis. Under normal conditions, mitochondrial Ca^{2+} (Ca^{2+}_m) regulates energy metabolism, whereas, at high concentrations, it stimulates mitochondria-mediated cell death pathways. Mitochondria respond to many different types of stress including oxidative and metabolic stresses. They are the main source of reactive oxygen species (ROS), a side product of respiration generated mainly at the electron transport chain (ETC) complexes I and III. Ca^{2+} overload along with high ROS and P_i , result in changes in mitochondrial membrane permeability and induces the opening of non-selective and high-conductance permeability transition pores (PTP) in the inner mitochondrial membrane (IMM). The PTP opening further compromises bioenergetics function and structural integrity of mitochondria leading to cell death (Halestrap et al., 1998; Petronilli et al., 2001; Rasola and Bernardi, 2011).

Importantly, under physiological conditions, the mitochondrial volume is regulated by ion fluxes, mainly by K^+ and Ca^+ fluxes across the IMM, (Garlid and Paucek, 2003; Szabo and Zoratti, 2014). Mild increases in matrix volume over the physiological range stimulate mitochondrial function and metabolism (Halestrap, 1989). Beneficial effects of matrix volume changes on mitochondrial metabolism and function may be due to structural and functional remodeling of the IMM, particularly, the fluidity of the membrane although precise mechanisms of these effects remain unknown.

Excessive mitochondrial swelling, which occurs mostly due to PTP opening, is a central player that can induce cell death through apoptosis or necrosis depending on availability of ATP. Notably, mitochondrial swelling is involved in the pathogenesis of many human diseases associated with oxidative stress such as ischemia (infarction)-reperfusion, hypoxia, inflammation and diabetes among others. Therefore, it is important to elucidate the mechanisms of mitochondrial swelling for understanding mitochondria-mediated cell death and development of new therapeutic strategies by targeting the mitochondrion. Regulation of the mitochondrial matrix volume may provide relief to stress, which would allow mitochondria to maintain their functional and morphological integrity (Kaasik et al., 2007);

aiding in sustaining cellular life. As mentioned above, PTP opening induces mitochondrial swelling. However, inhibition of the PTP does not prevent mitochondrial swelling completely, suggesting a role of PTP-independent mechanisms are involved in the swelling (Eliseev et al., 2002; Gogvadze et al., 2004). The mechanisms of swelling are not clear, though ions, pH_i , and membrane potential (Ψ_m) are the major players that regulate matrix swelling. Particularly, the concentration of K^+ and Ca^{2+} across the IMM have been known to play a central role in creating the electrogenic transition leading to mitochondrial swelling (O'Rourke, 2007).

In this review, we discuss previous studies on the potential mechanisms of mitochondrial swelling as well as a variety of theoretical approaches to modeling mitochondrial swelling. Modeling analysis of mitochondrial swelling, particularly, the mechanisms of transition from physiological (reversible) to pathological (irreversible) swelling is important for understanding mitochondria-mediated cell death and organ/tissue dysfunction in human diseases.

2. Mitochondrial swelling: causes and consequences

Mitochondrial swelling begins with changes in ion homeostasis of the matrix, which induces an osmotic imbalance between the cytosol and the matrix. As a result, increased colloidal osmotic pressure enhances the water influx leading to matrix swelling. Notably, changes in matrix volume have an important role in regulating mitochondrial function and metabolism under physiological conditions. However, an extensive increase of the matrix volume or matrix swelling compromises mitochondrial function and initiates mitochondria-mediated cell death. Main transport mechanisms that regulate ion homeostasis and matrix volume in mitochondria are shown in Figure 1.

2.1. Physiological role of changes in matrix volume

2.1.1. Role of K^+ and Ca^{2+} fluxes—Under physiological conditions, mitochondria maintain the matrix volume within a narrow range through the regulation of influx/efflux mechanisms for ions across the IMM. One of the main mechanisms regulating the matrix volume in unstressed mitochondria includes the movement of K^+ in or out of the matrix (*reviewed in* (Garlid and Paucek, 2003; Halestrap, 1994)). In 1986, Halestrap et al (Halestrap et al., 1986) found that Ca^{2+} stimulated an energy dependent K^+ uptake that provoked a swelling response in rat liver mitochondria. Under physiological conditions, K^+ -dependent Ca^{2+} influx induces an increase in matrix volume (Halestrap et al., 1986). Although the precise role of PTP in physiological swelling remains unclear (Mnatsakanyan et al., 2017), under pathophysiological conditions (e.g. oxidative stress), the PTP presumably stimulates a shift in osmotic pressure that leads to matrix swelling.

The main K^+ influx mechanisms are the mitochondrial Ca^{2+} regulated K^+ large conductance channel (mBK_{Ca}), a voltage-activated K^+ channel of the $\text{Kv}1.3$ type ($\text{mKv}1.3$) and mitochondrial ATP activated K^+ channels (mK_{ATP}). The K^+/H^+ exchanger (KHE) is responsible for K^+ efflux from the matrix. Opening of the mBK_{Ca} increases the permeability of the IMM to K^+ and which, in turn, decreases the mitochondrial Ca^{2+} buffering capacity due to membrane depolarization. The latter reduces the driving force for mitochondrial Ca^{2+}

uptake and thereby, prevents Ca^{2+} overload (Giorgi et al., 2012). In favor of this, K^+ influx mediated by the mBK_{Ca} improved respiratory function of mitochondria (Aon et al., 2010; Heinen et al., 2007a, 2007b). The beneficial effects of increased K^+ influx on respiratory function were dependent on mitochondrial swelling with maintained Ψ_{m} (Aon et al., 2010). On the other hand, increased matrix K^+ influx due to stimulation of mBK_{Ca} increased the ETC activity and mitochondrial respiration without altering Ψ_{m} (Heinen et al., 2007a, 2007b). The K^+ entry into the matrix occurs mostly through the electrogenic putative mK_{ATP} channel, whereas K^+ efflux mediated by KHE equilibrates the osmotic pressure across the IMM (Diwan, 1987). Hence, activation of the putative mK_{ATP} channel facilitates K^+ influx and increases the matrix volume whereas stimulation of the K^+ efflux through the KHE, in contrast, decreases the volume (Garlid, 1980). A myriad of intra- and extra-mitochondrial factors are involved in the regulation of the balance between K^+ influx and efflux mechanisms in order to maintain matrix volume homeostasis. The molecular identities of the putative mK_{ATP} and the KHE have not been established. The $\text{mKv}1.3$ was initially discovered in mitochondria of lymphocytes (Szabo et al., 2005). Later, it was identified in the IMM of different cells, where, similar to other K^+ channels, it likely participate in regulation of Ψ_{m} , matrix volume and ion homeostasis (*Reviewed in* (Szabo and Zoratti, 2014). A precise role of $\text{mKv}1.3$ under physiological conditions is not clear, however it plays a critical role in apoptosis, since knockout of the channel prevents apoptotic cell death (Bock et al., 2002). Also, a mitochondrial small-conductance Ca^{2+} -activated K^+ channel (mSK_{Ca}) was identified in the IMM of guinea pig (Stowe et al., 2013), rat and human (Yang et al., 2017) cardiomyocytes by using both biochemical and molecular biology techniques. Pharmacologically induced opening of the mSK_{Ca} prevented mitochondrial dysfunction and protected against cardiac ischemia-reperfusion injury (Stowe et al., 2013) and glutamate-induced oxytosis (Dolga et al., 2013).

In addition to K^+ , Ca^{2+} is involved in the regulation of mitochondrial ion homeostasis and matrix swelling. Mitochondria are known to play a vital role in buffering cytosolic and mitochondrial Ca^{2+} (Hoppe, 2010; Rizzuto et al., 2012). Mitochondrial Ca^{2+} signaling regulates metabolism and function of mitochondria providing a crosstalk between changes in matrix volume and Ca^{2+} influx/efflux mechanisms. Minute augmentations of $\text{Ca}^{2+}_{\text{m}}$ may induce a tiny swelling of the matrix accompanied by upregulation of ETC activity and oxidative phosphorylation. Adversely, once $\text{Ca}^{2+}_{\text{m}}$ reaches a critical threshold it triggers a pathological swelling effect caused by mitochondrial PTP opening (Szabo and Zoratti, 2014).

The main Ca^{2+} influx mechanisms include the mitochondrial Ca^{2+} uniporter (MCU), rapid mode of Ca^{2+} uptake (RaM) and mitochondrial ryanodine receptor (mRyR). The MCU is the main Ca^{2+} uptake channel, which is heavily dependent on the Ψ_{m} . Its activity is controlled by the mitochondrial Ca^{2+} uptake 1 (MICU1) protein, an essential regulator of Ca^{2+} uptake that prevents Ca^{2+} overload in mitochondria (Patron et al., 2013). Studies with genetic ablation of the MCU revealed its important role in $\text{Ca}^{2+}_{\text{m}}$ accumulation suggesting that the Ca^{2+} -induced PTP opening occurs due to MCU activation (Pan et al., 2013). Another mechanism regulating $\text{Ca}^{2+}_{\text{m}}$ is the RaM. The optimal rate of Ca^{2+} uptake through the RaM mechanism is greater than the MCU at low Ca^{2+} and the rate declines with increasing $\text{Ca}^{2+}_{\text{m}}$ (Santo-Domingo and Demareux, 2010). Also, a Ca^{2+} influx in mitochondria is regulated by

the mRyR. The role of RyR in Ca^{2+} -induced Ca^{2+} release (CICR) within the endo(sarco)plasmic reticulum has been known for a long time, and discovery of the mRyR in 2001 (Beutner et al., 2001) opened new perspectives in the understanding of Ca^{2+} metabolism in mitochondria. Unlike the RyR, mRyR acts as a Ca^{2+} influx transporter allowing rapid sequestration of Ca^{2+} at low concentrations. The mRyR is inhibited at high Ca^{2+} concentration suggesting that it plays a physiological role in fast Ca^{2+} dynamics (Beutner et al., 2005). Since the RAM and mRyR have either a slow resetting time and/or are inhibited by high Ca^{2+} , they have no significant effects on swelling.

The mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger (mNCX), $\text{H}^+/\text{Ca}^{2+}$ exchanger (mHCX) and PTP are the main mitochondrial Ca^{2+} efflux mechanisms (Szabo and Zoratti, 2014). The mNCX is a highly selective Ca^{2+} efflux exchanger, which is regulated by Na^+ , K^+ , cytosolic Ca^{2+} , and Ψ_m . It has been extensively debated whether the mNCX plays the role of an electrogenic or electroneutral channel, however an analysis of the mNCX in cardiac mitochondria has provided an evidence that the mNCX sustains a stoichiometry of 3 Na^+ :1 Ca^{2+} (Dash and Beard, 2008). However, later the same group demonstrated that the mNCX may undergo the transition from an electrogenic to an electroneutral state in a later publication (Blomeyer et al., 2013). The mNCX has been shown to function in a reverse mode under certain conditions that include the hyperpolarization of the mitochondria (Kim and Matsuoka, 2008; Smets et al., 2004). Although a vast amount of information about the function of the mNCX is known, its molecular identity has yet to be discovered (Castaldo et al., 2009). The NHE isoform 1 (mNHE) first identified in cardiac mitochondria (Javadov et al., 2011) has been shown to play a key role in Ca^{2+} -induced mitochondrial swelling and PTP opening (Villa-Abrille et al., 2011). It regulates the mNCX activity by providing the Na^+ efflux needed to maintain the concentration gradient of ions across the IMM.

The mHCX is known for its electroneutral capability to exchange 2 H^+ (out) for 1 Ca^{2+} (in). It is activated by the Ca^{2+} gradient allowing it to pump H^+ against the proton gradient. It should be noted that the exchanger can function in a reverse mode at high Ca^{2+}_m (Tsai et al., 2014). In addition, this study identified mHCX to be insensitive to ruthenium red and have an approximate K_m of 25 μM . In recent knockout experiments, the mHCX has been identified as part of the leucine zipper-EF-hand-containing transmembrane protein 1 (LETM1) family, though the molecular identity of the transporter remains unknown (Jiang et al., 2009). When compared to the mNCX, it was found that the LETM1 (mHCX) did not play a significant role in extrusion of Ca^{2+} , thus undermining its function in Ca^{2+} efflux (De Marchi et al., 2014). In addition, genetic studies (Graier et al., 2008; Trenker et al., 2007) demonstrated that uncoupling proteins (UCPs) 2 and 3 are important for mitochondrial Ca^{2+} uptake. However, mechanisms underlying the Ca^{2+} regulatory role of the UCPs remain to be elucidated. In addition to ion channels and transporters, aquaporins (AQP), the membrane proteins that transfer water across the membrane, can also contribute to mitochondrial swelling. The AQP8 found in the IMM was suggested to play an important role for rapid expansions of matrix volume and thus, regulate the activity of ETC complexes (complex IV) and oxidative phosphorylation (F_0F_1 -ATP-synthase) (Calamita et al., 2005). However, it remains unclear whether AQP8 is present in all mitochondria and whether it, in addition to regulation of osmosis in the mitochondrial matrix, participates in the transport of other molecules across the IMM (Lee and Thevenod, 2006).

2.1.2. Role of transient PTP opening—Growing body of studies demonstrate that the matrix volume can also be regulated through a low-conductance PTP opening, which makes the IMM permeable to solutes, mostly ions, with molecular weight less than 300 Da. PTP flickering in a low-conductance mode, induces negligible matrix swelling (Ichas and Mazat, 1998). Inhibition of cyclophilin D (CypD), a major PTP inhibitor, prevented the Ca^{2+} -induced swelling in liver mitochondria (Davidson and Halestrap, 1990) presumably due to normalization of Ca^{2+} levels in the matrix (Altschuld et al., 1992; Bernardi and Petronilli, 1996). Mice lacking CypD exhibited elevated levels of matrix Ca^{2+} presumably due to reduced physiological opening of the PTP (Elrod et al., 2010). Repetitive gating of the PTP can induce fluctuations in Ψ_m and the fast release of accumulated Ca^{2+} from the matrix thus serving as an emergency mechanism to regulate Ca^{2+} homeostasis and function of mitochondria (Huser and Blatter, 1999). In addition, mitochondria are excitable organelles that generate and convey electrical and Ca^{2+} signals, hence a low-conductance PTP opening can initiate mitochondrial depolarization spikes and generate Ca^{2+} waves through CICR from one mitochondrion to another (Ichas et al., 1997). Physiological role of PTP opening has been reviewed elsewhere (Brenner and Moulin, 2012; Javadov et al., 2017).

Thus, under physiological condition, the mitochondrial volume is regulated by ion fluxes through the IMM, mainly by the K^+ and Ca^{2+} fluxes. Mild increases in matrix volume over the physiological range have been shown to stimulate mitochondrial function and metabolism. Particularly, an increase in matrix volume stimulated fatty acid oxidation associated with increased ETC activity and oxidative phosphorylation in rat liver mitochondria (Halestrap and Dunlop, 1986). Furthermore, increased mitochondrial volume may stimulate gluconeogenesis through stimulation of pyruvate carboxylase (Owen and Halestrap, 1993). We found that ischemic preconditioning, when the heart is exposed to brief periods (3–5 min) of ischemia followed by reperfusion, increased the matrix volume of cardiac mitochondria and stimulated their respiratory function (Lim et al., 2002). This study suggests that increased matrix volume may represent an important adaptive response to protect the mitochondria against severe oxidative stress. The molecular mechanisms underlying the beneficial effects of mild mitochondrial swelling on mitochondrial metabolism remain unknown. An increase in matrix volume can expand the IMM and thus, affect the fluidity of the membrane and stimulate ETC activity (Halestrap, 1989). In favor of this, previous studies demonstrated the dependence of the ETC activity on changes in the IMM fluidity (Armston et al., 1982; Okayasu et al., 1985). In addition, volume-induced changes in the IMM fluidity can affect the activity of ion channels and other transporters in the inner membrane and thus, regulate mitochondrial function and metabolism.

2.1.3. Mitochondrial flash activity: potential target for matrix volume changes

—In addition to mitochondrial dynamics, changes in mitochondrial volume can affect the mitochondrial flash activity. The mitochondrial flashes reflect integrated snapshots that occur as transient and stochastic events in individual mitochondrion. They involve transient concurrent changes in superoxide generation, pH, and Ψ_m , and associated with the ETC activity and transient PTP opening (Wang et al., 2008). Mitochondrial flashes integrate the signals of ROS, pH, Ψ_m , and Ca^{2+} in individual mitochondrion, and thus, serve as a biomarker for mitochondrial activity under physiological and pathological conditions

(*Reviewed in* (Wang et al., 2016). Interestingly, transient PTP openings play a causative role in triggering mitochondrial flashes since inhibition of CyP-D prevented mitochondrial flashes in cardiomyocytes (Gong et al., 2015; Shang et al., 2016). Since PTP opening (transient or permanent) affect the matrix volume, we can suggest a potential link between volume changes and flash activity of individual mitochondria. Interestingly, most recent studies demonstrated L-OPA1 regulates mitochondrial flash biogenesis by regulation of pH/ Ψ_m coupling independently from membrane fusion (Rosselin et al., 2017). The authors proposed that OPA1 stabilizes structure of ETC supercomplexes which, in turn, enables mitochondria compensate a drop in Ψ_m by a matrix pH flash. In addition, PTP opening plays a causal role in disassembling of ETC supercomplexes in response to oxidative stress (Jang et al., 2016). Further studies are required for clarification of the cause-effect relationship between volume changes, cristae remodeling and flash activity in mitochondria.

2.1.4. Cristae remodeling and mitochondrial function—It should be noted, that there is a crosstalk between structural integrity of the IMM, particularly cristae ultrastructure, and functional activity of the membrane (*Reviewed in* (Mannella, 2006; Quintana-Cabrera et al., 2017; Rampelt et al., 2017). Cristae are the IMM invaginations (curvature) connected through tubular and narrow junctions, known as cristae junctions, to the boundary membrane (Frey and Mannella, 2000). Increases in matrix volume can affect dimensions (mainly, width) of the cristae junction and cristae lumen, and thus, stimulate: 1) functional association between ETC complexes (supercomplexes), and oxidative phosphorylation (Cogliati et al., 2013), 2) functional activity of membrane transporters, including ion exchangers and channels, and thereby, providing a regulatory crosstalk between ion influx and matrix swelling (Garlid, 1996; O'Rourke et al., 2005), 3) structural organization of cardiolipin, the main phospholipid which is localized in the inner leaflet of the IMM and plays a crucial role in maintaining of membrane proteins (Paradies et al., 2014), 4) release of cytochrome c, which can trigger apoptosis under pathological conditions (Scorrano et al., 2002), and 5) diffusion of proteins/metabolites across the IMM to the intermembrane space (IMS) (Frezza et al., 2006). Notably, cristae remodeling can affect the IMM architecture and dynamics without significant changes in mitochondrial volume (Rampelt et al., 2017). In addition to the matrix, physical interactions with the OMM apparently play a major role in maintenance of the IMM shape.

Topology of the IMM is also regulated by “mitochondria-shaping” proteins known as fission and fusion proteins (*Reviewed in* (Liesa et al., 2009), and changes in matrix volume can induce cristae remodeling through changes in mitochondrial dynamics. From all fission and fusion proteins only optic atrophy 1 (OPA1) is bound to the IMM and plays an essential role in fusion (Cipolat et al., 2004). Genetic ablation of OPA1 induces cristae remodeling and apoptosis independently on the fusion (Frezza et al., 2006). Conversely, overexpression of OPA1 prevented cytochrome c release and kept the cristae junctions tight during apoptosis suggesting a distinct function of the OPA1 in cristae remodeling (Frezza et al., 2006). OPA1 has been shown to regulate mitochondrial function, and assembly and stability of ETC supercomplexes through cristae remodeling (Cogliati et al., 2013). Overall, these studies provide evidence on the existence of tight relationship between mitochondrial function/ metabolism and cristae shape, which can be modulated by volume changes.

2.2. Pathological swelling of mitochondria: key event that leads to cell death

Matrix swelling develops due to the formation of an osmotic pressure that transports water into the matrix causing morphologic and functional changes in mitochondria. Osmotic pressure across the IMM is generated by changes in the concentration of solutes, mainly ions, which increase the osmotic pressure on the IMM (Nowikovsky et al., 2009). Since the IMM is highly selective with low permeability to ions, changes in ionic concentration may occur by the following 3 ways to induce mitochondrial swelling: 1) high concentration of ions in the cytoplasm, 2) dysfunction of ion transport associated with increased rate of ion influx and decreased rate of ion efflux across the IMM, and 3) an increase in permeability of the IMM. Changes in mitochondrial volume occurs in response to oxidative and energy stresses in the cell. Under these conditions, an increase in cytosolic Ca^{2+} and decrease in pH are the main factors that stimulate mitochondrial Ca^{2+} buffering activity (*Reviewed in* Bernardi and Di Lisa, 2015; Halestrap et al., 2004). Considering that the PTP should not open under a low pH_m (Bernardi et al., 1992), Ca^{2+}_m may play an important role in inducing an osmotic drive towards the matrix resulting in mitochondrial swelling. Alterations in ion transport mechanisms in mitochondria have been known to play a crucial role in matrix volume regulation. Wolf-Hirschhorn syndrome is an ideal example of a dysfunctional mitochondrial transporter leading to changes in matrix volume (Nowikovsky et al., 2009). The disease induced by a mutation in the LETM1 gene, which codes the mitochondrial KHE, is associated with swelling of mitochondria due to the retention of higher K^+ levels. In addition, increased IMM permeability was associated with mitochondrial dysfunction as evidenced by Ψ_m loss and ATP depletion (Nowikovsky et al., 2009).

A critical event that initiates mitochondria-mediated cell death is the excessive swelling of the mitochondrial matrix that occurs due to irreversible (high-conductance) PTP opening. PTP-induced cell death is involved in the pathogenesis of ischemia/reperfusion injury of different organs especially, heart and brain (Bernardi and Di Lisa, 2015; Halestrap et al., 2004; Kristian and Siesjo, 1998). During sustained opening of the PTP mitochondria become uncoupled and F_0F_1 -ATP synthase (ETC complex V) works in a reverse mode that instead of producing ATP, hydrolyzes it. PTP opening allows entrance of water, ions and other solutes with a molecular weight less than 1500 Da into the matrix and thus, alters ion homeostasis in the mitochondrial matrix due to equilibration of the solutes on both sides of the IMM. However, mitochondrial proteins retain in the matrix that, together with high ion levels, increase the colloidal osmotic pressure leading to matrix swelling. Since the surface area of the IMM is significantly larger than the outer mitochondrial membrane (OMM), matrix swelling induces the rupture of the OMM in the presence of a relatively intact IMM. The rupture of the OMM facilitates the release of pro-apoptotic proteins such as cytochrome c, Smac/Diablo, apoptosis inducing factor, endonuclease G from the IMS to the cytosol thus initiating apoptotic death pathways. Under severe stress when cells have insufficient ATP to complete the apoptotic process, cell death occurs through necrosis.

Thus, excessive mitochondrial swelling plays a causative role and therefore, it is important to provide mathematical modeling and kinetic analysis of mechanisms underlying the transition from reversible swelling to irreversible swelling. Proposed steps and consequences of mitochondrial swelling are illustrated in Figure 2. Understanding these mechanisms can

be helpful for the development of new potential therapeutic approaches to target mitochondrial swelling and prevent cell death.

3. Methods for measurement of mitochondrial volume

Elucidating of molecular mechanisms of mitochondrial structure and metabolism under physiological and pathological conditions requires an accurate estimate of the mitochondria, particularly, matrix volume. Unfortunately, methods for measurement of mitochondrial volume *in situ* (in intact organs or tissue) are not available. The main method widely used for estimation of the mitochondrial volume is based on the measurement of absorbance (optical depth) in isolated mitochondria. The amount of light scattered by mitochondria depends on the matrix volume, where a decrease of absorbance is proportional to an increase of mitochondrial volume (Tedeschi and Harris, 1958). Although the term of “absorption” is broadly used in mitochondria studies, actual light absorption capacity of mitochondria is unknown. Instead of that, a light scattering effect that creates a decrease of sample light transmission spectrum is determined. Light scattering induced by matrix swelling decreases the light transmission spectrum. In other words, matrix swelling changes the refraction coefficient, decreases the scattering efficiency and increases the light transmission properties of the mitochondria. Since pathological swelling of mitochondria leads to PTP opening and Ca^{2+} release, measurement of the CICR can reflect the extent of swelling (Selivanov et al., 1998). However, both techniques have several disadvantages. For example, these techniques can be used only on isolated mitochondria, which do not reflect *in vivo/in situ* behavior of mitochondria in cell/tissue. In addition, isolation procedure *per se* that includes several steps of centrifugation in sucrose medium can affect morphology and shape of mitochondria leading to misestimation of real volume changes.

An accurate method was developed for measurement of the mitochondrial volume in cultured liver cells without cell disruption using $^3\text{H}_2\text{O}$ and [^{14}C]-mannitol (Quinlan et al., 1983). In this approach, mannitol was used as an extramitochondrial marker and matrix volume was determined *in situ* based on the H_2O to mannitol ratio. Importantly, values of mitochondrial volumes measured using $^3\text{H}_2\text{O}$ and [^{14}C]-mannitol in intact cells (Quinlan et al., 1983) were similar to those found in isolated mitochondria (Halestrap and Quinlan, 1983) (0.40 and 0.46 $\mu\text{l}/\text{mg}$ of mitochondrial protein, respectively). A similar technique based on the intra- and extramitochondrial distribution of $^3\text{H}_2\text{O}$ and [^{14}C]-sucrose was used for measurement of the matrix volume changes in isolated mitochondria (Lim et al., 2002). This method is more precise than the light scattering technique. In favor of this, conformational changes in the ANT induced by inhibitors (e.g. carboxyatractyloside and bongkreic acid) cause distinct changes in absorbance and affect light scattering of mitochondria without any changes in matrix volume (Das et al., 2003; Klingenberg et al., 1971).

Microscopy techniques are used for the measurement of the mitochondrial volume in intact cells (fluorescent/confocal microscopy) and fixed cell/tissue samples (electron microscopy). However, these techniques also have a number of limitations in estimation of actual volume of mitochondria. For example, main disadvantages of confocal/light microscopy include low resolution, negative effect of light intensity (laser power, filters) on image quality, and

diffraction limits. The mitochondrial uptake of the majority of fluorescent probes and potentiometric dyes depends on the Ψ_m and thus, can affect actual fluorescent imaging of mitochondria. This problem has been partially solved by using a variety of fluorescent proteins such as the green fluorescent protein (GFP), for *in vivo* imaging and estimation, among other parameters, the size/number of mitochondria (Chudakov et al., 2005; Rizzuto et al., 1995). Furthermore, to overcome the diffraction limits in optical microscopy, new fluorescence microscopies with super-resolution that allow imaging on a nanometer levels have been recently developed (Huang et al., 2009; Patterson et al., 2010). The artifacts due to sample preparation (fixation) and the study of non-functional cells are the drawbacks for electron microscopy. New electron microscopy technologies significantly improved quality of imaging and provide more detailed information about structure/volume changes of mitochondria. High-resolution scanning electron microscopy and electron tomography are able to provide 3D imaging of mitochondria where changes in morphology can be seen at molecular levels (Mannella, 2006; Perkins et al., 1997). In addition, cryo-electron microscopy and cryo-electron tomography are used to examine the interior architecture of mitochondria including structural composition of multiprotein complexes, cristae structure, shape modeling and other structural and molecular features at very high (nanoscale) resolution (Bartesaghi and Subramaniam, 2009; Letts et al., 2016). Also, the artefacts induced by sample processing such as chemical fixation, dehydration, and staining are excluded in these techniques.

Thus, several methods are available for quantitative analysis of volume changes in mitochondria *in vitro*. Lack of techniques for in situ measurement of the mitochondrial volume is the main obstacle in this area. Although electron microscopy is highly effective for the elucidation of ultrastructural and molecular changes, it cannot provide information of actual changes of the mitochondrial volume. If we assume that the mitochondrion has a sphere shape, a 25% increase in matrix volume would produce only a 3% increase in diameter of mitochondria based on cubic relationship between the volume and diameter. Also, changes in the IMS, and cristae remodeling may increase mitochondria diameter without changes in matrix volume (Frey et al., 2002). Nevertheless, the microscopy techniques alone or in combination with other methods provide information on the changes in the shape, cristae morphology and matrix volume of mitochondria under both physiological and pathological conditions.

4. Modeling of mitochondria swelling dynamics

4.1. General view of different modeling approaches

The development of an adequate modeling analysis for biological systems is essential for understanding and predicting a system behavior under different conditions. Modeling of the mitochondrial swelling process is based on the following two conceptually different types of approaches. The first approach develops the biological dynamics and fitting (kinetics) models, which consist of modeling kinetic schemes of the mitochondrial swelling process and approximating the simulation to experimental results. This includes the use of the law of mass action and dividing the mitochondria into un-swollen, semi-swollen, and fully swollen fractions while averaging their volume. Typically, the volume obtained by averaging the

mitochondrial swelling fractions are converted to absorbance values to fit experimental data. The second approach is applied to develop the biophysical model, which takes into consideration the flux of one or more ions, changes in Ψ_m , mitochondrial respiration rates, and hydrodynamic flux across the IMM. This approach can be further divided into 2 groups: a) individual approach, which includes the detailed development of one transport mechanism, and b) holistic approach, which develops modeling based on two or more transport mechanisms. Main mechanisms involved in mitochondrial swelling are shown in Figure 3.

4.2. Biological dynamics and fitting models

Biological dynamics and fitting models simplify the analysis of swelling dynamics by ignoring the details of the biophysical and biochemical processes in living systems including mitochondria. Initially, a relationship between light scattering and matrix volume had to be developed before such fitting models could be created. The preliminary attempts to describe this relation were based on a linear relationship between absorbance and mitochondrial volume. However, the linear equation was only capable of describing the end portion of the nonlinear absorbance change that the data produced (Beavis et al., 1985; Tedeschi and Harris, 1955). The equation was further improved with the addition of a logarithmic relationship between the absorbance and volume change which was better capable of imitating the experimental absorption data (Massari, 1996). This kinetic model of mitochondrial swelling described the effects of different concentrations of PTP-inducers on the diameter and number of the pores. The approach included a first order differential equation that described reversible Ca^{2+} -induced permeabilization including the transition of non-swollen mitochondria to swollen mitochondria and *vice versa* as described before (Al-Nasser and Crompton, 1986). These populations were typically represented as a mitochondrial fraction that refers to the portion of the mitochondrial population found at a given transitional state such as un-swollen, swelling, or final swollen state. The mitochondrial fraction is assigned a given volume and the average of the total volumes assigned to the mitochondrial fractions would then be calculated. In the following study, the average volume of the total mitochondrial population only took into consideration the transition from the un-swollen to final swollen state. A logarithmic relationship between matrix volume and absorbance changes was developed to allow the model to be fitted to experimental data (Massari, 1996). The model was utilized to describe both the swelling pattern and complete swelling effect on the mitochondrial population provoked by Ca^{2+} and phenylarsine oxide (a PTP inducer). The model was capable of fitting the tail or ending portion of the experimental absorbance data, though it failed to replicate the initial portion. The authors stated that a linear kinetics could be expected with a stable membrane potential and in the absence of Ca^{2+} net movement suggesting that the model could be further improved. Increasing the complexity of the model to a second order relation could improve the fitting quality of the model.

Baranov et al. (Baranov et al., 2008) proposed a model, which was able to properly simulate the PTP-induced swelling process. The authors attempted to capture the Ca^{2+} autolytic activity from its reuptake upon PTP-induced Ca^{2+} release. In comparison with previous studies (Massari, 1996), the model was significantly improved by the addition of a lag phase.

The latter allowed the model to be fitted to the swelling effects induced by Ca^{2+} at lower concentrations (1–20 μM). Additionally, it proved to be more practical because of its capability to distinguish the different mechanisms by which Mg^{2+} and Ru-360 inhibited Ca^{2+} uptake. However, the model did not take into account the changes in Ψ_m , that can alter Ca^{2+} uptake rates and additional ion transport mechanisms and thus, modulate the swelling process. The authors described mitochondrial Ca^{2+} uptake by a set of processes including a modified kinetic rate, which regulates the Ca^{2+} uptake dynamics. However, all kinetic processes included in the study were considered as irreversible. This limitation allows for the production of a simulation with acceptable accuracy for only the middle part of the experimental swelling curves or the portion of the absorbance data where swelling occurs. However, it does not precisely fit the “tail” of the swelling dynamics (Baranov et al., 2008). In addition, although parameter variations were tested and discussed for inducers and inhibitors of Ca^{2+} uptake, the effects of Ca^{2+} -induced mitochondrial swelling at higher concentration ($> 20 \mu\text{M}$) were not studied in the model.

Ca^{2+} induced mitochondrial swelling was taken into account in the model developed by Eisenhofer et al. (Eisenhofer et al., 2010). The approach developed in this model is an extension of the aforementioned kinetic model (Massari, 1996). The mitochondrial fraction differential equation was extended from a first to second order differential equation including additional parameters. The parameters included a positive feedback within the swelling process (a), background swelling coefficient (b), a mean mitochondrial volume (k), and an average swelling time for a single mitochondrion to swell (τ). This equation was then applied to a secondary equation, which would calculate the average volume at a certain time point of the swelling process. At this time point, all mitochondria are divided into 3 states: un-swollen, swelling, and swollen states. This model introduced a set of variable-parameters that were adapted to changes in Ca^{2+} levels, thus allowing the prediction of the dynamics of swelling. The relationship between the averaged mitochondrial volume and time of swelling was determined by introducing the τ (Eisenhofer et al., 2010). This approach can be successively applied to fit experimental data of mitochondrial swelling, since it reveals a positive feedback behavior of mitochondrial swelling, and allows for analyses of the effects of swelling inducers and inhibitors. However, the model does not have clear biophysical basis as well as it does not include Ca^{2+} uptake in mitochondria (Eisenhofer et al., 2010). Therefore, the proposed approach can be considered as an abstract mathematical approach of the description of swelling process in mitochondria.

In conclusion, the biological dynamics and fitting models are based on a simpler approach that can be easily adopted to other systems/cells. Although the approach is limited due to a lack of consideration of the many factors that may affect swelling, it is promising in providing information about kinetic rates, pharmacological inhibition, and volume changes of swelling mitochondria. An extension of this type of modeling, including additional swelling factors, could better simulate in vivo mitochondrial swelling. However, it would vastly increase the complexity of the model.

4.3. Biophysical model

A biophysical model of the mitochondrial swelling process typically takes into account several processes in mitochondria such as mitochondrial respiration, ion transport, and membrane depolarization among others. Modeling of each of these processes creates sets of nonlinear differential equations, a solution of which is dependent on variable parameters related to the systems original and boundary conditions (e.g. Ca^{2+}_m , pH_m , Ψ_m).

4.3.1. Individual approach—In contrast to the holistic approaches that evaluates the interaction between different mechanisms, individual approaches are applied to describe the contribution of an individual transporter to the ionic homeostasis and thus, mitochondrial swelling. Additionally, this approach can describe the characteristics of transporters under different conditions.

A kinetic model of the Ca^{2+} transport across the IMM was reported by Dash et al. (Dash et al., 2009). The authors took into account the MCU-mediated Ca^{2+} uptake mechanism in respiring mitochondria. The model is based on a multi-state catalytic binding and Eyring's free-energy barrier including a transporter T , which forms a complex with Ca^{2+} denoted as $[Ca^{2+}..T]$. The process of complex formation is reversible in the cytoplasm, but equilibrium is strongly shifted to the complex formation. The transporter T is located in the uniporter system, and the authors proposed reversible processes of Ca^{2+} - T complex formation on the outer surface of the IMM as well as dissociation and formation of such complex on the inner surface of the IMM. The model also introduced the process of complex transformation of Ca^{2+} from the outer surface of IMM to its inner surface. Such modeling approach for Ca^{2+} transport by IMM correlated well with the ion transport process across the IMM. The model includes two conformational changes of the MCU binding site that are dependent on Ψ_m , a regulator of the Ca^{2+} uptake. The model showed that the charge distribution of the MCU does not linearly decrease with Ca^{2+} uptake. In other words, the Ca^{2+} translocation from the IMS to the matrix by the uniporter is not a linear process.

A similar modeling approach was used that included a set of equations to clarify the contributions of Ψ_m , P_i , and Mg^{2+} to the MCU-mediated Ca^{2+} uptake (Pradhan et al., 2010b, 2011). The model described the possible mechanisms of the inhibitory effect of Mg^{2+} on Ca^{2+} uptake. Based on the experimental data, it simulated a dual inhibitory role of Mg^{2+} which acts as a competitive and non-competitive inhibitor. Three model types based on 3 different Ca^{2+} and Mg^{2+} binding assumptions were described to acquire the data fitting. It might be interesting to develop an extended model that could describe the role of electrogenic transfer of Ca^{2+} in volume dynamics.

A biophysical model for the kinetics of mNCE was developed for understanding of mitochondrial Ca^{2+} homeostasis (Pradhan et al., 2010a). The model is thermodynamically balanced and adequately describes a number of independent experimental data sets under a variety of experimental conditions. It takes into consideration a multistate catalytic binding mechanisms for carrier-mediated facilitated transport and Eyring's free energy barrier theory. The model is able to predict the effects of Ψ_m and pH on the electrogenic exchange activity of mNCE.

Bazil and Dash (Bazil and Dash, 2011) continued modeling of individual IMM transporters such as the RaM, where a 4 state (i.e. the transporter could assume 4 conformational states for Ca^{2+} uptake) approach of the transporter was created and analyzed to experimental data from the liver and the heart. The model analysis identified functional differences between both tissues such as RaM in cardiac mitochondria take longer to recover (90 sec) than liver mitochondrial RaM (1 sec). The model was based on a set of ordinary and algebraic differential equations and the experimental data was fitted utilizing the least squared method. Interestingly, despite the simplicity of the model, the data produced from the model highly correlated with experimental data.

In conclusion, the individual approach provides great information about transport mechanisms such as elucidating their functional role and acquiring a deeper understanding of the inhibitory mechanisms displayed in these transporters. It plays an important role in explaining contradictory experimental data that may come from underlying characteristics of the transporter. An integration of the models developed via this approach should provide a holistic model that could probably describe mitochondrial ion dynamics.

4.3.2. Holistic approach—Kinetic models based on holistic approaches take into account 2 and more factors (processes) that can contribute to swelling. A kinetic model developed by Selivanov et al. (Selivanov et al., 1998) for the analysis of Ca^{2+}_m dynamics is based on the application of the Goldman equation to explain Ca^{2+} oscillations and Ca^{2+} sparks generated by the CICR mechanism in mitochondria associated with PTP flickering at a low conductance mode. The Goldman equation was used to describe various ion fluxes across the IMM by taking into consideration four mechanisms including the MCU, PTP, ETC, and a weak acid transporter. In this study, the contributions of the mNCCX and mHCX were ignored because of the followings: a) Na^+ was excluded from the media in experiments that would reduce the mNCCX activity, and b) the role of mHCX in Ca^{2+} transport was found to be negligible (Ichas et al., 1997; Ichas et al., 1994). The model proposed that Ca^{2+} *per se* does not trigger PTP opening during mitochondrial CICR, however, if the concentration of Ca^{2+} in the matrix is sufficient, PTP opening is observed due to changes in matrix pH (pH_m) or Ψ_m (Selivanov et al., 1998). Ca^{2+} uptake decreases Ψ_m and increases pH_m , i.e. these two factors can be considered as triggers of PTP opening. It should be noted, that changes in pH_m *per se* have a substantial effect on the buffering capacity of Ca^{2+} within the matrix through modulation of the activity of NHX and HCX (Santo-Domingo and Demareux, 2010). In addition, pH_m can affect mitochondrial Ca^{2+} influx/efflux mechanisms (proteins) and thus, change the Ca^{2+} sequestering capacity of mitochondria. For example, the pH dependence of mitochondrial Ca^{2+} uptake was shown in Jurkat cells (Zabłocki et al., 2005) suggesting a potential role of H^+ in the regulation of mitochondrial Ca^{2+} buffering capacity. Further studies are required to understand the role of pH_m in mitochondrial Ca^{2+} buffering as well as establish a cause-effect relationship between changes in the Ca^{2+} sequestering capacity, Ψ_m and pH_m . Based on experimental data, it was concluded that Ψ_m does not trigger PTP opening, and pH_m was included in the model as the trigger of PTP opening (pH > 7.0 opens whereas pH < 7.0 closes the PTP). Numerical analysis of the model demonstrated that time oscillations of external Ca^{2+} , pH, and Ψ_m are highly correlated with experimental data reported by others (Ichas et al., 1997; Ichas et al., 1994).

Pokhilko et al. (Pokhilko et al., 2006) developed a mathematical model of mitochondrial ion homeostasis. The model proposed three modes of Ca^{2+} transport and was applied to experimental data for the analysis of different modes of Ca^{2+} uptake by mitochondria. Modeling analysis demonstrated that the kinetics of Ca^{2+} uptake is regulated by the total amount of Ca^{2+} in the system, and the Ca^{2+} handling can be defined in three conditions: (1) Low total Ca^{2+} levels ($< 140 \text{ nmol Ca}^{2+}/\text{mg protein}$), where all available Ca^{2+} is accumulated in the matrix without activation of the PTP. (2) Intermediate total Ca^{2+} levels ($140\text{--}160 \text{ nmol Ca}^{2+}/\text{mg protein}$), where the accumulation of Ca^{2+} generates periodic opening and closure of the PTP and oscillations of ion fluxes. (3) High total Ca^{2+} levels ($> 160 \text{ nmol Ca}^{2+}/\text{mg protein}$), PTP is permanently opened, the effect of membrane depolarization is observable and small ions are moved out from the matrix. The model included the correlation of Ψ_m with ion transport across the IMM based on the Nernst equation. The equation is applied to quasistationary conditions, i.e. equilibrium in Ca^{2+} levels between cytoplasm and mitochondria creating a much faster transport mechanism.

Data simulation at low and intermediate levels of Ca^{2+} provided good modeling of the matrix volume dynamics (Pokhilko et al., 2006). The model describes the PTP opening and transport of K^+ , H^+ , Ca^{2+} and anions. It displayed Ca^{2+} retention, oscillation and release at low, intermediate, and high Ca^{2+} levels, respectively. Increasing Ca^{2+} uptake rates induced faster PTP opening and the simulation suggests that the mitochondria can accumulate up to $140 \text{ nmol of Ca}^{2+}/\text{mg of protein}$ without PTP opening. However, the volume simulated by high Ca^{2+} failed to correspond with experimental data when mitochondrial volume decreased with the opening of high-conductance PTPs. Additionally, the model demonstrated an increase of pH_m by 0.3 units at high total $[\text{Ca}^{2+}]$ in the matrix. This intuitively would not make sense considering that the matrix content could be diluted by cytoplasmic medium ($\text{pH } 7$) upon PTP opening. Although the model does not completely simulate the experimental data, it introduced a wide variety of parameters such as ion changes, pH_m , Ψ_m , and volume fluxes that were not considered in other models at the time.

Bazil et al (Bazil et al., 2010b) developed a model using multiple non-linear differential equations and differential algebraic equations (DAE). The model included several factors including ion fluxes (K^+ cycle, mNCX), the TCA cycle, ETC, and oxidative phosphorylation, and the modeling process was represented by reversible and thermodynamically balanced reactions. The authors took into account the effect of mitochondrial swelling based on mitochondrial bioenergetics, which highly correlated with experimental data. However, the developed model was mostly directed to the analysis of mitochondrial bioenergetics, and swelling was considered as a small perturbation. The swelling portion of the model was simplified by focusing on the K^+ cycle excluding the PTP as well as detailed mechanisms of Ca^{2+} fluxes such as RaM , mRyR and mBK_{Ca} channels that are involved in the mitochondrial swelling dynamics. In a study conducted soon after, an improved version of the model was presented, including a PTP induced swelling mechanism and 3 states (subpopulations) of mitochondria: the aggregated, the orthodox, and post-transition state (Bazil et al., 2010a). The model displays the aggregated state as the functional mitochondria before PTP opening. Once the conditions favoring PTP opening are met, an irreversible transition from the aggregated to the orthodox state occurs that includes

the collapse of the Ψ_m and matrix swelling. Additionally, the model includes PTP closure when the mitochondria transfer from the orthodox to the post-transition state. This is a recovery state where mitochondria attempt to reestablish their Ψ_m and ionic concentration after the loss of matrix contents. The model provides valuable information about PTP opening rate (with and without Mg^{2+} inhibition), the mitochondrial swelling process induced by the PTP, and Ca^{2+} retention capacity. Overall, the model predicted that increasing the KHE may prevent PTP-induced swelling and its devastating consequences and suggested an alternative approach for targeting the PTP induction.

Recent studies provided more detailed mathematical analysis of Ca^{2+}_m dynamics to describe Ca^{2+} influx and efflux mechanisms in mitochondria (Bazil et al., 2013; Tewari et al., 2014). Individual roles of MCU as the Ca^{2+} buffering pathway and the putative mitochondrial RyR as the bioenergetics pathway were taken into consideration to distinguish two modes of Ca^{2+} uptake (Tewari et al., 2014). Although these models provide substantial information on the mechanisms of ion dynamics, their extension is necessary for the precise description of Ca^{2+} fluxes, mitochondrial swelling and PTP opening dynamics. In 2013, an interesting model was developed to elucidate the dynamics of the MCU and mNCX based on the Ca^{2+} buffering capacity in and out of the matrix (Blomeyer et al., 2013). Usually, Ca^{2+} dynamics and mitochondrial buffering capacity is characterized as the rise of matrix Ca^{2+} in an equal proportion to the decrease of external Ca^{2+} . The model revealed the existence of a non-reciprocal relationship between the matrix and external Ca^{2+} . The differences in the matrix Ca^{2+} and external Ca^{2+} dynamics is explained by the Ca^{2+} buffering system in the matrix. Ca^{2+} -phosphate precipitation was discussed as a mechanism of matrix Ca^{2+} buffering, where environmental conditions such as pH_i could drastically mediate the effectiveness of the mitochondrial buffering capacity due to a shift in Ca^{2+} -phosphate precipitation capacity. Additionally, the authors observed a Na-dependent relationship between external Ca^{2+} and activation of the mNCX (Blomeyer et al., 2013). This study provided experimental data that supported modeling analysis on the dynamic Ca^{2+} buffering power of the matrix (Bazil et al., 2013) although the study did not take into consideration the role of PTP opening in the maintenance of the Ca^{2+} buffering capacity in mitochondria.

A mathematical model that described mitochondrial energetics, pH_m regulation, and ion homeostasis was developed to assess the cross talk between excitation-contraction coupling and ATP synthesis (Wei et al., 2011). The model elucidated the dependence of mitochondrial respiration and Ψ_m on the ion homeostasis. It simulated oxygen consumption and ATP synthase activity under different conditions such as hypoxia and uncoupling. The model described the changes in Na^+ , P_i and H^+ , but did not include mitochondrial volume changes. Further studies are necessary for the creation of a mitochondrial swelling model that could examine mitochondrial dysfunction in a large scale including both physiological and pathophysiological conditions. The model did not take into consideration K^+ transport that was shown to affect both mitochondrial respiration and swelling.

Interaction between secondary ions (e.g. Mg^{2+}) and transport mechanisms can be mediated through allosteric regulation of the transport mechanisms for primary ions such as Ca^{2+} . In a recent study, an analysis of the effects of Mg^{2+} on mitochondrial Ca^{2+} buffering capacity was conducted by elucidating its effects on Ca^{2+} influx/efflux mechanisms (Blomeyer et al.,

2016). A fluorescent labelling technique was applied to evaluate Mg^{2+} sensitive Ca^{2+} uptake and the effects on Ca^{2+} buffering capacity. It must be noted that all Ca^{2+} influx and efflux mechanisms were assumed to occur through the MCU and mNCX, respectively. As discussed previously (Dash et al., 2009), the MCU can function under two distinct modes including the slow and fast Ca^{2+} uptakes. The study found that Mg^{2+} reduced Ca^{2+} influx by the MCU in a slow Ca^{2+} uptake mode, and Ca^{2+} efflux by the mNCX. Interestingly, Mg^{2+} had no effect on the MCU-mediated Ca^{2+} influx at the fast Ca^{2+} uptake mode. Additionally, Ca^{2+} buffering capacity of mitochondria was compromised with the allosteric effect of Mg^{2+} on the MCU (slow Ca^{2+} uptake mode) and mNCX. Apparently, allosteric regulation of the ion channels/transporters should be taken into consideration for a detailed modeling analysis of ion transport mechanisms in the mitochondria. It would be interesting to extend the analysis to include mitochondrial K^+ uptake on Ca^{2+} buffering capacity.

As mentioned above (section 2.1.4), mitochondrial swelling is tightly associated with structural changes in the IMM integrity that affect both functional and metabolic activity of mitochondria. Hence, elucidation of the relationship between swelling and morphology with the progression of mitochondrial dysfunction is important for understanding the pathogenesis of aging, diabetes, cancer, neurodegenerative and cardiovascular diseases. A modified Nernst-Planck equation was applied by Song et al. (Song et al., 2013) for the analysis of the correlation between the IMM structure and function of mitochondria to produce ATP. The authors developed a theoretical model to test the effects of IMM morphology on the Ψ_m . The modeling analysis suggested that differences in proton-generated forces in the cristae and a non-cristae portion of the inner membrane can significantly change the IMM morphology. It was also shown that morphology-dependent electrical potential gradient along the outer side of the IMM controls proton concentration in cristae, and a high surface-to-volume ratio of an individual crista increases the proton motive force (Song et al., 2013). Thus, increased surface of an individual crista can stimulate ATP synthesis. This model compiles morphology of mitochondrial crista at the meso-scale with mitochondrial function. Although the modeling analysis does not take into consideration mitochondrial swelling, it can be a starting point for the development of a new model that would investigate the mechanism of interaction between mitochondrial swelling, IMM morphology and mitochondrial function in detail.

In conclusion, the holistic approach unites different mechanisms that regulate the mitochondrial volume. Although the approach is highly effective in providing insight into a multivariable system it is difficult to verify the model experimentally and develop it based on the multifactorial analysis. Furthermore, although a major part of experiments and model analyses has been done on mitochondria under physiological conditions (equilibrium state), very little emphasis has been given to the analysis of mitochondria under pathological conditions when ion flux mechanisms and PTP induction are significantly affected.

5. Alternative modeling approaches to mitochondrial swelling dynamics

As mentioned in previous sections, mitochondrial Ca^{2+} transport is a complex process, which is strongly coupled with Ψ_m and pH_m . All 3 factors (Ca^{2+} , Ψ_m and pH_m) affect the PTP induction and dynamics. Matrix swelling induced by differences in ion concentrations

between the cytoplasm and the matrix modulates the Ψ_m , IMM morphology, and pH_m due to dilution. Accordingly, the dilution process can also proportionally increase dynamics of metabolic processes in the matrix. Thus, the matrix behavior is significantly dependent on mitochondrial swelling dynamics induced by Ca^{2+} transport between the cytoplasm and matrix. In the following sections, we will focus on the elucidation of ion dynamics across the IMM.

5.1. Volume dynamics based on osmotic pressure

Under the given cytosolic environment hydraulic pressure acting on the mitochondria may be neglected and only changes in the osmotic pressure can be considered to cause water flux. The identification of an equation that relates the changes of osmotic pressure with the changes in ionic concentration across the IMM has not been established yet.

An interesting model was developed for analysis of transmembrane ion/molecule transport on the basis of ionic electro-diffusion and osmotic water flows through the plasma membrane (Mori, 2012). A system of partial differential equations describes the dynamics of ion concentration and fluid flow with boundary conditions on the membrane surface that were deformed by swelling. The model takes into account the energy conservation law, where the sum of the entropic, elastic and electrostatic free energy is dissipated through viscous, electro-diffusive and osmotic flows. The 3D theoretical model was approximated by 1D modeling analysis through the use of numerical analysis. However, it is not clear how a water cross-membrane flow factor was considered for analysis of osmotic pressure created by differences in ion concentrations between the cytosol and the external media. Accordingly, osmotic pressure used for analysis of the cell swelling for *i*-th ion can be determined as follows:

$$P_{os} \approx k_B T N \left[\frac{\Delta C_i^{in/out}}{M_i} + A_{2w} (\Delta \bar{C}^{in/out})^2 \right] \quad (1)$$

Where P_{os} is the osmotic pressure, M_i is the ion molecular/atomic mass,

$\Delta C_i^{in/out} = C_i^{in} - C_i^{out}$ and $A_{2w} = \frac{1}{(\Delta \bar{C}^{in/out})^2} \sum A_{ij} \Delta C_i^{in/out} \Delta C_j^{in/out}$ is the weight averaged second virial coefficient. Taking into account ion transport, $\Delta C_i^{in/out}(t)$ is the time-dependent parameter, i.e. osmotic pressure is a time-dependent value that induces deformation of the plasma membrane. $\Delta C_i^{in/out}(t)$. This simple analysis demonstrates that the dynamics of cell morphology is an important factor in cell life.

In conclusion, potential effects of changes in membrane structure were not taken into consideration in previous studies related to modeling analysis of the ion transport across the membrane. However, mathematical tools developed in previous studies (Bazil and Dash, 2011; Dash et al., 2009; Pradhan et al., 2010a) can be adapted to the theoretical study of the mitochondrial swelling dynamics induced by ion transport across the IMM.

5.2. Diffusion limit of non-electrically charged species uptake by the IMM

Ion flux by the IMM was already determined in the previous section, and here we will discuss the transport of non-electrically charged species (NECS) through the IMM taking into consideration the role of PTP opening. The relationship (extended form) for NECS transport across the IMM considering PTP opening is expressed as follows:

$$\vec{J}_{NECS}(\vec{r}) = -D_{NECS} \left(\vec{\nabla} \cdot C_{NECS}^{out}(\vec{r}) \right) - \frac{z_{NECS} F}{k_B T} \mu_{NECS} C_{NECS+}^{out}(\vec{r}) \left(\vec{\nabla} \cdot \psi \right) + C_{NECS}^{out}(\vec{r}) v(\vec{r}) \quad (2)$$

where $v(\vec{r})$ is the velocity (cm/s) with which the volume of cytoplasmic solution moves along the \vec{r} vector; D_{NECS} is the diffusion coefficient. IMM surface in our case is considered as a “black surface”, where the permeability coefficient of NECS uptake equal to one (assuming NECS atomic mass < 1.5 kDa). Since convection fluxes are not created in the bio-cells, the last term can be neglected. Likewise, the second term can also be neglected due to the lack of charges and thus, the last relationship can be rewritten as Fick’s law of diffusion:

$$\vec{J}_{NECS}(\vec{r}) = -D_{NECS} \left(\vec{\nabla} \cdot C_{NECS}^{out}(\vec{r}) \right) \quad (3)$$

This relationship can be presented as follows:

$$\left| \vec{J}_{Ca^{2+}}(r=R_{IMM}) \right| = D_{NECS} \frac{C_{NECS}^{out}(r \rightarrow \infty)}{\delta_0} \quad (4)$$

where δ_0 is an unknown parameter, which is dependent of NECS mobility coefficients in the cytoplasm. The last approximation is explained with the fact that the real stationary diffusion profile of the NECS concentration is presented by curve (1), while approximated profile is presented by linear line (2). The last relationship can be used to estimate NECS flux, which is passed to the matrix.

An approximate approach was proposed for estimation of the time characteristics of nonstationary system development to quasistationary conditions in the diffusional limit case.

The following equation was obtained: $\delta(t) = 2 \sqrt{D_{NECS} t}$ Thus, $\tau = \frac{\delta_0^2}{4D_{NECS}}$ is a time characteristics of creation of quasistationary conditions.

Thus, diffusion of the NECS across the IMM during PTP opening can be considered for analysis of the osmotic driving force created by transport. Since most models take into consideration only the water flux through the movement of ionic species, this could provide insight into the water flux provided by other solutes such as glucose.

5.3. Application of thermodynamics

Many approaches developed earlier for analysis of the transport dynamics by IMM are based on the application of thermodynamics such as the Goldman's and Nernst's. By considering

solute flux to be at equilibrium, the following may be considered: $K = \frac{k_1}{k_{-1}} = e^{-\frac{\Delta G}{k_B T}}$, the k_1 and k_{-1} were defined as the rate constants of direct influx and efflux processes, respectively. The detailed analysis demonstrates that in general case $A_1 = A_{-1}$ (A_1 , A_{-1} are pre-exponential factors of the respective k_1 and k_{-1} constants). Thus, we can write in general

case, $\frac{A_1}{A_{-1}} e^{-\frac{E_{a,1} - E_{a,-1}}{k_B T}} = e^{-\frac{z|e|\Delta\Psi_{IMM}}{k_B T}}$, where $E_{a,1}$ and $E_{a,-1}$ are activation energies direct and

inverse uptake processes. The ratio of $\frac{A_1}{A_{-1}}$ can be estimated using a typical way as the ratio

of cell volume (V_c) to mitochondrial volume (V_m). Thus, the relationship the ratio of $\frac{A_1}{A_{-1}}$ helps determine the enthalpy of the uptake process, which is defined by the activation energy difference as follows:

$$\Delta H = E_{a,1} - E_{a,-1} = k_B T \ln \left(\frac{V_m}{V_c} \right) + z |e| \Delta\Psi_{IMM} \quad (5)$$

A thermodynamic approach can be used to analyze enthalpy of the uptake process as a function of Ψ_m . On the other hand, the time characteristic equation (section 5.2.) can be applied to the analysis of the case, when the time of equilibrium creation by, for example Ca^{2+} , is much shorter than the characteristics time of Ψ_m dynamics.

Basic statements of non-equilibrium thermodynamics were developed by Prigogine (Prigogine, 1961) that were further applied to the analysis of different non-equilibrium physical, chemical and biophysical/biochemical processes. We already discussed the simplest reversible process of such transport that can be further elaborated for Ca^{2+} transport. For equilibrium conditions, the equilibrium concentrations of Ca^{2+} "in" and "out" of the matrix can be defined as $(C_{\text{Ca}^{2+}}^{\text{in}})_{eq}$ and $(C_{\text{Ca}^{2+}}^{\text{out}})_{eq}$, respectively. If the system is under non-equilibrium conditions but it is close to equilibrium conditions, the respective concentration ratio can be presented as follows (Glansdorff and Prigogine, 1978):

$$\frac{C_{\text{Ca}^{2+}}^{\text{in}}}{C_{\text{Ca}^{2+}}^{\text{out}}} = K_{eq} e^{\frac{A}{k_B T}} \quad (6)$$

Where, $A = \mu_{\text{Ca}^{2+}}^{\text{out}} - \mu_{\text{Ca}^{2+}}^{\text{in}}$ is a difference of the chemical potentials of the Ca^{2+} ion "out" and "in" the mitochondrial matrix. The last relationship describes the non-equilibrium ratio of Ca^{2+} concentrations, when deviation from equilibrium conditions are very small. This is a simple case of the development and application of non-equilibrium thermodynamics tool, however, such tool can be further developed for large deviations from equilibrium

conditions. Note that the problem discussed above can also be used to obtain the Goldman equation.

6. Conclusions

In the present review, we discussed the different potential approaches, which can be applied to the analysis of mitochondrial swelling. Apparently, Ca^{2+} and K^+ play an important role in physiological and pathological swelling of mitochondria. The transport of Ca^{2+} and K^+ across the IMM is associated with influx/efflux mechanisms for other ions such as Na^+ , Cl^- and H^+ (not completely addressed in the text) suggesting a complex interplay between mitochondrial swelling and ion homeostasis. This interplay maintains function and metabolism of mitochondria under normal physiological conditions. However, excessive mitochondrial swelling resulted from alterations in ion homeostasis in the cytoplasm and mitochondrial matrix has detrimental consequences leading to cell death. Mitochondrial swelling is involved in the pathogenesis of many human diseases such as neurodegenerative and cardiovascular diseases, diabetes, etc. Therefore, modeling analysis of the swelling process is important for understanding the mechanisms of mitochondria-mediated cell dysfunction. Analysis of existing literature showed that there are a limited number of studies dedicated to the modeling of the mitochondrial swelling dynamics. In this review, we attempted to discuss different modeling approaches that could explain the underlying mechanisms of swelling. Based on this analysis, we assume that a full model that could highly simulate in vivo swelling should include several processes that regulate ion transport in mitochondria. The main processes contributing to mitochondrial swelling are the rate of ion influx/efflux across the IMM and changes in Ψ_m , pH_m , and redox state of mitochondria. Also, membrane structure, metabolic state as well as heterogeneity of mitochondria that can significantly change ionic transport dynamics should be taken into consideration. Kinetic models and different approaches to modeling discussed in this review can be useful for the development of new models of swelling in mitochondria as well as other systems (cell and organ levels).

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The abbreviations used

AQP8	aquaporin-8
Ca^{2+}_m	mitochondrial Ca^{2+}
CICR	Ca^{2+} -induced Ca^{2+} release
CypD	cyclophilin D
ETC	electron transport chain
mHCX	mitochondrial $\text{H}^+/\text{Ca}^{2+}$ exchanger

IMM	inner mitochondrial membrane
IMS	intermembrane space
KHE	K ⁺ /H ⁺ exchanger
KO	knockout
Kv1.3	voltage regulated K ⁺ channel
mBK_{Ca}	mitochondrial Ca ²⁺ regulated K ⁺ large conductance channel
mK_{ATP}	mitochondrial ATP activated K ⁺ channels
mKv1.3	mitochondrial voltage-activated K ⁺ channel of the Kv1.3 type
MCU	mitochondrial Ca ²⁺ uniporter
mRyR	mitochondrial RyR
Ψ_m	mitochondrial membrane potential
mSK_{Ca}	mitochondrial small-conductance Ca ²⁺ -activated potassium channel
mNCX	mitochondrial Na ⁺ /Ca ²⁺ exchanger
mNHE	mitochondrial Na ⁺ /H ⁺ exchanger
OMM	outer mitochondrial membrane
pH_m	matrix pH
PTP	permeability transition pore
RaM	rapid mode of Ca ²⁺ uptake
ROS	reactive oxygen species
RyR	ryanodine receptor
TCA	tricarboxylic acid
UCP	uncoupling proteins

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Highlights

- Several regulatory mechanisms are involved in the maintenance of mitochondrial volume
- Mitochondrial swelling plays a central role in cell life and cell death
- Modeling of mitochondrial swelling is important for understanding the mechanisms of metabolic and functional changes in the cell under physiological and pathological conditions
- Different kinetic models and approaches for simulation and prediction of *in vivo* mitochondrial swelling are discussed

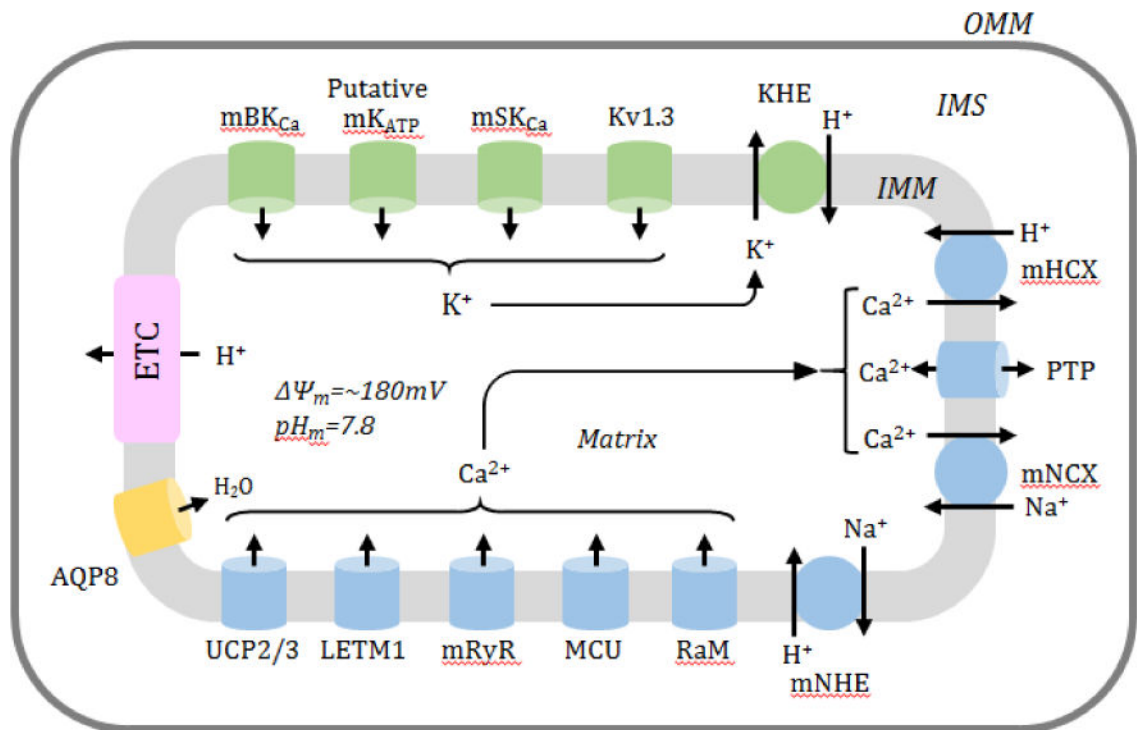


Figure 1. Main ion flux mechanisms that regulate ion homeostasis and matrix volume in mitochondria

A schematic diagram represents the main Ca^{2+} (blue) and K^{+} (green) influx and efflux mechanisms that regulate the mitochondrial volume. Main Ca^{2+} influx mechanisms include the mitochondrial Ca^{2+} uniporter (MCU), rapid mode of Ca^{2+} uptake (RaM), mitochondrial ryanodine receptor (mRyR), leucine zipper-EF-hand-containing transmembrane protein 1 (LETM1) and uncoupling proteins 2 and 3 (UCP2/3). Ca^{2+} efflux mechanisms are important for balancing of Ca^{2+} as well as ion homeostasis in the matrix, and include $\text{Na}^{+}/\text{Ca}^{2+}$ exchange (mNcX), $\text{H}^{+}/\text{Ca}^{2+}$ exchange (mHcX) and mitochondrial permeability transition pore (PTP). Transport of K^{+} is equally important for mitochondrial metabolism and function and changes in K^{+} correlate with changes in matrix volume. K^{+} influx mechanisms include the mitochondrial Ca^{2+} regulated K^{+} large conductance channel (mBK_{Ca}), mitochondrial ATP activated K^{+} channel (mK_{ATP}), mitochondrial small-conductance Ca^{2+} -activated potassium channel (mSK_{Ca}), and a voltage-activated K^{+} channel of the Kv1.3 type (mKv1.3), whereas K^{+} efflux mechanisms are limited to the $\text{K}^{+}/\text{H}^{+}$ exchanger (KHE). Additionally, the mitochondrial $\text{Na}^{+}/\text{H}^{+}$ exchanger (mNHE) and aquaporin 8 (AQP8) have been included in the regulation of ion homeostasis in mitochondria.

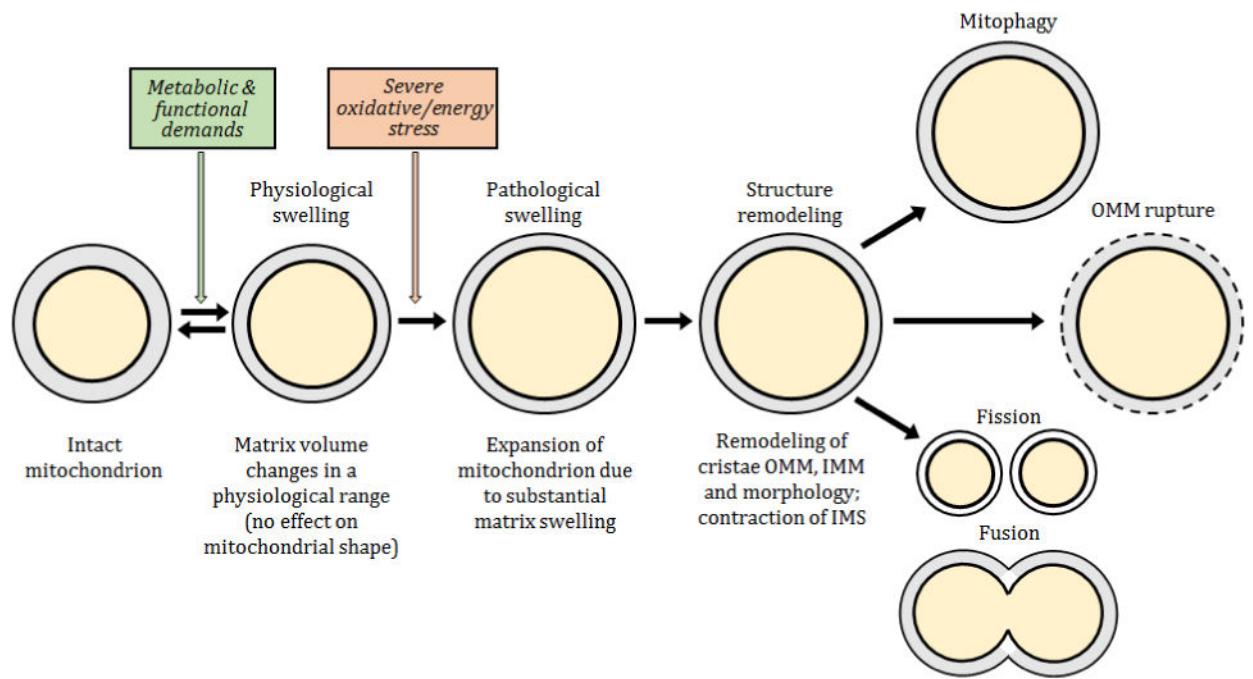


Figure 2. Proposed steps and consequences of mitochondrial swelling

Mitochondria are dynamic organelles that are constantly adapting to changes in the environment. Increased cellular energy demands induces slight swelling of the mitochondrial matrix that stimulates ETC activity and oxidative phosphorylation. However, severe energy or oxidative stress provokes a transition of mitochondria from a functional to a dysfunctional metabolic state accompanied by pathological swelling. Consequently, in response to matrix swelling, structural remodeling of mitochondria that results in the following consequences including mitophagy, rupture of the OMM and/or mitochondrial fusion and fission

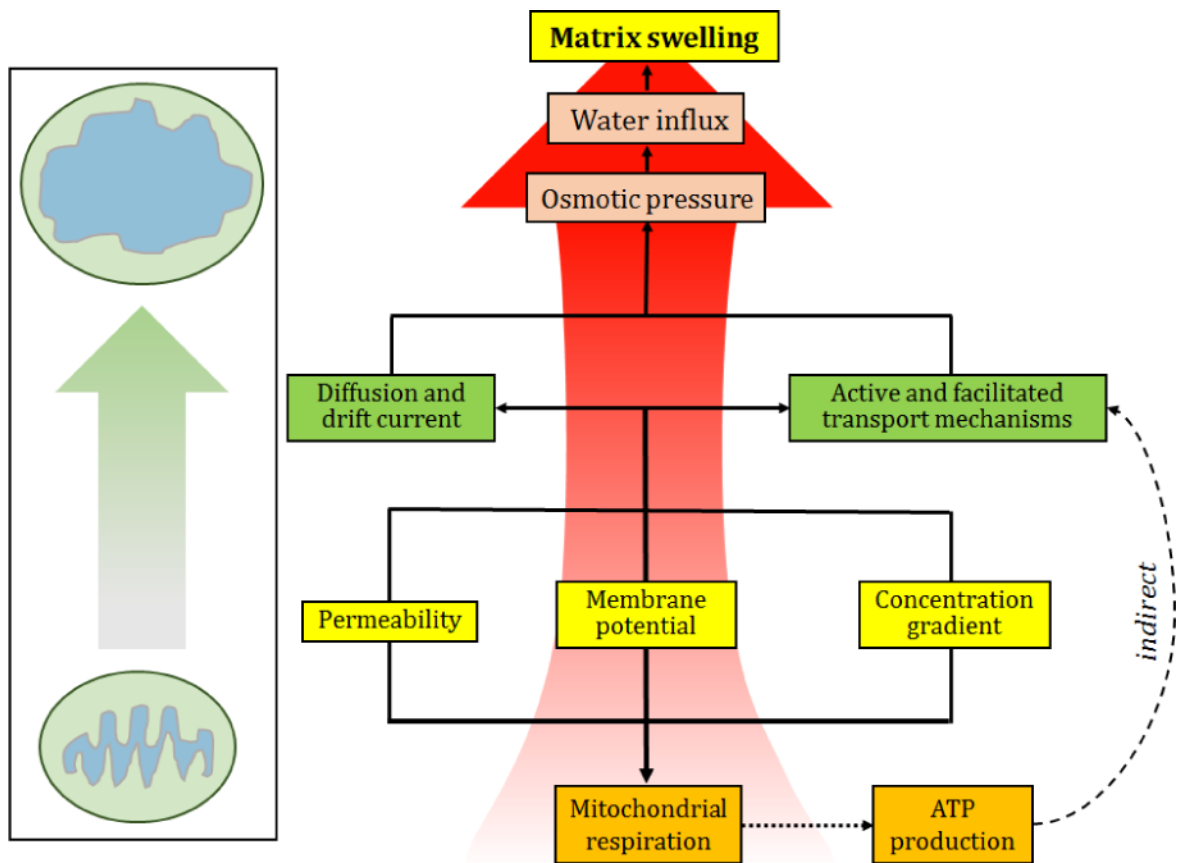


Figure 3. Main mechanisms for mitochondrial matrix swelling

Changes in the Ψ_m and ion concentration gradient can affect the ion transport through the IMM. ATP production capacity as well as signalling pathways regulating ion channels/transporters can be involved in maintaining ion homeostasis/matrix volume. As a result, an imbalance in ionic transport mechanisms or diffusion process can cause changes of osmotic pressure in the matrix. Increased osmotic pressure can induce matrix swelling due to increased net water influx.

Table 1

Main parameters used for modeling analysis of mitochondrial swelling

Variable	Definition	Value	Units
	<i>Pressure</i>		
π_{IMM}	Osmotic pressure changes across the IMM	–	atm
	<i>Permeability and diffusion coefficients</i>		
P_{IMM}	Permeability of the IMM	–	cm ²
$P_{IMM,i}$	Electroneutral and electrogenic permeability coefficient of ions (e.g. H ⁺ , K ⁺ , HCO ₃ ⁻ , and PO ₄ ³⁻)	–	min ⁻¹ mg ⁻¹ (protein) min ⁻¹ mV ⁻¹ mg ⁻¹ (protein)
$P_{max,uncoupler}$	Maximum uncoupler (e.g. FCCP) H ⁺ permeability coefficient	–	nmol mg ⁻¹ (protein) min ⁻¹
$D_{m,i\pm}, D_{C,i\pm}$	Diffusion coefficients of ions (e.g. H ⁺ , K ⁺ , HCO ₃ ⁻ , and PO ₄ ³⁻) in matrix and cytoplasm	–	cm ² s ⁻¹
	<i>Energy parameters</i>		
Ψ_m	Electric potential difference across the IMM	-110 – -150	mV (Nicholls and Ward, 2000; Petronilli et al., 1993)
μ_H	Chemical potential difference across the IMM	1.29	kJ mol ⁻¹ (Nicholls and Ward, 2000)
$\Psi_{m^+} \mu_H/(zF)^{(1)}$	The proton motive force	180	mV (Nicholls and Ward, 2000)
	<i>Rate parameters</i>		
$k_{m,ATP}$	Rate constant of ATP synthesis/hydrolysis in the matrix	7.5×10 ⁴	nmol mg ⁻¹ (protein) min ⁻¹ (Bazil et al., 2010a)
V_{max}	Maximal transporter rate	–	min ⁻¹ mg ⁻¹ (protein)
K_m	Michaelis constant for ion transporters and matrix enzymes	–	mM
k_o	The channel open probability rate constant (e.g PTP)	–	min ⁻¹
k_{cl}	The channel closing probability rate constant (e.g PTP)	–	min ⁻¹
k_A	Rate constant for the production of respiration activating molecules in the matrix	–	min ⁻¹
K_{eq}	Equilibrium constants for ion transporters and matrix enzymes	–	mM
K_d	Dissociation constant for ion binding proteins	–	mM
$k_{uncoupler}$	Apparent uncoupler (e.g. FCCP) $k_{1/2}$ constant	–	min ⁻¹
	<i>Electrical properties</i>		
C_{mito}	Mitochondrial membrane capacitance	1.45×10 ⁻³	nmol (ion) mg ⁻¹ (protein) mV ⁻¹ (Magnus and Keizer, 1997)
$g_{ion\ channel}$	Conductance of ionic channels in the IMM	–	nS
E_{ion}	Reversal potential based on the Nernst equation	–	mV
I_{ion}	The current produced by electrogenic channels	–	pA
M	Electric mobility of ions in the matrix	–	cm ² mV ⁻¹ min ⁻¹
ρ_0	Average surface charge density of the matrix	1.5×10 ⁻³	cm ⁻² (Song et al., 2013)
	<i>Oxidative phosphorylation</i>		
E_{ATP}	Energy used to synthesize 1 mole of ATP	42.68–58.58	kJ (Kocherginsky, 2009; Song et al., 2013)
ρ_{ATPase}	Density of F ₀ F ₁ -ATP synthase	2500	mol μm ⁻² (Song et al., 2013)

Variable	Definition	Value	Units
G	Gibb's free energy of reaction for ATP hydrolysis	-4.51	kJ mol^{-1} (Bazil et al., 2010a)
<i>Geometrical parameters</i>			
d_{mit}	Averaged diameter of mitochondria	0.237	μm (Song et al., 2013)
S_{OMM}	Surface area of the OMM	0.736	μm^2 (Song et al., 2013)
d_{crista}	Diameter of a crista junction	20–40	nm (Mannella, 2006)
L	Length of a crista junction	30–200	nm (Mannella, 2006)
S_{IMM}	Surface area of the IMM	0.708	μm^2 (Song et al., 2013)
V_{Mat}	Mitochondrial matrix water volume	–	$\mu\text{l mg}^{-1}$ (protein)
<i>Impermeable molecules</i>			
MPC	Matrix protein content	–	$\mu\text{g } \mu\text{l}^{-1}$