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Transport of Calcium Ions into Mitochondria

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Abstract: To uptake calcium ions of mitochondria is of significant functional connotation for cells, because calcium ions in mitochondria are involved in energy production, regulatory signals transfer, and mitochondrial permeability transition pore opening and even programmed cell death of apoptosis, further playing more roles in plant productivity and quality. Cytoplasmic calcium ions access into outer mitochondrial membrane (OMM) from voltage dependent anion-selective channel (VDAC) and were absorbed into inner mitochondrial membrane (IMM) by mitochondrial calcium uniporter (MCU), rapid mitochondrial calcium uptake (RaM) or mitochondrial ryanodine receptor (mRyR). Although both mitochondria and the mechanisms of calcium transport have been extensively studied, but there are still long-standing or even new challenges. Here we review the history and recent discoveries of the mitochondria calcium ions channel complex involved calcium assimilation, and discuss the role of calcium ions into mitochondria.

Keywords: Mitochondria, Channels, Calcium ions uptake, Uniporter.

1. INTRODUCTION

Ringer (1883) found that calcium ions play an important role in the contraction of myocardial cells, thus opening the field of calcium signal [1]. Study on the calcium ions in the mitochondria can be traced back to 1960s, when it was found that mitochondria could quickly absorb calcium [2, 3]. The concentration of calcium ions could be up to 10 times or more, the results led to suggestions that mitochondria could regulate calcium ion concentration in cytoplasm [2, 4]. More studies found that calcium is a highly flexible second messenger, in control of all the key reactions in eukaryotic cells [5]. Calcium signal controls both the millisecond short biological processes, such as muscle contraction movement or signal transfer between nerve cells, as well as regulation of long-term processes [6], such as cell proliferation and organ development.

Calcium ions can not only be absorbed and released by mitochondria, but also the progresses of calcium absorption and release maintain cytoplasmic calcium homeostasis and regulate signal modification in cells. Calcium ions as an important second messenger participate in salt, cold, osmotic and other abiotic stress responses [7-9], activate a variety of transporters and dehydrogenase activity [10-13]. Chilling injury, osmotic stress and contact stimulus activated calcium transient signals for 10-20 s, long calcium signal (> 120s) of mitochondria was induced by hydrogen peroxide [14]. In root hairs, mitochondria were involved in the cytoplasm calcium ions homeostasis.

Disruption of actin microfilaments led to the release of a large number of calcium ions into the cytosol [15]. Actin related protein complex Arp2/3 in Arabidopsis thaliana responded to salt stress by regulating calcium signaling [16]. In addition, the essence of ATP synthesis is the shuttle behavior of calcium ions from cell gap into another cell mitochondria [17]. So the mechanism of calcium transport in mitochondria is of great significance to living things. Although the endoplasmic reticulum (ER) and its special form sarcoplasmic reticulum (SR) in muscle cells is considered to be the main calcium storage organelle [18, 19], the mitochondria also play a key function of absorption and storage of calcium ions [20]. Absorption of calcium in the mitochondria is very important for cells, which could help to form the regulatory signals, not only to adjust the concentration of calcium ions but also to provide more energy or to start some functions such as cell apoptosis [21]. A mitochondrion from outside to inside can be divided into four functional areas: the outer mitochondrial membrane (OMM), the mitochondrial intermembrane space, the inner mitochondrial membrane (IMM) and the mitochondrial matrix. Calcium ions mainly distribute in the intermembrane space and the matrix. But because of OMM high permeability, the concentration of calcium ions in intermembrane is equivalent to cytoplasm. In general smooth cells, the concentration of calcium ions in mitochondrial matrix was about 100-200 nmol/L. But when the calcium signals were activated, the concentration of calcium would increase up to 1-2 μmol/L in cytoplasm, and up to 10-500 μmol/L in mitochondrial matrix [22-24]. How the calcium ions in the cells gap get into mitochondria matrix, there seems to be two steps, firstly, throng voltage dependent on anion-selective channel (VDAC) on OMM

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into intermembrane space [25-27], secondly, by mitochondrial calcium uniporter (MCU), rapid mitochondrial calcium uptake (RaM) or mitochondrial ryanodine receptor (mRyR) on IMM into mitochondrial matrix [28-31].

2. CALCIUM IONS TRANSPORT OR STORAGE INTO MITOCHONDRIA MATRIX

2.1. Calcium Ions Transport Through the OMM

The VDAC on the OMM is a kind of selective ion channel regulated by the OMM electric potential, totally has three types of proteins: VDAC1, 2, and 3, all could interact with different proteins to transform ions, anions and other molecules [25, 32-34], of course including transshipment calcium, but whether VDAC starting to transfer calcium ions depends on the concentration of calcium ions out of mitochondria [35]. -+

The transient over-expression of VDAC in HeLa cells and skeletal myotubes enhanced the amplitude of the agonist dependent calcium ions concentration increases in mitochondrial matrix by allowing the fast diffusion of calcium ions from ER release sites to the IMM, while knockout of VDAC led to the calcium ions concentration decrease [36]. Calcium ions passed through VDAC without any physical limits, but the capacity of VDAC transferring calcium ions was closely related to the number of VDAC on OMM [26]. The number of VDAC and the factors of regulating VDAC switch are both necessary for VDAC to transfer calcium ions into OMM.

2.2. Calcium Ions Transport Through the IMM

There are three main ways of calcium ions transport into the mitochondrial matrix from the intermembrane space, the first one is dependent on MCU one-way absorption, the second one is dependent on RaM absorption, and the last one is dependent on mRyR absorption. In addition, there may be other ways of calcium ions transport into mitochondria.

2.2.1. MCU One-way Absorption

MCU one-way absorption is a main way of calcium ions transport into the inner mitochondrial membrane. Although we knew it existence from 1960s [2, 3], but to identify the protein spent more than 50 years. In 2010 Mootha team identified one protein named mitochondrial calcium uptake 1 (MICU1), a 54 kDa mitochondrial inner membrane protein. When silencing MICU1, mitochondria lost the function of uptaking calcium ions. But this protein has only one transmembrane domain, suggesting that it is not the core protein of MCU. MICU1 has two calcium binding domain EF-hands, the free peptide on the end of EF-hand can bind calcium ions, indicating that MICU1 has the function of assisting MCU to perform the ability of transporting calcium ions [37].

The protein of MCU was identified in 2011 [38, 39], which has two transmembrane domains. It is localized to the inner membrane, and is a specific transporter of calcium ions. It appears to oligomerize within mitochondrial inner membrane as part of a larger molecular weight complex, consistent with the presence of MICU1. Furthermore, short interfering RNA (siRNA) silencing MCU in HeLa cells markedly reduced mitochondrial calcium ions uptake. MCU overexpression doubled the matrix calcium concentration increase evoked by inositol 1, 4, 5-trisphosphate-generating agonists, thus significantly buffering the cytosolic elevation [39]. There was a difference between WT and MCU knockout in the mice skeletal muscle under conditions of maximum work: the MCU knockout mice had reduced abilities to generate maximal power, and found that MCU gene knockout has certain embryonic lethality [40]. The above results indicate that MCU is closely related to calcium ions absorption, muscle movement and embryo development.

The member of one way absorption channels i.e. mitochondria calcium unipoter, MICU2 was searched with bioinformatics in human and mouse genome, belonging to MICU1 homologous proteins [41]. MICU3 is also a membrane protein involved in MCU channel but does not widely exist. It was found only in skeletal muscle cells and nerve cells, replaced the protein of MICU2 [42]. Four MCUs were oligomerized within mitochondrial inner membrane, MICU1 and MICU2 interacted with each other to regulate MCU transfer calcium ions (Fig. **1**). MICU2 inhibits the function of MCU under the low concentration of calcium ions in cytoplasm but when the concentration of calcium ions goes up enough, the EF hands of MICU1 and MICU2 bound calcium ions. The inhibition of MICU2 is relieved and MICU1 is activated, at the same time the channel of MCU opens [43, 44].

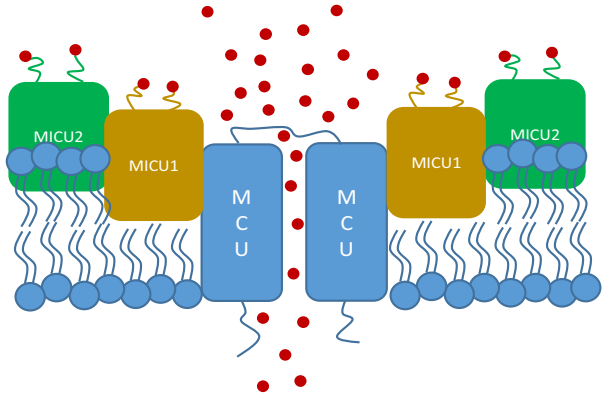

Fig. (1). Schematic representation of MCU based on published data [44]. At high calcium ions levels, the EF hands of both MICU2 and M1CU1 are bound with calcium ions, quieting inhibitory of MICU2 and activating stimulatory MICU1, the MCU opens, and calcium is taken up rapidly into the mitochondria.

The homologous proteins of MCU are similar in structure, but have different functions in different tissues, the expression level of MCU and the homologous proteins are always different in different organizations, perhaps because different tissues need different amount of calcium ions [45]. MCUb is a MCU homologous protein which has similar structure with MCU, but when the four oligomeric MCUs were completely replaced by MCUb in the one way absorption, the complex would lost the capacity of transferring calcium ions. If only one MCU was replaced by MCUb, reducing a lot of transport ability. The ratio of mRNA expression level of MCU and MCUb is 3:1 to 40:1 in different tissues.

Such as in myocardial cells, the value is 3:1, which is far below than in skeletal muscles which is 40:1. The concentration of calcium ions in skeletal cells is 28 times more than in myocardial cells [46]. This indicates an existence that the homologous proteins of MCU always exist in the same species, and with similar structure, but the functions may have significant difference.

So far, the research on MCU mainly focus on animals, the function of MCU in plants is rarely reported, perhaps with the common understanding generally believed that mitochondria are organelles that provide energy, and a lot of animal movement behavior need more energy than plants. But this is not to say that the plant cell does not need energy. MCU on calcium ion transport is not only related to the release of energy, but also signal transfer. In the near future research on plant MCU will gradually go deeply.

2.2.2. RaM-rapid Mitochondrial Calcium Uptake

Ram calcium absorption widely exists in heart, liver and brain tissues of the mammals and birds. The absorption velocity of calcium ions is 1000 times more than MCU [47], but the driver of RaM absorption calcium ions is similar to MCU, all dependent on the voltage of mitochondria inner membrane, and can be blocked by ruthenium red. Usually, RaM absorption occurred on the initial calcium absorption of the MCU, so some researchers speculated that RaM is merely a different form of MCU [48]. Moreover, RaM could be inhibited by high concentration of calcium ions out of mitochondria, but when the concentration is below than a value, like in mice liver cells is 100 nmol/L, the activation process of RaM absorption calcium ions is in a millisecond [47]. Therefore, according to the characters of high rate transferring calcium ions and repeatedly activated by low concentration calcium ions, the mitochondria which are out of the micro region can regulate the ratio of ATP synthesis through RaM when MCU had lost its capacity of calcium ions absorption in order to satisfy the needs of the cell energy metabolism [48]. In addition, RaM from different tissue sources was different especially in inhibitor and activator [49]. At present, the progress of research on molecular mechanism of RaM is delayed.

2.2.3. mRyR Mitochondrial Calcium Uptake

The RyR is a kind of channel that discharges the calcium ions in SR/ER into the cytoplasm [50]. Three kinds of RyR shear forms have been identified, RyR1, 2 and 3. They oligomerize a tetramer to exercise the function of calcium ion channel [50]. But, whether RyR exist on mitochondria or not is unclear. A 600 KD protein was found in the mitochondrial cristae, similar to RyR1, Beutner team named it mRyR [31]. mRyR might be regulated by the concentration of calcium ions in cytolymph, absorbed calcium ions under normal physiological conditions, and released calcium ions when mitochondrial calcium overloaded [51, 52]. In addition, mRyR found on mitochondrial cristae of rat spleen sinus epithelium is similar to RyR3 [53]. However, Salnikov believed that mRyR did not exist on the rat heart mitochondria [54].

A speculation is that RaM is the main channel of absorption calcium ions in rat heart mitochondria in the initial stage when calcium ions concentration is low; when the outer mitochondrial calcium ions concentration increased gradually, RaM will be inhibited to absorb calcium ions, and the work of transferring calcium ions mainly be replaced by the mRyR and MCU. However, due to the existence of mRyR is still controversial, whether the model established or not is questionable.

3. CONCLUSION AND OUTLOOK

Over the past few years, some interesting progress has been seen in the mechanism of molecular biology and functional characteristics about mitochondrial calcium absorption. The physiological function of mitochondria and even whole cells are regulated by different mechanisms of calcium ions transport between cytoplasm and mitochondria. *In vivo* experiments have demonstrated the importance of mitochondrial calcium ions absorption in cell function. A lot of pathways of calcium transport in or out of mitochondria have been determined with biochemical and electrophysiological methods. From those experiments ion selective specificity, activator, inhibitor, kinetics, conductivity and other features have been confirmed. Therefore, we have a certain degree of understanding about these processes. Due to the development and advancement of molecular biology and bioinformatics technology, the molecular basis of calcium transport pathway have also been discovered. But there is a tough situation, knowledge derived from experiments in permeabilized cells or isolated mitochondria cannot be readily transposed to the more complex situation of intact cells. The results from overexpression or silencing of MCU in normal cells has also brought a doubt, because the same results could not be obtained in isolated mitochondria with the same method. So Future experiments should use similar and unbiased screens to identify the elusive uniporter and to establish how many molecules are involved in mitochondrial calcium ion uptake, and calcium transport in mitochondria must be closely related and interacted with other organelles calcium transport. The interaction and influence will also become the focus of future research. So far, the research of MCU are mainly concentrated in animals, the function of MCU reported rarely in plants, this may be related to general recognition, generally considered the mitochondria organelles is to provide energy, many sports behavior of animals need more energy than plants. But this does not mean plant cells do not need energy. Actually, when plants under salt stress, more calcium ions will accumulate in the cells [55], and will go through MCU into mitochondria, so that with salt stress the mitochondrial calcium ion concentration will be very high, but high concentration calcium induces the generation of mitochondrial reactive oxygen species (ROS) [56], this affect the efficiency of ATP synthesis in mitochondria, resulting in root and leaf due to lack of energy supply, and affect a variety of ion transport and metabolism in plant cell (Fig. **2**), and MCU for calcium ion transport not only relates to the mitochondria for the release of energy, more direct is the transmission of signals, calcium is widely recognized as the second messenger [57-59]. So we believe the research of MCU in the plants will be also gradually in-depth in the near future.

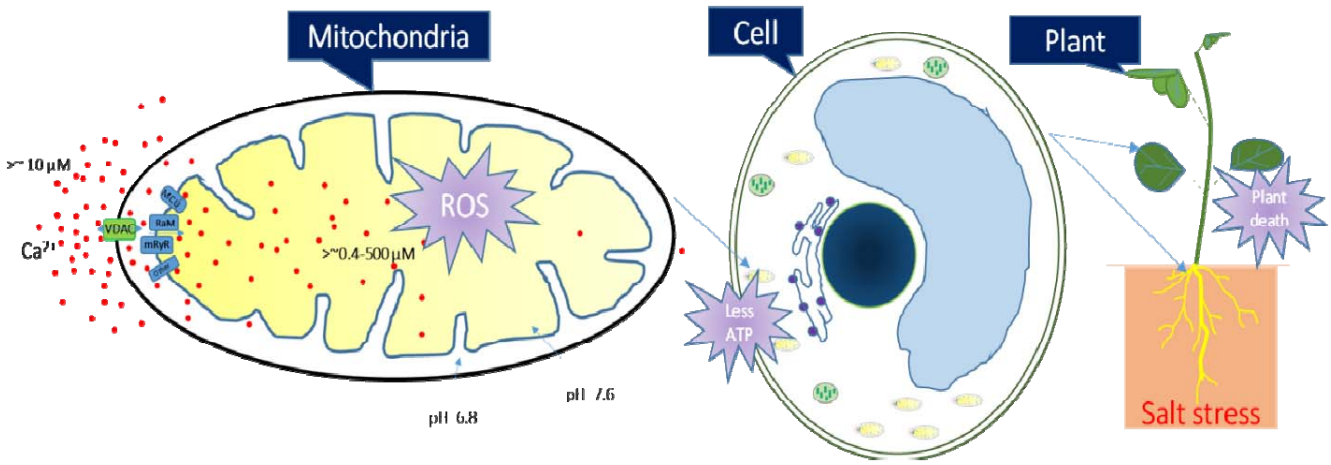

Fig. (2). Schematic representation of calcium ion in plant cell under salt stress. Under salt stress, more calcium ions will accumulate in the cells and will go through MCU into mitochondria, and with salt stress the mitochondrial calcium ion concentration will be very high, but high concentration calcium induces the generation of mitochondrial reactive oxygen species, which affect the efficiency of ATP synthesis in mitochondria, resulting in root and leaf due to lack of energy supply, and affect a variety of ion transport and metabolism in plant cell.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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