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Review

Characteristics and possible functions of mitochondrial Ca^{2+} transport mechanisms

Thomas E. Gunter [a,*], Shey-Shing Sheu [b]

[a] Department of Biochemistry and Biophysics and Mitochondrial Research and Innovation Group, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA
[b] Department of Pharmacology and Physiology and Mitochondrial Research and Innovation Group, University of Rochester School of Medicine and Dentistry, 601 Elmwood Avenue, Rochester, NY 14642, USA

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ABSTRACT

Mitochondria produce around 92% of the ATP used in the typical animal cell by oxidative phosphorylation using energy from their electrochemical proton gradient. Intramitochondrial free Ca^{2+} concentration ($[Ca^{2+}]_m$) has been found to be an important component of control of the rate of this ATP production. In addition, $[Ca^{2+}]_m$ also controls the opening of a large pore in the inner mitochondrial membrane, the permeability transition pore (PTP), which plays a role in mitochondrial control of programmed cell death or apoptosis. Therefore, $[Ca^{2+}]_m$ can control whether the cell has sufficient ATP to fulfill its functions and survive or is condemned to death. Ca^{2+} is also one of the most important second messengers within the cytosol, signaling changes in cellular response through Ca^{2+} pulses or transients. Mitochondria can also sequester Ca^{2+} from these transients so as to modify the shape of Ca^{2+} signaling transients or control their location within the cell. All of this is controlled by the action of four or five mitochondrial Ca^{2+} transport mechanisms and the PTP. The characteristics of these mechanisms of Ca^{2+} transport and a discussion of how they might function are described in this paper.

1. Introduction

Since the discovery of transport of Ca^{2+} by mitochondria from mammals and other higher vertebrates in the early 1960's [1,2], we have learned a considerable amount about how Ca^{2+} is transported into and out of mitochondria, and sometimes have been able to infer why it is done as it is. Mitochondria in higher eukaryotes today carry out many functions (cytosolic $[Ca^{2+}]$ buffering, partial control of apoptosis [3,4], β oxidation of fatty acids [5], role in the urea cycle [5], role in synthesis and metabolism of iron-containing proteins [6,7], etc.) in addition to oxidative phosphorylation. However, production of ATP by oxidative phosphorylation was surely the initial and is still the primary function of mitochondria. Oxidative phosphorylation produces around 92% of the ATP used in the typical mammalian cell. In times past, production of sufficient ATP in times of stress must have exerted considerable evolutionary pressure on the rate limiting steps of oxidative phosphorylation so that these steps have been accelerated by evolutionary change to a point where there is no single rate limiting step. Today, rate limitation is shared by a number of steps [8–10], which has led to a situation in which activation of a single partially rate limiting step doesn't do much to increase the rate of the overall process. Yet it is vital to all life to produce and use ATP slowly when only a little energy is needed but to be able to produce and use it very quickly to escape danger. Therefore, it is crucial to be able to control the rate of oxidative phosphorylation. What is needed to do this is a single signal which can accelerate all of the partially rate limiting steps simultaneously and a transient increase in intramitochondrial free Ca^{2+} concentration ($[Ca^{2+}]_m$) is a very good choice. Ca^{2+} is one of the most common second messengers, modulating many processes which increase the use of energy including muscle contraction. By using the same type of signal, a transient increase in intracellular and intramitochondrial $[Ca^{2+}]$, to increase both the use of energy and energy production, evolution produced what may be the world's first on time delivery system. For more complete descriptions of the arguments which underlie these concepts see earlier reviews [11–13].

We have known for some time of the elaborate set of mechanisms and processes controlling Ca^{2+} transport both inward and outward across the mitochondrial inner membrane — three mechanisms or modes of influx and two of efflux. There is also the mitochondrial permeability transition (MPT) mediated by the permeability transition pore (PTP), which is Ca^{2+}-induced and makes the membrane leaky to all small, freely-diffusible ions and molecules [14–17]. The most studied of the transport mechanisms is the mitochondrial Ca^{2+} uniporter, the mechanism mediating the Ca^{2+} influx which led to the initial discovery of Ca^{2+} uptake by mitochondria [1,2]. Another mechanism or mode of uptake is called the rapid mode or RaM, which was discovered much more recently and has received the least attention of all of these transport mechanisms [18]. The third Ca^{2+} uptake mechanism is the mitochondrial ryanodine receptor (mRyR) that was identified recently in excitable cells [19]. The two

* Corresponding author. Fax: +1 585 275 6007.
E-mail address: Thomas_gunter@urmc.rochester.edu (T.E. Gunter).

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mechanisms of Ca^{2+} efflux are the Na^+-dependent and the Na^+-independent mechanisms, which were discovered in the 1970's [20,21]. During the years when these transport mechanisms were discovered and initially studied, it was felt that the outer mitochondrial membrane was freely permeable to small ions through the voltage dependent anion channel (VDAC); however, more recent data has suggested that VDAC can provide a barrier to free diffusion under some conditions [22]. Curiously, VDAC provides a higher Ca^{2+} permeability in the closed states [23]. Finally, the MPT, also discovered in the 1970's [14–16,24–26], was initially associated with pathological consequences, but is today viewed as possibly also having physiological roles.

2. Isolated mitochondria vs mitochondria in cells

We would like to understand how Ca^{2+} affects mitochondria in the living animal, but even studies in tissue are very difficult. Partly because there were no convenient probes or techniques available prior to the mid 1980's that allowed studies of these mechanisms at the cell or tissue levels, the earlier work in identifying mitochondrial Ca^{2+} transport mechanisms was carried out using isolated mitochondria, usually from liver, heart, or brain. Today, with the development of many fluorescent probes, which can be selectively directed to specific parts of the cell, including the mitochondria, endoplasmic reticulum, etc., most of the work is being carried out at the cell level [27]. However, there are advantages and disadvantages to working at the isolated mitochondrial and at the cell levels that should be considered logically. An obvious advantage of working at the cell level over the isolated mitochondrial level is that mitochondrial structures and the concentrations of components of medium around the mitochondria are more like those in vivo than they are with an isolated mitochondrial suspension. However, they are not necessarily the same as those in vivo. The metabolism of isolated cells in culture, particularly of immortalized cell lines, is often very different from that of similar types of cells in vivo [28–31]. It is common for transformed cell lines to produce more ATP through glycolysis than similar types of cells would in tissue. There are other organelles and structure at the cell level that could show important interactions with mitochondria; however, even this cell structure may be modified from that found in vivo. Concentrations of trace constituents of the intracellular medium are undoubtedly more similar to those found in vivo than to the artificial media used with isolated mitochondria. As an example of a component that can significantly modify the behavior of both the uniporter and the RaM, physiological levels of spermine or other polyamines [32–34] can cause large changes in the transport characteristics of these mechanisms. These polyamines are always present in experiments carried out in cells, but with a few exceptions, they haven't been in experiments with isolated mitochondria. A major negative factor that affects all work with isolated mitochondria is that the process of isolating mitochondria from tissue not only selects for healthy mitochondria, but also breaks up filaments of mitochondria which then reseal in a non physiological way. While this resealing is rapid, there may be some deenergization and exchange of internal for external medium involved in this process. We know that mitochondrial fission and fusion are normal physiological processes that occur continually with mitochondrial populations inside of cells [35,36]; however, the fragmentation which takes place during mitochondrial isolation is different from that which takes place physiologically.

Not all of the advantages lie with work at the cell level, however. Even where fluorescent probes can be successfully targeted to specific organelles such as mitochondria, only free ion concentration can be estimated through the fluorescence techniques. We refer to the results of measuring $[Ca^{2+}]$ in mitochondria inside cells as estimates because it is very difficult to accurately calibrate the K_d for binding of Ca^{2+} to the fluorescent probe inside of mitochondria inside cells, particularly for nonratiometric probes [37]. It is sufficiently difficult that very few investigators in the field even attempt to do this. Absolute errors in concentration can be over a factor of two [37]. By using radioactive isotopes as well as fluorescent probes, both free and total ion concentrations can be measured. This provides easily measured, direct transport data with isolated mitochondria, while this type of experiment is much more difficult and contains more caveats with intact cells. This can be an important advantage for working with isolated mitochondria. Other types of measurements such as membrane potential, oxidation rate, intramitochondrial pH, etc. are also valuable for an understanding of mitochondrial Ca^{2+} influx or efflux and these measurements can often be made simultaneously and much more accurately with a larger variety of techniques with isolated mitochondria than with mitochondria within cells. As a result, a considerable amount of quantitative data is available from the literature from measurements on isolated mitochondria while measurements on mitochondria within cells are generally more qualitative. This quantitative data is very useful for calculating estimates of necessary energies or effects of processes that are difficult to measure directly in intact cells. While more recent data at the intact cell level has provided very important insights and modifications to the earlier data obtained from experiments on isolated mitochondria, particularly with respect to the interactions of mitochondria with endoplasmic reticulum and other cell structures [27,38], the concepts of mitochondrial Ca^{2+} transport today are still similar to the concepts drawn from the isolated mitochondrial data. At the very least, measurements on isolated mitochondria tell us what type of behavior can be mediated by the mitochondria themselves, independently of the presence of the rest of the cell, e.g. very rapid uptake through the RaM mechanism. It is useful to have both types of information and to evaluate results with the limitations of the techniques used in mind.

3. Characteristics of the influx mechanisms: the uniporter

The mitochondrial Ca^{2+} uptake mechanisms appear reversible; however, it is still proper to refer to them as “influx mechanisms” unless the mitochondria are deenergized because of the effects of the strong, internally negative mitochondrial membrane potential on the direction of transport.

Mitochondria from all vertebrate organs tested show Ca^{2+} uptake via a mechanism which has similar characteristics in all of these types of mitochondria, those of a Ca^{2+} uniporter [17]. A uniporter is a mechanism which facilitates passive transport of Ca^{2+} down its electrochemical gradient without coupling Ca^{2+} transport to the transport of another ion. This mode of transport begins during embryonic development at a time that is species- and possibly organ-dependent [39]. Mitochondria in yeast, plants and lower animal species are also often able to sequester Ca^{2+}; however, the characteristics of transport in these species can differ significantly from those of the mitochondrial Ca^{2+} uniporter of higher animals [40]. Recognition that this mechanism of Ca^{2+} uptake was a uniporter came in stages. The first was Mitchell's recognition that the mitochondrial inner membrane pumped protons outward to produce an electrochemical proton gradient composed of a large, internally-negative membrane potential and an internally alkaline pH gradient [41,42]. An internally negative membrane potential could also be produced by ATP hydrolysis on the F_1 ATPase or by efflux of intramitochondrial K^+ using the ionophore valinomycin. Both of these means of producing an internally negative potential also induced Ca^{2+} uptake by the mitochondria [43,44]. In an elegant series of swelling experiments, that identified the combinations of ions which, with Ca^{2+}, would permit swelling under passive conditions, Selwyn et al. showed that the influx of Ca^{2+} was not directly coupled to the transport of another ion [45]. Finally, it was shown that the membrane potential dependence of Ca^{2+} uptake via this influx mechanism closely fit the predictions of the equations for electrochemical diffusion [17,46]. Taken together, these observations