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Review article

NCLX: The mitochondrial sodium calcium exchanger

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ABSTRACT

The free Ca^{2+} concentration within the mitochondrial matrix ($[Ca^{2+}]_m$) regulates the rate of ATP production and other $[Ca^{2+}]_m$ sensitive processes. It is set by the balance between total Ca^{2+} influx (through the mitochondrial Ca^{2+} uniporter (MCU) and any other influx pathways) and the total Ca^{2+} efflux (by the mitochondrial Na^+/Ca^{2+} exchanger and any other efflux pathways). Here we review and analyze the experimental evidence reported over the past 40 years which suggest that in the heart and many other mammalian tissues a putative Na^+/Ca^{2+} exchanger is the major pathway for Ca^{2+} efflux from the mitochondrial matrix. We discuss those reports with respect to a recent discovery that the protein product of the human FLJ22233 gene mediates such Na^+/Ca^{2+} exchange across the mitochondrial inner membrane. Among its many functional similarities to other Na^+/Ca^{2+} exchanger proteins is a unique feature: it efficiently mediates Li^+/Ca^{2+} exchange (as well as Na^+/Ca^{2+} exchange) and was therefore named NCLX. The discovery of NCLX provides both the identity of a novel protein and new molecular means of studying various unresolved quantitative aspects of mitochondrial Ca^{2+} movement out of the matrix. Quantitative and qualitative features of NCLX are discussed as is the controversy regarding the stoichiometry of the NCLX Na^+/Ca^{2+} exchange, the electrogenicity of NCLX, the $[Na^+]_i$ dependency of NCLX and the magnitude of NCLX Ca^{2+} efflux. Metabolic features attributable to NCLX and the physiological implication of the Ca^{2+} efflux rate via NCLX during systole and diastole are also briefly discussed.

Contents

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1. Introduction

Considerable evidence suggests that the free $[Ca^{2+}]$ in the mitochondrial matrix ($[Ca^{2+}]_m$) is important in the regulation of mitochondrial metabolism. This is due largely to the $[Ca^{2+}]_m$ sensitivity of key steps in energy production [1,2]. Such $[Ca^{2+}]_m$ dependent components include the Krebs cycle dehydrogenases that supply substrate to the electron transport chain (ETC) [1–5], F_1F_0-ATPase (Complex V) [6] and additional components of the ETC [7], the uncoupling proteins [8–11], the putative permeability transition pore (PTP) [12–14] and other proteins [15,16]. This review seeks to present a current view of the molecular and biophysical properties of NCLX and its role in regulating $[Ca^{2+}]_m$.

$[Ca^{2+}]_m$ is set in the steady state by the balance of Ca^{2+} leak into the matrix by any Ca^{2+} entry pathway and the efflux by any Ca^{2+} extrusion/pump mechanism. Passive Ca^{2+} entry is favored by the very large potential across the inner mitochondrial member, $\Delta\Psi_m$ (−150 to −200 mV). The proton-motive potential powers the ATP synthase so that it can make ATP and is a combination of the inner membrane potential ($\Delta\Psi_m$) and the pH gradient across the inner membrane (the matrix is more alkaline, a pH of 7.8 compared to 7.2 in the cytosol). The large $\Delta\Psi_m$ also favors entry of Ca^{2+} down its electrochemical gradient. The primary Ca^{2+} entry pathway is the mitochondrial Ca^{2+} uniporter (MCU), a channel thought to be highly selective for Ca^{2+} [17,18]. Other features related to the MCU are now in dispute and will be presented here only briefly. These features that are currently the topic of active investigation by us and others relate to the abundance of the MCU in the inner membrane, its open probability, its conductance, its dependence on cytosolic and matrix regulators and its gating [19–25]. Nevertheless, two groups identified a compelling candidate protein that appears to have all of the properties consistent with an MCU [26,27] and appears to be the same protein. The Ca^{2+} efflux from the mitochondrial matrix in excitable-cells appears to be mediated by the recently identified mitochondrial Na^+/Ca^{2+} exchanger (NCLX) [28]. This Ca^{2+} extrusion from the matrix is powered by the electrochemical gradient for Na^+ entry into the mitochondrial matrix from the cytosol. The energy available to NCLX for mitochondrial Ca^{2+} extrusion thus depends on the concentrations of Na^+ in the cytosol ($[Na^+]_i$) and matrix ($[Na^+]_m$), on $\Delta\Psi_m$ and also on the stoichiometry of NCLX. In non-excitable cells (e.g., liver cells) Ca^{2+} efflux is also mediated by a H^+/Ca^{2+} exchanger of unknown molecular identity [29].

The mitochondrial Na^+/Ca^{2+} exchanger gene was identified by Cai et al. [30] and Palty et al. [31] and its initial function was characterized as described below. It is called NCLX an abbreviated term for (Na/Li/Ca exchanger) because Palty et al. found that it can transport either lithium (Li^+) or sodium (Na^+) in exchange for Ca^{2+} while the plasma membrane exchangers NCX and NCKX do not transport Li^+. Two important issues that motivate current mitochondrial research but are still poorly understood are; (1) the amount of Ca^{2+} that can be transported by NCLX when it is fully activated and (2) the extent and kinetics of NCLX transport rate and how transport is influenced by cytosolic and matrix regulatory factors. Here we present an overview of the molecular and physiologic function of NCLX. Since mitochondria from different tissues exhibit great differences in their permeability to Ca^{2+} [23], unless stated otherwise we focused here on the quantitative information from investigations of cardiac cells or isolated cardiac mitochondria.

2. Critical features of NCLX

2.1. The movement of Ca^{2+} across the inner mitochondrial membrane

The $[Ca^{2+}]_i$ transient, activated by the cardiac action potential (AP), underlies the cardiac contraction. Ca^{2+} enters the cell primarily through voltage-gated L-type Ca^{2+} channels, triggers and synchronizes Ca^{2+} sparks to produce the global or cell-wide $[Ca^{2+}]_i$ transient (70–80 μmol of Ca^{2+} per liter of cytosol) during the "systolic period". The elevated $[Ca^{2+}]_i$ is reduced as Ca^{2+} is reacquired by the sarcoplasmic reticulum (SR) or extruded from the cell and this reduction of $[Ca^{2+}]_i$ underlies the relaxation phase of the cardiac contraction; the "diastolic period" [32]. One of the potential sources and sinks for Ca^{2+} is the large array of mitochondria in the cytosol. During systole, cytosolic $[Ca^{2+}]_i$ increases cell-wide from about 100 nM to approximately 0.5 to 1.0 μM. A fraction of the elevated $[Ca^{2+}]_i$ enters the mitochondrial matrix where it activates ATP generation [3,33]. The exact amount of Ca^{2+} that enters the mitochondria is a highly debated topic (see below). However, in the steady state this Ca^{2+} influx must be balanced by $Ca^{2\pm}$ efflux. Or put another way, under steady-state conditions the same amount of Ca^{2+} that enters each mitochondrion during a $[Ca^{2+}]_i$ transient must be extruded from it before the next $[Ca^{2+}]_i$ transient. Therefore, if the mitochondrial Ca^{2+} uniporter (MCU) is the only pathway for Ca^{2+} to enter the mitochondrial matrix and the mitochondrial Na^+/Ca^{2+} exchanger (NCLX) is the only pathway for mitochondrial Ca^{2+} extrusion, then MCU influx must equal the NCLX efflux. In reviewing this topic two central questions will be discussed: (1) what is the magnitude of this Ca^{2+} efflux and (2) what are its kinetics. Exploring these questions leads us to a quantitative analysis of the influence of NCLX stoichiometry and of $[Na^+]_i$ on the magnitude and kinetics of NCLX Ca^{2+} efflux. See below.

To investigate the magnitude and kinetics of mitochondrial Ca^{2+} fluxes, two approaches have been used by investigators. The first [34–37] seeks primarily to measure and calibrate $[Ca^{2+}]_i$ and $[Ca^{2+}]_m$ in cardiac cells. The second seeks to examine cellular and mitochondrial dynamics in diverse cell types under a much wider range of conditions [5,38–42]. These two approaches come to very different conclusions regarding Ca^{2+} uptake by mitochondria. They either suggest that very little Ca^{2+} enters the mitochondria under physiological conditions or that a great deal of Ca^{2+} enters mitochondria, respectively.

The first group of studies (see [35–37,43]) finds that 99% of the Ca^{2+} that enters the cytosol from the extracellular space or from the SR during systole is removed by the joint action of the Na^+/Ca^{2+} exchanger (NCX) located on the sarcolemma (SL) and the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) located on the sarcoplasmic reticulum (SR) membrane. The remaining 1% is removed equally by the mitochondria (entry into the matrix through MCU channels) and the plasmalemmal/sarcolemmal Ca^{2+}-ATPase pump, PMCA [34,35]. If true, under physiological conditions the amounts of Ca^{2+} that are transiently removed from the cytosol during systole by entering the mitochondrial matrix is inconsequential with respect to excitation–contraction coupling in the heart [44]. This would suggest that the highest measured mitochondrial $[Ca^{2+}]_m$ transient will be small (i.e., 10 to 30 nM [36,37]).

The second group indicates that much higher mitochondrial Ca^{2+} fluxes and changes in $[Ca^{2+}]_m$ occur during the cytosolic $[Ca^{2+}]_i$ transient. Results from these studies suggest large changes in $[Ca^{2+}]_m$ which parallel changes in $[Ca^{2+}]_i$. These results rely in part on the loading of inorganic dyes (e.g., Fluo-3 AM and Rhod-2 AM) into the mitochondrial matrix. Since application of the known MCU inhibitor, RU-360, prevents changes in the mitochondrially that this signal is now reporting changes

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