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Life after the birth of the mitochondrial Na^+/Ca^{2+} exchanger, NCLX

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Powered by the mitochondrial membrane potential, Ca^{2+} permeates the mitochondria via a Ca^{2+} channel termed Ca^{2+} uniporter and is pumped out by a Na^+/Ca^{2+} exchanger, both of which are located on the inner mitochondrial membrane. Mitochondrial Ca^{2+} transients are critical for metabolic activity and regulating global Ca^{2+} responses. On the other hand, failure to control mitochondrial Ca^{2+} is a hallmark of ischemic and neurodegenerative diseases. Despite their importance, identifying the uniporter and exchanger remains elusive and their inhibitors are non-specific. This review will focus on the mitochondrial exchanger, initially describing how it was molecularly identified and linked to a novel member of the Na^+/Ca^{2+} exchanger superfamily termed NCLX. Molecular control of NCLX expression provides a selective tool to determine its physiological role in a variety of cell types. In lymphocytes, NCLX is essential for refilling the endoplasmic reticulum Ca^{2+} stores required for antigen-dependent signaling. Communication of NCLX with the store-operated channel in astroglia controls Ca^{2+} influx and thereby neuro-transmitter release and cell proliferation. The refilling of the Ca^{2+} stores in the sarcoplasmic reticulum, which is controlled by NCLX, determines the frequency of action potential and Ca^{2+} transients in cardiomyocytes. NCLX is emerging as a hub for integrating glucose-dependent Na^+ and Ca^{2+} signaling in pancreatic β cells, and the specific molecular control of NCLX expression resolved the controversy regarding its role in neurons and β cells. Future studies on an NCLX knockdown mouse model and identification of human NCLX mutations are expected to determine the role of mitochondrial Ca^{2+} efflux in organ activity and whether NCLX inactivation is linked to ischemic and/or neurodegenerative syndromes. Structure-function analysis and protein analysis will identify the NCLX mode of regulation and its partners in the inner membrane of the mitochondria.

NCLX, MCU, mitochondrial Ca^{2+} signaling, Na^+/Ca^{2+} exchanger, Na^+ signaling

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The mitochondrion is not only the energy supplier of the cell, but also a major Ca^{2+} signaling hub. Powered by the steep mitochondrial membrane potential, Ca^{2+} permeates through a Ca^{2+} channel traditionally called the Ca^{2+} uniporter [1,2], and then is pumped out by a $3Na^+/1Ca^{2+}$ exchanger [3,4], or in some cells by a H^+/Ca^{2+} exchanger [5,6]. Although the functional elements of mitochondrial Ca^{2+} shuttling were discovered many years ago, their physiological relevance remained questionable for many years, because of their low Ca^{2+} affinity at the range of micromolar compared with the magnitude of Ca^{2+} transients recorded in the cytosol of most cell types. The pioneering work of Tulio Pozzan and Rossario Rizzuto, who were first to monitor *in situ* mitochondrial Ca^{2+} using the mitochondrial-targeted Ca^{2+} sensor, aequorin, showed that almost any Ca^{2+} response is propagated by a mitochondrial Ca^{2+} transient [7]. The apparent discrepancy between the low affinity of the mitochondrial Ca^{2+} influx and its frequent role in Ca^{2+} re-

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sponse has been resolved by high-resolution imaging, which revealed that cellular Ca^{2+} responses are highly heterogeneous and in fact Ca^{2+} reaches micromolar levels at cellular microdomains, also called hot spots, particularly at the vicinity of the plasma membrane and endoplasmic reticulum (ER) [8,9]. This major breakthrough was followed by a dramatic surge in interest and studies aimed at determining the role of mitochondria in cellular Ca^{2+} signaling. These studies, which are described in many excellent reviews, found that the mitochondria are a major and critical cellular Ca^{2+} signaling hub, mediating crosstalk particularly with the ER and plasma membrane domains [10–12]. By controlling cellular Ca^{2+} signals, the mitochondria control principal physiological processes ranging from hormonal secretion to cardiac and neuronal activity. In addition, mitochondrial Ca^{2+} overload is a preceding hallmark event in necrotic or apoptotic cell death during ischemic and neurodegenerative syndromes. Thus, fully coordinated activity of the uniporter and exchanger is required to prevent these processes [13]. A major limitation, however, in investigating in detail the molecular events linked to the role of mitochondria in physiological processes and how they break down during diseases was that the identities of the mitochondrial uniporter and exchanger were unknown. An additional hurdle was that the inhibitors used to determine their role were not highly selective and interacted with other major Ca^{2+} channels or transporters at similar concentrations required to block the exchanger.

The last four years were a period of dramatic changes and progress, because of the molecular identification of the exchanger and uniporter. Novel insight is emerging on their molecular regulation, and precise molecular tools are now available to control their activity and expression. The uniporter was identified following an ingenious mitochondrial proteomic study that sought to fish out mitochondrial proteins linked to mitochondrial Ca^{2+} shuttling found in mammals but not in yeast, for example. This screen first led to the identification of the Ca^{2+} uniporter inhibitor, MICU1 [14], and then to the identification of the Ca^{2+} uniporter itself, MCU, by the groups of Mootha and Rizzuto [1,2]. Many excellent reviews have been published on the physiological and molecular aspects of MCU in health and diseases [15–17]. Here, we will first describe how NCLX was identified, and then describe novel molecular and physiological aspects that were discovered after its identification.

1 Identification of the mitochondrial NCLX

The functional phenomenon of Na^+-dependent Ca^{2+} release underlying the exchanger activity was first discovered 40 years ago by Ernesto Carfoli in cardiac mitochondria [3]. A unique ionic property of this exchanger that was later used for its molecular identification was that in contrast to other Na^+/Ca^{2+} exchanger superfamily members, such as NCX and NCKX that are inert to Li^+, it could catalyze Li^+- and Na^+-dependent Ca^{2+} exchange at similar rates. This study was later followed by purification and functional reconstitution of a putative 60-kD polypeptide thought to be related to the mitochondrial exchanger [18]. However, this did not lead to the molecular identification of the mitochondrial exchanger. The identification of the mitochondrial Na^+/Ca^{2+} exchanger gene began when we attempted to find the gene responsible for a Na^+/Zn^{2+} exchange activity in HEK 293 cells and neurons. The rationale behind our strategy was that the Na^+/Zn^{2+} exchanger may be a member of the Na^+/Ca^{2+} exchanger superfamily. We cloned a putative candidate termed FLJ22233. Phylogenetic analysis of this candidate indicated that it is more ancestral than the NCX and NCKX families, but it shares with them the same $\alpha1$ and $\alpha2$ domains that form the catalytic cation transport site. We then expressed this putative gene in cells and asked if its expression is linked to Zn^{2+} transport. To our disappointment at the time, FLJ22233 expression was not linked to Zn^{2+} transport. We then turned to Ca^{2+} and found that it mediated Na^+/Ca^{2+} exchange. One of the classic controls for analyzing Na^+-dependent Ca^{2+} transport is to replace Na^+ with Li^+. The latter ion is inert to NCX and NCKX. To our surprise, we found that Li^+ supported Ca^{2+} efflux in cells expressing FLJ22233 almost at the same potency as that of Na^+. We then realized that the mitochondrial Na^+/Ca^{2+} exchanger was not selective to Li^+ [19]. We then set out to determine whether FLJ22233, now called NCLX ($Na^+/Li^+/Ca^{2+}$ exchanger), is found in the mitochondria by Western blot and electron microscopy-based immunological analysis and found that NCLX is not only enriched in the mitochondrial fraction but is also found in the inner mitochondrial membrane. Subsequent functional experiments using the inner mitochondrial-targeted Ca^{2+} sensor, mito-pericam, showed that mitochondrial expression of NCLX triggered an accelerated Ca^{2+} efflux that was fully blocked by this exchanger's inhibitor, CGP-37157. Importantly, the endogenous mitochondrial Ca^{2+} efflux activity could be blocked by reducing NCLX expression by RNA_i using siNCLX or shNCLX constructs, or by expressing an NCLX mutation at the catalytic $\alpha1$ domain. The effect of the latter is presumably mediated by a strong dominant negative effect of the mutation, which is related to the dimeric functional organization of NCLX. Finally, our results indicated that the mitochondrial Ca^{2+} efflux mediated by NCLX fully depends on the transpresence of Na^+, and that mitochondrial Ca^{2+} efflux by NCLX is coupled to Na^+ influx. Remarkably, Li^+ could effectively replace Na^+ in promoting mitochondrial Ca^{2+} efflux. Thus, the fortuitous mislocalization of NCLX to the cell membrane induced by overexpression in HEK 293 cells led to the identification that NCLX is the long-sought mitochondrial Na^+/Ca^{2+} exchanger [4]. In the following, we will describe some of the studies using the molecular tools derived from NCLX identification and discuss their physiological implications.

2 NCLX controls antigen-dependent Ca^{2+} response in lymphocytes

Ca^{2+} is a major second messenger in lymphocytes. Binding of antigens to their receptors triggers a robust Ca^{2+} response that is controlled by the crosstalk between the store-operated Ca^{2+} pathway, which consists of two major components. The first is STIM, a Ca^{2+} sensor in the ER that upon depletion of the ER stores forms dimers that activate the store-sensitive plasma membrane Ca^{2+} channel, Orai. The second component is the ER, a major store of Ca^{2+} that releases Ca^{2+} upon activation of the inositol trisphosphate (IP3) receptor Ca^{2+} channel on the ER. Interestingly, the mitochondria network is highly dynamic in lymphocytes and often moves toward the immunological synapse participating in Ca^{2+} signaling in these cells [20]. In their study, Kim et al. used DT40, a haploid lymphocytic cell line with a specific NCLX knockdown. They found that following NCLX knockdown the mitochondrial Ca^{2+} efflux was completely blocked, thus confirming the role of NCLX as a mitochondrial exchanger and a major Ca^{2+} efflux pathway. Remarkably, they also found that the cytosolic antigen-dependent Ca^{2+} response was fully blocked. By monitoring the cytosolic and ER Ca^{2+} response, they concluded that the reduction in the cytosolic Ca^{2+} signal was linked to impaired refilling of the ER store. Indeed, they demonstrated a tight physical interaction between the ER and mitochondria in lymphocytes. These findings are consistent with previous studies that showed a direct Ca^{2+} transport route between the mitochondria and ER, suggesting that NCLX activity is an important component in mitochondria to ER Ca^{2+} passage [21,22]. Depletion of the ER Ca^{2+} stores is likely to activate the store-operated Ca^{2+} channel. It will be interesting to determine how this pathway is affected by the activity of NCLX.

3 NCLX and Ca^{2+} signaling in astrocytes

Ca^{2+} signaling is also central in controlling astrocyte function and in mediating astrocyte-neuron communication. However, although the activity of the mitochondrial exchanger in response to glutamate signaling has been suggested [23], its role in shaping global astrocytic Ca^{2+} signaling or in controlling their executive functions is unknown. To determine whether NCLX is expressed and whether its expression can be molecularly controlled in astrocytes, Parnis et al. [24] conducted Western blot analysis of siControl- versus siNCLX-treated astrocytes and found that it localized in the astrocyte's mitochondria and its expression could be molecularly controlled. To determine its role in controlling the ER versus the store-operated Ca^{2+} channel, the cytosolic Ca^{2+} response was monitored in the presence or absence of extracellular Ca^{2+}. Remarkably and in contrast to the results obtained in lymphocytes, the knockdown of NCLX expression changed the cytosolic Ca^{2+} response only in the presence of extracellular Ca^{2+}, indicating that it primarily shapes the store-operated Ca^{2+} response. Indeed, a parallel experiment monitoring the mitochondrial Ca^{2+} response indicated that the store-operated Ca^{2+} response evoked a much greater mitochondrial Ca^{2+} response compared with that by an ER-evoked signal. The diverse effects of NCLX activity in lymphocytes compared with astrocytes indicate that NCLX in particular and the mitochondria in general have a highly versatile role in controlling Ca^{2+} signaling in various cell types. This study further determined how NCLX is linked to the executive function of glial cells. A major finding of this study was that while NCLX does not play a major role in controlling the mechanically evoked Ca^{2+} wave that primarily originates in the ER store, it has a profound effect on astrocytic glutamate release. The latter function is required for glia-neuron communication. Because the secretory process depends on Ca^{2+} signaling at the plasma membrane domain, it involves the dominant role of NCLX in regulating the store-operated Ca^{2+} response. In addition, NCLX activity or expression is linked to the proliferation and migration of astrocytes. Similarly to secretory processes, this function strongly depends on Ca^{2+} signaling that initiates at the plasma membrane domain. An open and intriguing question is the role of this exchanger in neurons, and the same strategy can now be used to study the neuronal role of mitochondrial Na^+/Ca^{2+} exchange.

4 The mitochondrial Na^+/Ca^{2+} exchange in Ca^{2+} signaling is linked to insulin secretion

The link between metabolic activity and Ca^{2+} signaling is fundamental in pancreatic β cells. The textbook dogma for insulin secretion is that the enhanced formation of ATP by glucose entry leads to closure of the K^+-ATP channel, culminating in the activation of the voltage-gated Ca^{2+} channel, and the subsequent rise in Ca^{2+} triggers insulin secretion [25]. Although the mitochondrial Ca^{2+} signal has a profound role in controlling ATP production, this aspect is much less understood. Two conflicting papers were published on the role of the mitochondrial exchanger in insulin secretion. The first study reasoned that because this exchanger lowers mitochondrial Ca^{2+} blocking, its activity can be used to enhance ATP production and thereby insulin secretion. Consistent with this hypothesis, application of the mitochondrial exchanger blocker, CGP-37157, in cell culture and *in vivo* augmented metabolic activity and insulin secretion, indicating that blocking the exchanger can be used as a therapeutic strategy [26]. Although CGP-37157 is a potent blocker of the mitochondrial Na^+/Ca^{2+} exchanger, a growing number of studies have reported that it is a high-affinity blocker of the L-Type Ca^{2+} channels [27]. Consistent with this concern, a

study that monitored the glucose-dependent cytosolic Ca^{2+} response suggested that application of CGP-37157 lowered the Ca^{2+} signal in pancreatic β cells by blocking the L-Type Ca^{2+} channel [28].

To address the limitation and concern of using exchanger blockers, the role of NCLX was revisited using the RNA interference and dominant negative molecular tools aimed to control its expression and activity. Lowering NCLX expression and activity in the pancreatic β cell line, MIN6, or in primary pancreatic β cells was followed by a reduction in mitochondrial Ca^{2+} efflux. Notably, reducing NCLX expression or activity was followed by a reduction in the glucose-dependent mitochondrial Ca^{2+} efflux. Remarkably, knocking down NCLX activity was followed by attenuation of the rate of the glucose-dependent Ca^{2+} rise, indicating that mitochondrial Ca^{2+} shuttling is required for enhancing the glucose-dependent cytosolic Ca^{2+} response. Analysis of the metabolic rate indicated that knocking down the expression of NCLX reduced the basal metabolic rate and had no effect on the glucose-dependent rise in metabolic activity. Thus, these results indicate that knockdown of NCLX activity, in contrast to results obtained by blockers, does not have the potential to boost glucose-dependent ATP production [29]. Consistent with the role of NCLX in upregulating the glucose-dependent cytosolic Ca^{2+} rise, knockdown of NCLX expression reduced the initial rate of glucose-dependent insulin secretion. The latter effect is consistent with the need for a robust Ca^{2+} rise to activate the first phase of insulin secretion [30].

While most of the attention is focused on the role of the exchanger in Ca^{2+} signaling, it is important to remember that it is also a major Na^+ transporter that passes at least three Na^+ cations per one Ca^{2+} cation and that it constitutes the major mitochondrial Na^+ uptake pathway. Another major but poorly studied Na^+ pathway in pancreatic β cells is the voltage-gated Na^+ channels. Using TTX, a selective toxin blocker of these channels, and by molecularly controlling the expression of the mitochondrial uniporter, MCU, and of NCLX, we asked whether a Na^+-dependent crosstalk between the plasma membrane and the mitochondrial domain controls the glucose-dependent Ca^{2+} signaling. Glucose application was followed by a rise in cytosolic Na^+ that was blocked by TTX, indicating that this channel mediates a glucose-dependent cytosolic Na^+ signaling. The cytosolic Na^+ signal was followed by mitochondrial Na^+ uptake that was blocked by knockdown of NCLX expression, indicating that it is the major Na^+ influx pathway in mitochondria. Na^+ channels also elicit a strong glucose-dependent Ca^{2+} rise by depolarizing the cells and thereby strongly upregulating the firing rate and Ca^{2+} influx of the L-Type Ca^{2+} channels. This cytosolic Ca^{2+} response was propagated by mitochondrial Ca^{2+} uptake that was followed by enhanced metabolic activity and ATP production. The parallel activation of the mitochondrial NCLX paces the duration of the mitochondrial Ca^{2+} transient. Thus, the voltage-gated Na^+ channels play a dual role in pancreatic β cells in triggering Na^+ and Ca^{2+} signals. Both signals propagate into the mitochondria and control the metabolic activity, thereby controlling metabolic activity and secretion [31].

5 The role of NCLX in neurons and the use of exchanger inhibitors

Reversal of the plasma membrane exchanger in cardiomyocytes and neurons can trigger a cytosolic Ca^{2+} overload that is linked to cardiac and brain damage. Studies using the mitochondrial exchanger blocker have assigned a similar role to the mitochondrial Na^+/Ca^{2+} exchanger in this process. These studies, which used CGP-37157, suggested that using this blocker protected neurons in brain slices undergoing ischemic insults. To determine whether the CGP-37157 effect is linked to its effect on the exchanger or on the L-Type Ca^{2+} channel, the effect of CGP-37157 was compared with that of the classical dihydropyridine blockers of the L-Type Ca^{2+} channel on mitochondrial and cytosolic responses under normal and toxic conditions. While CGP-37157 effectively reduced the mitochondrial Ca^{2+} efflux, it also reduced the cytosolic Ca^{2+} rise and neuronal cell death. The latter effect could be fully reproduced by nifedipine, a classical L-Type Ca^{2+} channel blocker that does not modulate NCLX activity [32]. This study, therefore, warrants reconsideration of the role of the exchanger in neuronal survival by molecular tools aimed at controlling NCLX expression or by an NCLX knockdown mouse model.

6 The cardiac role of NCLX

Because of the rapid pacing and energy requirements of the heart, the mitochondrial Ca^{2+} transport via the Na^+/Ca^{2+} exchanger that couples Na^+ and Ca^{2+} exchange is of particular interest. Several intriguing studies have focused on the role of this exchange in ischemia and suggested that the rise in cytosolic Na^+ encountered during ischemic insult may enhance the exchange activity, thus depleting mitochondrial Ca^{2+}, leading to downregulation of the metabolic rate and ATP production [33,34]. Studies performed on tissue fractions obtained from cardiac tissue have, however, suggested that CGP-37157 also interacts with the ryanodine receptor, SERCA, and the L-Type Ca^{2+} channel in this tissue [35]. Therefore, molecular control is particularly important for determining the mitochondrial exchanger role in this tissue. A recent study has started to address this concern by molecularly controlling NCLX expression in the cardiomyocyte cell line, HL-1 [36]. This study has shown that siNCLX can be effectively used to control NCLX expression and to control its activity in cardiac mitochondria. The knockdown of NCLX expression was followed by a rise in basal free mi-

tochondria Ca^{2+} level. Remarkably, knockdown of NCLX was followed by a change in the kinetic parameters of action potential and Ca^{2+} transients. In particular, the cycle length of action potential and Ca^{2+} transients was prolonged. The authors suggested that the NCLX effect on these kinetic parameters is linked to its role in controlling the refilling of the sarcoplasmic reticulum (SR) Ca^{2+} stores. Knockdown of NCLX expression was followed by a strong decrease in refilling the SR Ca^{2+} stores, thus lowering the SR free Ca^{2+} content and SR ability to take up Ca^{2+}. Thus, this study indicates that the interaction of the mitochondrial exchanger with the SR Ca^{2+} stores plays a general role in cardiac pacing. There are, however, major morphological and functional differences among cell line, primary cardiomyocytes and cardiac tissue, as well as major morphological and functional differences regarding the interaction of SR with the cytosolic and plasma membrane. In addition, whether mitochondria regulate cytosolic Ca^{2+} on a bit-to-bit basis is controversial. Therefore, viral vector and mouse knockdown models will be required to address the physiological role of NCLX in the heart and its importance during pathophysiological syndromes.

7 The role of the mitochondrial Na^+/Ca^{2+} exchanger, NCLX, versus H^+/Ca^{2+} exchanger in mitochondrial Ca^{2+} signaling

Mitochondrial Ca^{2+} efflux is thought to be mediated by two pathways, the Na^+/Ca^{2+} exchanger, NCLX, and the H^+/Ca^{2+} exchanger. Leucine zipper, EF-hand, transmembrane mitochondrial protein 1 (Letm1) emerged as a candidate transporter for mediating H^+/Ca^{2+} exchange [6]. While Letm1 was first shown to mediate K^+/H^+ exchange [37], subsequent studies suggested that it mediates an electrogenic ruthenium red-sensitive H^+/Ca^{2+} exchange [6]. Subsequent studies performed on purified and reconstituted Letm1 at a higher density indicated that it mediates a very slow (two cycles s^{-1}) electroneutral ruthenium red-insensitive $2H^+/Ca^{2+}$ exchange [5]. To compare their role in Ca^{2+} transport, NCLX and Letm1 were expressed in HeLa cells and their mitochondrial transport rate was determined. Overexpression of NCLX increased the rate of mitochondrial Ca^{2+} efflux, while overexpression of Letm1 had no apparent effect [38]. The estimated turnover rate of NCLX is at least 1000 cycles per second, and its electrogenic $3Na^+/Ca^{2+}$ transport mode indicates that it can effectively use the steep-180 mV mitochondrial membrane potential to pump Ca^{2+} out of the mitochondria. The low electroneutral mode of operation (two cycles s^{-1}) of Letm1 suggests that it has a much lower capacity of mediating mitochondrial Ca^{2+} efflux. Thus, the physiological relevance of Ca^{2+} transport mediated by Letm1 remains to be determined. Further analysis of primary liver tissue, where mitochondrial H^+/Ca^{2+} exchange is most convincing, can also be highly informative for addressing the elusive role of this transporter and to determine whether the major activity is K^+/H^+ or $2H^+/Ca^{2+}$ exchange.

8 Conclusion and future directions

The original identification of NCLX as a mitochondrial exchanger is now supported by several studies that molecularly linked it to the mitochondrial Na^+/Ca^{2+} exchange in multiple cell types ranging from neurons to pancreatic β cells, lymphocytes and cardiomyocytes. Several studies have also highlighted the risk of solely relying on the pharmacological inhibition of this exchanger by CGP-37157. While the latter is a highly potent blocker of the mitochondrial exchanger, it is also an effective inhibitor of other major Ca^{2+} pathway components, most notably the L-Type Ca^{2+} channel. Several studies have indicated that the controversy regarding the role of the exchanger in pancreatic β cells or during brain ischemia may at least be partly related to the effect of CGP-37157 on the Ca^{2+} channel. Molecular 3D modeling of NCLX based on the resolved structure of other NCX membranes can provide a platform for novel agonists and antagonists that may be more selective.

Despite the considerable progress made, there are many unresolved questions regarding the mode of NCLX activity and its physiological role that need to be addressed. Some examples are as follows:

(i) Mode of NCLX regulation. The NCX members of the Na^+/Ca^{2+} exchange family use an allosteric Ca^{2+}-binding domain called CBD that plays a critical role in controlling the activity of this exchanger [39]. NCLX does not have a similar domain; however, previous studies have suggested that the mitochondrial Na^+/Ca^{2+} exchange may be regulated by kinases such as protein kinase C and Pink-1 [40]. *In silico* analysis of the NCLX sequence indicated that it has several putative kinase-binding or phosphorylation sites, and biochemical analysis can now be combined with physiological monitoring of NCLX activity, to determine the major regulatory factors and their mode of regulation.

(ii) The NCLX counter partner MCU interacts with several accessory units, among them MICU1 and MCU1 that have a profound effect on its activity. In fact, MCU is part of a multi-protein complex [1]. Considering the major role of mitochondrial Ca^{2+} efflux, it is therefore plausible that NCLX is part of this or another complex that controls mitochondrial Na^+/Ca^{2+} exchange. Therefore, proteomic analysis of mitochondrial proteins that interact with NCLX and functional studies of their physiological importance are of high priority.

Analysis of MCU mouse knockdown and human subjects with MCU mutations suggests that it is of particular importance for skeletal muscle and brain activity [41,42]. It will be interesting to see whether a similar phenotype will be found for NCLX or whether NCLX deficiency will be

linked to a distinct set of functions. Another intriguing concept is whether mutations that hinder NCLX expression and/or activity are associated with human diseases, particularly those associated with mitochondrial Ca^{2+} overload. The identification of NCLX finally enables us to address this issue and work is in progress to understand its physiological role and pathophysiological implications.

1 Baughman JM, Perocchi F, Girgis HS, Plovanich M, Belcher-Timme CA, Sancak Y, Bao XR, Strittmatter L, Goldberger O, Bogorad RL, Koteliansky V, Mootha VK. Integrative genomics identifies MCU as an essential component of the mitochondrial calcium uniporter. Nature, 2011, 476: 341–345

2 De Stefani D, Raffaello A, Teardo E, Szabo I, Rizzuto R. A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature, 2011, 476: 336–340

3 Carafoli E, Tiozzo R, Lugli G, Crovetti F, Kratzing C. The release of calcium from heart mitochondria by sodium. J Mol Cell Cardiol, 1974, 6: 361–371

4 Palty R1, Silverman WF, Hershfinkel M, Caporale T, Sensi SL, Parnis J, Nolte C, Fishman D, Shoshan-Barmatz V, Herrmann S, Khananshvili D, Sekler I. NCLX is an essential component of mitochondrial Na^+/Ca^{2+} exchange. Proc Natl Acad Sci USA, 2010, 107: 436–441

5 Tsai MF, Jiang D, Zhao L, Clapham D, Miller C. Functional reconstitution of the mitochondrial Ca^{2+}/H^+ antiporter Letm1. J Gen Physiol, 2014, 143: 67–73

6 Jiang D, Zhao L, Clapham DE. Genome-wide RNAi screen identifies Letm1 as a mitochondrial Ca^{2+}/H^+ antiporter. Science, 2009, 326: 144–147

7 Rizzuto R, Simpson AW, Brini M, Pozzan T. Rapid changes of mitochondrial Ca^{2+} revealed by specifically targeted recombinant aequorin. Nature, 1992, 358: 325–327

8 Rizzuto R, Pozzan T. Microdomains of intracellular Ca^{2+}: molecular determinants and functional consequences. Physiol Rev, 2006, 86: 369–408

9 Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca^{2+} responses. Science, 1998, 280: 1763–1766

10 Rizzuto R, Duchen MR, Pozzan T. Flirting in little space: the ER/mitochondria Ca^{2+} liaison. Sci STKE, 2004, 2004: re1

11 11. Rizzuto R, Pinton P, Brini M, Chiesa A, Filippin L, Pozzan T. Mitochondria as biosensors of calcium microdomains. Cell Calcium, 1999, 26: 193–199

12 Rizzuto R, De Stefani D, Raffaello A, Mammucari C. Mitochondria as sensors and regulators of calcium signalling. Nat Rev Mol Cell Biol, 2012, 13: 566–578

13 Duchen MR. Roles of mitochondria in health and disease. Diabetes, 2004, 53(Suppl 1): S96–102

14 Perocchi F, Gohil VM, Girgis HS, Bao XR, McCombs JE, Palmer AE, Mootha VK. MICU1 encodes a mitochondrial EF hand protein required for Ca(2+) uptake. Nature, 2010, 467: 291–296

15 Patron M, Raffaello A, Granatiero V, Tosatto A, Merli G, De Stefani D, Wright L, Pallafacchina G, Terrin A, Mammucari C, Rizzuto R. The mitochondrial calcium uniporter (MCU): molecular identity and physiological roles. J Biol Chem, 2013, 288: 10750–10758

16 Raffaello A, De Stefani D, Rizzuto R. The mitochondrial Ca(2+) uniporter. Cell Calcium, 2012, 52: 16–21

17 Marchi S, Pinton P. The mitochondrial calcium uniporter complex: molecular components, structure and physiopathological implications. J Physiol, 2014, 592: 829–839

18 Paucek P, Jaburek M. Kinetics and ion specificity of Na(+)/Ca(2+) exchange mediated by the reconstituted beef heart mitochondrial Na(+)/Ca(2+) antiporter. Biochim Biophys Acta, 2004, 1659: 83–91

19 Palty R, Ohana E, Hershfinkel M, Volokita M, Elgazar V, Beharier O, Silverman WF, Argaman M, Sekler I. Lithium-calcium exchange is mediated by a distinct potassium-independent sodium-calcium exchanger. J Biol Chem, 2004, 1: 1

20 Kim B, Takeuchi A, Koga O, Hikida M, Matsuoka S. Pivotal role of mitochondrial Na^+-Ca^{2+} exchange in antigen receptor mediated Ca^{2+} signalling in DT40 and A20 B lymphocytes. J Physiol, 2012, 590: 459–474

21 Csordas G, Varnai P, Golenar T, Sheu SS, Hajnoczky G. Calcium transport across the inner mitochondrial membrane: molecular mechanisms and pharmacology. Mol Cell Endocrinol, 2012, 353: 109–113

22 Csordás G, Golenár T, Seifert EL, Kamer KJ, Sancak Y, Perocchi F, Moffat C, Weaver D, de la Fuente Perez S, Bogorad R, Koteliansky V, Adijanto J, Mootha VK, Hajnóczky G. MICU1 controls both the threshold and cooperative activation of the mitochondrial Ca^{2+} uniporter. Cell Metab, 2013, 17: 976–987

23 Reyes RC, Parpura V. Mitochondria modulate Ca^{2+}-dependent glutamate release from rat cortical astrocytes. J Neurosci, 2008, 28: 9682–9691

24 Parnis J, Montana V, Delgado-Martinez I, Matyash V, Parpura V, Kettenmann H, Sekler I, Nolte C. Mitochondrial exchanger NCLX plays a major role in the intracellular Ca^{2+} signaling, gliotransmission, and proliferation of astrocytes. J Neurosci, 2013, 33: 7206–7219

25 Ashcroft SJ, Weerasinghe LC, Randle PJ. Interrelationship of islet metabolism, adenosine triphosphate content and insulin release. Biochem J, 1973, 132: 223–231

26 Lee B, Miles PD, Vargas L, Luan P, Glasco S, Kushnareva Y, Kornbrust ES, Grako KA, Wollheim CB, Maechler P, Olefsky JM, Anderson CM. Inhibition of mitochondrial Na^+-Ca^{2+} exchanger increases mitochondrial metabolism and potentiates glucose-stimulated insulin secretion in rat pancreatic islets. Diabetes, 2003, 52: 965–973

27 Thu le T, Ahn JR, Woo SH. Inhibition of L-type Ca^{2+} channel by mitochondrial Na^+-Ca^{2+} exchange inhibitor CGP-37157 in rat atrial myocytes. Eur J Pharmacol, 2006, 552: 15–19

28 Luciani DS, Ao P, Hu X, Warnock GL, Johnson JD. Voltage-gated Ca^{2+} influx and insulin secretion in human and mouse beta-cells are impaired by the mitochondrial Na^+/Ca^{2+} exchange inhibitor CGP-37157. Eur J Pharmacol, 2007, 576: 18–25

29 Nita II, Hershfinkel M, Fishman D, Ozeri E, Rutter GA, Sensi SL, Khananshvili D, Lewis EC, Sekler I. The mitochondrial Na^+/Ca^{2+} exchanger upregulates glucose dependent Ca^{2+} signalling linked to insulin secretion. PLoS One, 2012, 7: e46649

30 Barg S, Ma X, Eliasson L, Galvanovskis J, Göpel SO, Obermüller S, Platzer J, Renström E, Trus M, Atlas D, Striessnig J, Rorsman P. Fast exocytosis with few Ca^{2+} channels in insulin-secreting mouse pancreatic B cells. Biophys J, 2001, 81: 3308–3323

31 Nita II, Hershfinkel M, Kantor C, Rutter GA, Lewis EC, Sekler I. Pancreatic beta-cell Na^+ channels control global Ca^{2+} signaling and oxidative metabolism by inducing Na^+ and Ca^{2+} responses that are propagated into mitochondria. FASEB J, 2014, 28: 3301–3312

32 Ruiz A, Alberdi E, Matute C. CGP37157, an inhibitor of the mitochondrial Na^+/Ca^{2+} exchanger, protects neurons from excitotoxicity by blocking voltage-gated Ca^{2+} channels. Cell Death Dis, 2014, 5: e1156

33 Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, O'Rourke B. Elevated cytosolic Na^+ decreases mitochondrial Ca^{2+} uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. Circ Res, 2006, 99: 172–182

34 Boyman L, Williams GS, Khananshvili D, Sekler I, Lederer WJ. NCLX: the mitochondrial sodium calcium exchanger. J Mol Cell Cardiol, 2013, 59: 205–213

35 Neumann JT, Diaz-Sylvester PL, Fleischer S, Copello JA. CGP-37157 inhibits the sarcoplasmic reticulum Ca^{2+} ATPase and activates ryanodine receptor channels in striated muscle. Mol Pharmacol, 2011, 79: 141–147

36 Takeuchi A, Kim B, Matsuoka S. The mitochondrial Na^+-Ca^{2+} exchanger, NCLX, regulates automaticity of HL-1 cardiomyocytes. Sci Rep, 2013, 3: 2766

37 Nowikovsky K, Froschauer EM, Zsurka G, Samaj J, Reipert S, Kolisek M, Wiesenberger G, Schweyen RJ. The LETM1/YOL027

gene family encodes a factor of the mitochondrial K^+ homeostasis with a potential role in the Wolf-Hirschhorn syndrome. J Biol Chem, 2004, 279: 30307–30315

38 de Marchi U, Santo Domingo J, Castelbou C, Sekler I, Wiederkehr A, Demaurex N. NCLX, but not LETM1, mediates mitochondrial Ca^{2+} extrusion thereby limiting Ca^{2+}-induced NAD(P)H production and modulating matrix redox state. J Biol Chem, 2014, 289: 20377–20385

39 Blaustein MP, Lederer WJ. Sodium/calcium exchange: its physiological implications. Physiol Rev, 1999, 79: 763–854

40 Gandhi S, Wood-Kaczmar A, Yao Z, Plun-Favreau H, Deas E, Klupsch K, Downward J, Latchman DS, Tabrizi SJ, Wood NW, Duchen MR, Abramov AY. PINK1-associated Parkinson's disease is caused by neuronal vulnerability to calcium-induced cell death. Mol Cell, 2009, 33: 627–638

41 Pan X, Liu J, Nguyen T, Liu C, Sun J, Teng Y, Fergusson MM, Rovira II, Allen M, Springer DA, Aponte AM, Gucek M, Balaban RS, Murphy E, Finkel T. The physiological role of mitochondrial calcium revealed by mice lacking the mitochondrial calcium uniporter. Nat Cell Biol, 2013, 15: 1464–1472

42 Logan CV, Szabadkai G, Sharpe JA, Parry DA, Torelli S, Childs AM, Kriek M, Phadke R, Johnson CA, Roberts NY, Bonthron DT, Pysden KA, Whyte T, Munteanu I, Foley AR, Wheway G, Szymanska K, Natarajan S, Abdelhamed ZA, Morgan JE, Roper H, Santen GW, Niks EH, van der Pol WL, Lindhout D, Raffaello A, De Stefani D, den Dunnen JT, Sun Y, Ginjaar I, Sewry CA, Hurles M, Rizzuto R; UK10K Consortium, Duchen MR, Muntoni F, Sheridan E. Loss-of-function mutations in MICU1 cause a brain and muscle disorder linked to primary alterations in mitochondrial calcium signaling. Nat Genet, 2014, 46: 188–193