

Molecular Identity and Functional Properties of the Mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ Exchanger*

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The mitochondrial membrane potential that powers the generation of ATP also facilitates mitochondrial Ca^{2+} shuttling. This process is fundamental to a wide range of cellular activities, as it regulates ATP production, shapes cytosolic and endoplasmic reticulum Ca^{2+} signaling, and determines cell fate. Mitochondrial Ca^{2+} transport is mediated primarily by two major transporters: a Ca^{2+} uniporter that mediates Ca^{2+} uptake and a $\text{Na}^+/\text{Ca}^{2+}$ exchanger that subsequently extrudes mitochondrial Ca^{2+} . In this minireview, we focus on the specific role of the mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger and describe its ion exchange mechanism, regulation by ions, and putative partner proteins. We discuss the recent molecular identification of the mitochondrial exchanger and how its activity is linked to physiological and pathophysiological processes.

The movement of calcium ions in and out of mitochondria is mediated through two membranes. The first is the outer membrane, which is relatively permeable to ions and small molecules; via this membrane, Ca^{2+} transport is facilitated by the non-selective voltage-dependent anion channel (1). The second is the inner mitochondrial membrane, where Ca^{2+} transport is tightly regulated and ion translocation depends on the activity of specialized calcium transporters. Inward calcium flux across this membrane is mediated primarily via a highly selective pathway termed the mitochondrial Ca^{2+} uniporter (MCU).³ The MCU displays the electrophysiological characteristics of a gated inwardly rectifying Ca^{2+} channel with low affinity and high Ca^{2+} selectivity (2). Conversely, outward calcium flux is mediated primarily by a mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) and also by a $\text{H}^+/\text{Ca}^{2+}$ exchanger. The steep membrane potential (mitochondrial $\Delta\psi$) of ~ 180 mV (negative inside) across the mitochondrial inner membrane is the main driving force for mitochondrial Ca^{2+} entry. It allows mitochondria to

rapidly take up Ca^{2+} from strategically located microdomains of high Ca^{2+} near the plasma membrane and endoplasmic reticulum (ER), generated during Ca^{2+} -signaling events. As Ca^{2+} enters the mitochondria, it is partially buffered through the formation of calcium phosphate complexes. The ability to take up substantial amounts of Ca^{2+} underlines two principal roles of mitochondria; it enables mitochondria to shape the spatiotemporal profile of cytosolic Ca^{2+} signals and to simultaneously transmit these events into the mitochondrial matrix. In the matrix, Ca^{2+} up-regulates the activity of Ca^{2+} -sensitive dehydrogenases of the Krebs cycle and the F_0F_1 -ATP synthase (3), thereby controlling the rate of ATP production. A pathological aspect of this process is linked to mitochondrial Ca^{2+} overload that may trigger the activation of the mitochondrial permeability transition pore, which in turn releases apoptotic and necrotic signal factors, leading to cell death (4). To restore resting mitochondrial Ca^{2+} levels, massive amounts of accumulated Ca^{2+} are extruded from the mitochondria mainly through the activity of the mitochondrial NCX. This process of delayed mitochondrial Ca^{2+} release plays a dual role in refilling the ER Ca^{2+} stores and in shaping the amplitude and duration of the cytosolic Ca^{2+} signal. Because of its strict Na^+ dependence, the mitochondrial NCX links between cytosolic Na^+ elevations and mitochondrial Ca^{2+} release. Despite the well recognized importance of mitochondrial Ca^{2+} shuttling in cellular biology, documented for almost half a century, the molecular identities of the major mitochondrial Ca^{2+} -shuttling transporters, *i.e.* the Ca^{2+} uniporter and the NCX, remained elusive. In the last 2 years, promising candidates of the NCX, the $\text{Na}^+/\text{Ca}^{2+}/\text{Li}^+$ exchanger (NCLX) (5) and the MCU (6, 7), were discovered. Here, we briefly describe several functional, molecular, kinetic, and regulatory aspects of the mitochondrial NCX, review its molecular identification, and discuss its physiological and pathophysiological roles. The interested reader is also referred to excellent reviews describing in more detail the physiological implications of mitochondrial Ca^{2+} shuttling (8, 9) and the role of the mitochondrial NCX (10, 11).

Mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ Exchange, Discovery, Ionic Selectivity, and Inhibitors

Carafoli *et al.* (12) were the first to discover that Na^+ induces the release of Ca^{2+} from heart mitochondria (Fig. 1). Mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange was found to be rather selective toward Ca^{2+} , as Mg^{2+} (12) and Mn^{2+} (13) were not transported by the exchanger. On the other hand, the exchanger is only moderately selective for Na^+ , as Li^+ can substitute for this ion (Fig. 1) to evoke mitochondrial Ca^{2+} release, whereas Cs^+ , K^+ , and Rb^+ fail to do so (12, 14). Importantly, this functional fingerprint differs from the Li^+ inert transport mediated by plasma membrane NCX transporters and was later used to identify the mitochondrial NCX. Mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange is regulated by various ions and molecules. Micromolar concentrations of cytosolic Ca^{2+} induced a partial ($\sim 70\%$) inhibition of mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange (15), and the cooperative inhibitory profile indicates that Ca^{2+} interacts with

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³ The abbreviations used are: MCU, mitochondrial Ca^{2+} uniporter; NCX, $\text{Na}^+/\text{Ca}^{2+}$ exchanger; NCLX, $\text{Na}^+/\text{Ca}^{2+}/\text{Li}^+$ exchanger; ER, endoplasmic reticulum; SR, sarcoplasmic reticulum; SERCA, sarco/endoplasmic reticulum Ca^{2+} -ATPase; TISP, tetanic-induced synaptic potentiation; PD, Parkinson disease; HD, Huntington disease; AD, Alzheimer disease.

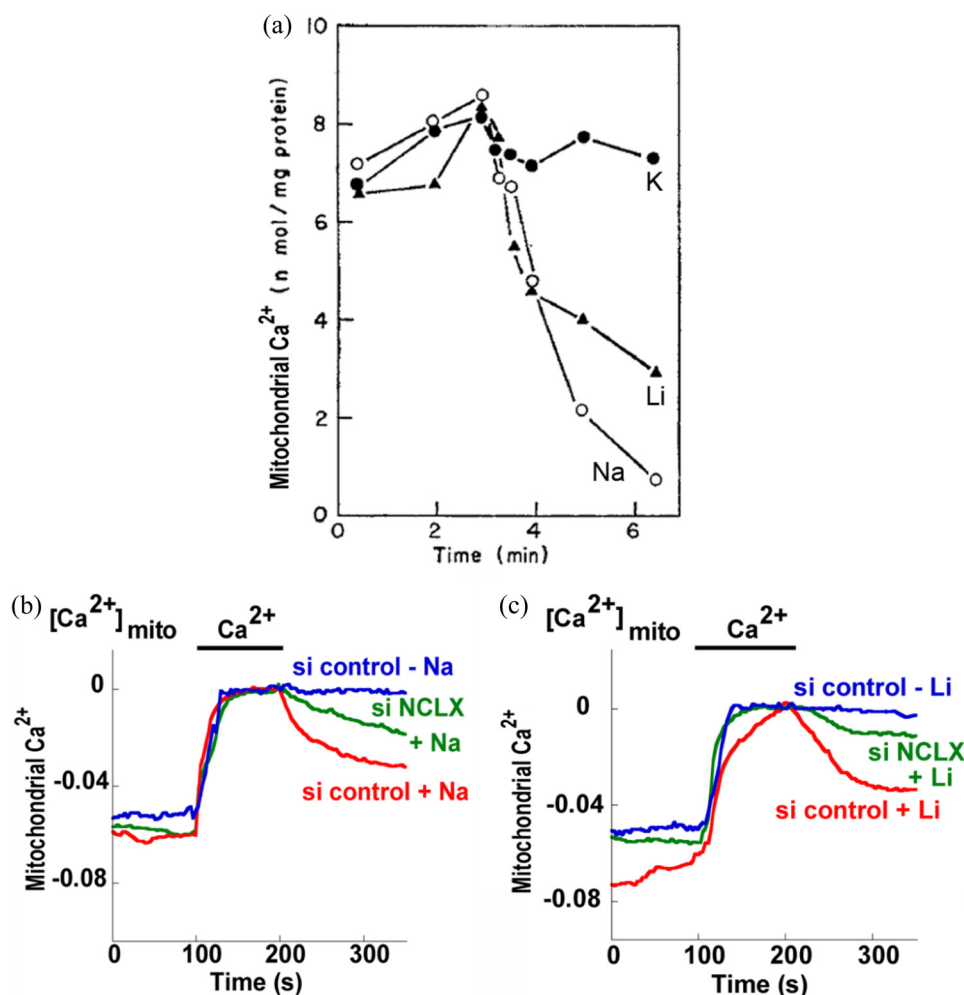


FIGURE 1. **Functional and molecular identification of the mitochondrial NCX.** *a*, the first evidence for Na^+ - or Li^+ -dependent mitochondrial Ca^{2+} exchange. Original data were taken from Ref. 12. Li^+ (\blacktriangle), Na^+ (\circ), or K^+ (\bullet) was added to isolated mitochondria while monitoring Ca^{2+} efflux. *b* and *c*, note that Na^+ promoted the mitochondrial efflux of Ca^{2+} , demonstrating a $\text{Na}^+/\text{Ca}^{2+}$ exchange pathway in this organelle. Unlike the plasma membrane NCX family members, the mitochondrial exchanger also mediated $\text{Li}^+/\text{Ca}^{2+}$ exchange, indicating that it has distinct functional properties. NCLX was identified as the mitochondrial exchanger. Studies were conducted on cells expressing the mitochondrial Ca^{2+} sensor mito-pericam. Knocking down the expression of mitochondrial NCLX using NCLX siRNA (*si NCLX*) abolished either Na^+ -dependent (*b*) or Li^+ -dependent (*c*) mitochondrial Ca^{2+} efflux. Thus, NCLX shares similar functional properties with the mitochondrial NCX. Original data were taken from Ref. 5. *si control*, control siRNA.

several cytoplasm-facing sites of the exchanger. NCLX does not share the hallmark cytosolic Ca^{2+} regulatory site of plasma membrane NCX proteins.⁴ Calcium-binding sites are, however, often elusive, and further molecular analysis is required to determine whether they are present on NCLX. Mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange is also regulated by a narrow range of pH 7.5–7.6, but whether this type of regulation is mediated by a pH sensor on the exchanger or by an interplay with other mitochondrial ion transporters such as the mitochondrial Na^+/H^+ exchanger is unclear (16). Potentiation of NCX activity is induced by K^+ ions (17, 18), by BSA (18), and by short chain alkanols (Fig. 2) (19). In contrast, inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange is induced by several ions such as Zn^{2+} , Co^{2+} , Sr^{2+} , Ni^{2+} , Mg^{2+} , Ba^{2+} , and La^{3+} (15, 20–23). Several compounds inhibit mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange. Among them are CGP-37157 (24, 25), tetraphenylphosphonium (21), trifluoperazine (15), diltiazem (24), verapamil (26), clonazepam (24), amiloride (27), and cyclosporin A (28). The most selective and

effective inhibitor of mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange is the CGP-37157 compound (24, 25). The affinity of CGP-37157 is at least 10-fold higher than that of any other blocker, and when tested in intact cells, CGP-37157 did not interfere with contraction and did not affect Ca^{2+} fluxes through L-type Ca^{2+} channels or the activity of the ER Ca^{2+} pump sarco/endoplasmic reticulum Ca^{2+} -ATPase (SERCA), in isolated mitochondria and cardiomyocytes (29, 30). Furthermore, an at least 10-fold higher concentration of CGP-37157 is required to inhibit the plasma membrane NCX isoforms (31). Due to these properties, most subsequent studies employed CGP-37157 at 10 μM or below. Recent studies suggest, however, that even low concentrations of CGP-37157 may modulate other Ca^{2+} transporters. For example, when the effect of CGP-37157 was determined in cardiac sarcoplasmic reticulum (SR) microsomes reconstituted into lipid bilayers, it augmented Ca^{2+} flux via the ryanodine receptors and blocked the SR Ca^{2+} pump SERCA (32). This finding suggested that CGP-37157 displays, as do other benzothiazepines, a complex pattern of interference with transporters involved in Ca^{2+} signaling. A noteworthy example is the

⁴I. Sekler, personal communication.

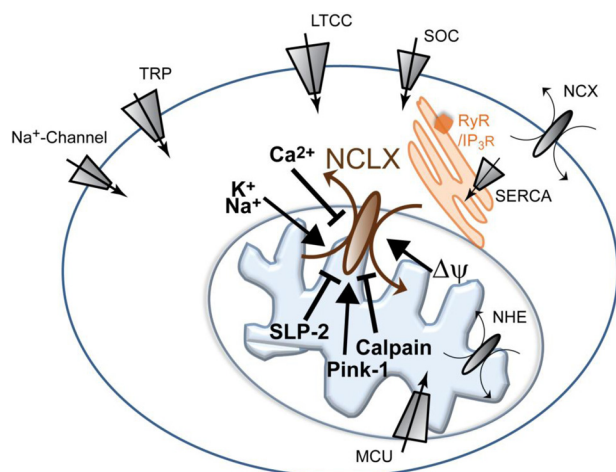


FIGURE 2. Schematic representation of ions, transporters, and kinases that interact or modulate the activity of the mitochondrial NCX. Functional cross-talk between the ER and plasma membrane fluxes of Na^+ and Ca^{2+} regulates the activity of the mitochondrial NCX, which in turn controls the Ca^{2+} content in the ER or plasma membrane microdomain and Ca^{2+} signaling in the cytosol. NHE, Na^+/H^+ exchanger; RyR, ryanodine receptor; IP_3R , inositol trisphosphate receptor; SOC, store-operated calcium channel; LTCC, L-type Ca^{2+} channel; TRP, transient receptor potential channel.

interaction of this compound with the L-type Ca^{2+} channel, which led to conflicting findings on the role of the exchanger in regulating insulin secretion (33, 34). Thus, studies aiming to elucidate the physiological role of the exchanger should consider employing additional and independent functional criteria, for example, monitoring Na^+ dependence of the exchanger or using molecular tools for modulating the expression or activity of NCLX.

Molecular Identity of the Mitochondrial NCX

In 1992, Garlid and co-workers (35) reported the successful purification of an ~ 110 -kDa mitochondrial polypeptide that, upon reconstitution into proteoliposomes, exhibited NCX and Li^+/Na^+ exchanger activity. Antibodies made against this protein blocked the reconstituted $\text{Na}^+/\text{Ca}^{2+}$ activity and identified the ~ 110 -kDa fragment along with an additional non-active ~ 60 -kDa fragment. However, although these promising findings were corroborated by subsequent work (18), they were not followed up at the molecular level, and 2 decades would pass before the exchanger gene was finally identified. The failure to identify the exchanger led to the hypothesis that plasma membrane NCX isoforms are targeted to and contribute to Ca^{2+} transport in the mitochondria. This hypothesis was supported by the presence of NCX1–3 isoforms in isolated mitochondrial fractions and *in situ* (36, 37). There were, however, several lines of evidence against this theory. The localization of NCX family members to the mitochondria was controversial (38), antibodies against the mitochondrial NCX did not react with polypeptides from cardiac sarcolemma vesicles (35), and enhanced plasma membrane NCX expression did not correlate with mitochondrial exchange activity (39). Perhaps most importantly, the cation selectivity and inhibitor sensitivity of the mitochondrial NCX were clearly distinct from those of all plasma membrane NCX family members (18).

The molecular identification of the mitochondrial NCX came about when we sought to identify a gene mediating $\text{Na}^+/\text{Zn}^{2+}$ exchange activity, monitored primarily on neuronal cell membranes (40). On the basis of this mechanism, we reasoned that it belongs to the NCX superfamily and cloned a putative member termed FLJ22233 (41). Phylogenetic analysis of this gene indicated that it did not belong to either one of the major mammalian $\text{Na}^+/\text{Ca}^{2+}$ subfamilies, NCX and the $\text{Na}^+/\text{Ca}^{2+}/\text{K}^+$ exchanger (SLC8 and SLC24), and that it diverged earlier in evolution to form a distinct subfamily with a single mammalian member (42, 43). The FLJ22233 gene product mediated $\text{Na}^+/\text{Ca}^{2+}$ but not $\text{Na}^+/\text{Zn}^{2+}$ exchange. Unexpectedly and in contrast to other NCXs, this transporter mediated both Li^+ - and Na^+ -dependent Ca^{2+} exchange activity and was therefore named NCLX (5). The $\text{Li}^+/\text{Ca}^{2+}$ exchange of NCLX prompted us to ask whether it is the mediator of this activity in mitochondria. Immunoblot analysis and immunoelectron microscopy analyses in cultured cells and native tissue revealed NCLX localization in the inner membranes of mitochondria, and increased expression of NCLX enhanced mitochondrial Ca^{2+} efflux (5). Furthermore, NCLX-mediated mitochondrial Ca^{2+} efflux was fully blocked by CGP-37157, by NCLX siRNAs, or by a catalytic mutant that had strong dominant-negative effect on mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange. Finally, Ca^{2+} efflux mediated by mitochondrial NCLX was Li^+ - or Na^+ -dependent (Fig. 1). Altogether, the localization and functional analyses indicate that NCLX is the long-sought mitochondrial NCX (5). These findings gained independent support by a recent study showing that NCLX was localized to mitochondria in B lymphocytes and that mitochondrial Na^+ -dependent Ca^{2+} exchange in these cells was abolished by genetic knock-out or by siRNA-mediated knockdown of NCLX expression (44).

Regulation of the Mitochondrial Exchanger by Protein Interactions

Several studies suggested that interaction of the mitochondrial NCX with other proteins (18, 35) regulated its activity (Fig. 2) (45–49). At least two protein kinases (PKC and PINK1), a member of the stomatin family (SLP-2), and the anti-apoptotic protein Bcl-2 modulate the activity of the mitochondrial NCX. A report by Yang *et al.* (49) showed that tetanic-induced synaptic potentiation (TISP) is dependent on the activity of the mitochondrial NCX, which is in turn regulated by PKC. Surprisingly, although TISP is Ca^{2+} -dependent, it occurs in the absence of extracellular Ca^{2+} . To clarify the functional basis for this type of synaptic potentiation, Yang *et al.* showed that an increase in Na^+ concentration at nerve terminals triggers Ca^{2+} release from mitochondria and that inhibition of the mitochondrial NCX or PKC activity abolishes TISP. Direct interaction between PKC and the mitochondrial exchanger appears plausible, as the mitochondrial localization of PKC, as well as its physical interaction with transport proteins on both the outer and inner mitochondrial membranes, has been reported (47). Gandhi *et al.* (46) showed that the loss of PINK1 function in neurons leads to profound inactivation of mitochondrial $\text{Na}^+/\text{Ca}^{2+}$ exchange, triggering mitochondrial Ca^{2+} overload and neuronal death in a model of Parkinson disease. Similarly, Zhu *et al.* (39) found that $\text{Na}^+/\text{Ca}^{2+}$ exchange activity is inhibited in

isolated mitochondria from hearts of transgenic mice overexpressing Bcl-2. A similar inhibitory effect was recently found for the mitochondrial protein SLP-2 (48). A study by Da Cruz *et al.* (48) showed that enhancing SLP-2 expression causes inhibition, whereas depleting SLP-2 expression results in augmentation of mitochondrial Na⁺/Ca²⁺ exchange activity. Finally, the calcium-dependent μ -calpain mediates cleavage of the mitochondrial NCX in isolated mitochondria (45). Analyses of NCLX sequence have not show a clear PINK1 or Bcl-2 phosphorylation motif,⁴ suggesting that the interaction of these proteins with the exchanger may be indirect. The molecular identification of the exchanger as described below invites future studies to elucidate the molecular basis of these important regulatory mechanisms.

Stoichiometry of the Mitochondrial NCX

Whether the mitochondrial NCX action is electrogenic is a key issue, as it determines the transmembrane Ca²⁺ and Na⁺ gradients. Early studies indicated that the velocity of Ca²⁺ efflux is a function of [Na⁺]⁴ and that it is stimulated by energy-linked respiration (14, 26, 50), suggesting an electrogenic process in which three Na⁺ ions are exchanged for one Ca²⁺ per cycle. Subsequent studies further indicated that the thermodynamic requirements for the Ca²⁺ gradient established by the mitochondrial NCX require that it mediates electrogenic transport (51, 52). In contrast, several studies presented evidence against electrogenicity of the exchanger, as its activity does not produce a change in mitochondrial $\Delta\psi$ (53), additional evaluation of the Na⁺/Ca²⁺ kinetics suggested that the velocity of Ca²⁺ efflux is in fact a function of [Na⁺] (27, 54),⁴ and mitochondrial Na/Ca²⁺ exchange activity was not affected by an electroneutral H⁺/Ca²⁺ uncoupler (A23187), thereby favoring the electroneutral model (55). A recent analysis by Kim and Matsuoka (56) successfully measured changes in mitochondrial $\Delta\psi$ induced by mitochondrial Na⁺/Ca²⁺ exchange following blockage of the respiratory chain. They found that, under these conditions, activation of the mitochondrial exchanger resulted in hyperpolarization when the exchanger acted in reverse mode (Ca²⁺_{in}/Na⁺_{out}) and in depolarization when the exchanger was active in direct Ca²⁺ forward mode (Na⁺_{in}/Ca²⁺_{out}). These findings are consistent with a stoichiometry of at least three Na⁺ ions/one Ca²⁺ ion per transport cycle and agree with a recent theoretical model also favoring a similar exchange mode (56, 57). Interestingly, when expressed in HEK293 cells, a fraction of NCLX reaches the plasma membrane and allows direct measurement of a Ca²⁺-dependent outward current, which is consistent with a stoichiometry of three to four Na⁺ ions/one Ca²⁺ ion (41, 58). Taken together, a growing number of studies now support an electrogenic mode of three to four Na⁺ ions/one Ca²⁺ ion per transport cycle mediated by the mitochondrial exchanger, and electrophysiological analysis of its activity may provide another important tool for studying its function and regulation.

Cytosolic Na⁺ and Mitochondrial NCX Activity

The mitochondrial NCX is also the major pathway for Na⁺ influx into mitochondria. Mitochondrial Na⁺ is subsequently balanced by a Na⁺/H⁺ exchanger, an important mitochondrial

transporter whose molecular identity remains to be elucidated. The K_m of the mitochondrial exchanger for Na⁺ is ~ 7 – 10 mM, remarkably tuned to the resting cytosolic Na⁺ concentration. This implies that even a small change in cytosolic Na⁺ will strongly modulate its activity. Such effects were indeed documented in several cell types and were linked to various physiological processes, including synaptic activity (49), energy balance (59, 60), and phagocytosis (61). Another interesting demonstration of Na⁺-induced mitochondrial Ca²⁺ release was recently reported in a study showing that, in smooth muscle cells, activation of TRPC6 following ER Ca²⁺ store depletion leads to a marked increase in cytosolic Na⁺, followed by subsequent release of mitochondrial Ca²⁺ (62). This Ca²⁺ release may contribute to salt-sensitive hypertension. Whether this phenomenon is extended to other cell types or other TRPC family members is an intriguing possibility with broad and important implications in cellular physiology. In cardiac myocytes, elevated cytosolic Na⁺ is followed by strong acceleration of mitochondrial Ca²⁺ efflux, which inhibits the mitochondrial metabolic rate and formation of reactive oxygen species (59, 63). In melanoma cells, a rise in cytosolic Na⁺ mediated by a splice variant of the Na⁺ channel Na_v1.6 promotes mitochondrial calcium release, which accelerates the invasiveness of these tumor cells (60). Activation of mitochondrial Na⁺/Ca²⁺ exchange by an increase in intracellular Na⁺ is also a prime suspect in several cellular pathologies such as heart failure, cancer, and hypertension. Collectively, these studies highlight a role of cytosolic Na⁺ as a dynamic and important regulator of the mitochondrial NCX.

Role of the Mitochondrial NCX in the Regulation of ER Ca²⁺

Over recent years, many studies (reviewed in Refs. 64–67) have revealed a dynamic and structural association between mitochondria and ER compartments where specialized and restricted calcium shuttling occurs (Fig. 2). The release of Ca²⁺ through the mitochondrial NCX supports a major pathway by which mitochondria feed Ca²⁺ into the ER. Mitochondria act both to recycle Ca²⁺ leaving the ER, preventing the depletion of neighboring ER regions (68), and to facilitate the shuttling of Ca²⁺ from plasma membrane store-operated Ca²⁺ channels en route to the ER and cytosol (69–71). The physiological significance of the mitochondrial NCX in keeping Ca²⁺ shuttling between the ER and mitochondria was demonstrated by various studies showing, for example, that it is critical for maintaining and controlling the frequency of oscillatory Ca²⁺ waves (72–75) or for proper protein folding (76). Recently, this activity was addressed for the first time using both pharmacological and genetic manipulation tools, demonstrating that, in B lymphocytes, the mitochondrial Na⁺/Ca²⁺ exchanger NCLX maintains Ca²⁺ recycling between mitochondria and ER, thereby playing a pivotal role in B cell responses to antigen (56). Finally, *in vivo* measurements of mitochondrial Ca²⁺ recycling in mouse skeletal muscle during contraction also revealed an important role for the NCX in Ca²⁺ shuttling between the mitochondria and SR (77).

Mitochondrial Na⁺/Ca²⁺ Exchange in Brain and Secretory Systems

The dominant role of the exchanger is to counterbalance mitochondrial Ca²⁺ uptake following transient cytosolic elevations by subsequent release to maintain resting mitochondrial Ca²⁺ at the required physiological levels. The released mitochondrial Ca²⁺ plays a major role in modulating the cytosolic Ca²⁺ response, as it generates a “shoulder”-like extension that shapes the magnitude and duration of the cytosolic Ca²⁺ signal (78–83). The high density of mitochondria in synaptic regions and the importance of Ca²⁺ signaling in these sites prompted many studies to focus on the role of the mitochondrial exchanger in synaptic transmission. Indeed, in various neuronal preparations, synaptosomal neurotransmitter release was modulated by CGP-37157. These effects were reproduced by inducing changes in intracellular Na⁺, thus linking the mitochondrial exchanger to several forms of synaptic plasticity (49, 81, 84, 85) and arguing against a non-selective effect of CGP-37157. Similarly, other forms of secretion are also modulated by the activity of the mitochondrial exchanger such as exocytosis of gonadotropins from rat gonadotrophs (75) and secretion of glutamate by cortical astrocytes (86).

There is a large body of literature describing the connection between brain disorders and abnormalities in mitochondrial Ca²⁺ handling, and only few examples are briefly described here. Cytosolic and mitochondrial Ca²⁺ overloads are principal pathophysiological events during brain ischemia. The majority of studies suggest a neuroprotective role for blocking the mitochondrial exchanger activity (11). A harmful effect of mitochondrial Na⁺/Ca²⁺ exchange activity following ischemic-like conditions was shown in rat hippocampal slices, where a prolonged neuronal cytosolic Ca²⁺ rise triggered by toxic levels of NMDA was augmented by elevation of cytosolic Na⁺ and blocked by CGP-37157 (87). Similarly, in hypoxic slices, increased release of free fatty acids (a marker for tissue damage) was reduced by CGP-37157 (88). The increase in mitochondrial Ca²⁺ by CGP-37157 in immature neurons during NMDA insult also induces a neuroprotective release of mitochondrial NO (89). In contrast, however, Scanlon *et al.* (90) reported that application of CGP-37157 does not profoundly alter glutamate-mediated changes in mitochondrial function following toxic NMDA insults. Also, mitochondrial NCX activity is required for the neuroprotective effect of cannabinoids during neuronal Ca²⁺ overload (91). Thus, whether mitochondrial Na/Ca²⁺ exchange activity is harmful or neuroprotective during ischemia requires additional investigation. A molecular control for the exchanger expression or activity employing, for example, NCLX knock-out mice may help in clarifying some of the uncertainties related to the role of the mitochondrial exchanger in these syndromes.

Dysfunction of neuronal metabolism is a major factor in many forms of seizure activity. Seizure-like events are accompanied by frequent stimulation of sodium channels that are likely to contribute to intracellular Na⁺ loads (92) and have been shown to be translated into mitochondrial Ca²⁺ transients conveyed by the MCU and NCX (93). These findings indicate that, through modulation of mitochondrial Ca²⁺, epi-

leptic activity results in changes in mitochondrial function that may contribute to subsequent neuronal injury. Impairments in Ca²⁺ homeostasis and mitochondrial function are also hallmark events in Parkinson (PD), Huntington (HD), and Alzheimer (AD) diseases. Although an elegant study showed that striatal neurons expressing the HD disease-related mutant huntingtin exhibit impaired mitochondrial Ca²⁺ shuttling following mitochondrial Ca²⁺ loads, the specific role of the mitochondrial exchanger remained unclear (94). Chin *et al.* (95) found a possible link between neuronal damage and mitochondrial Ca²⁺ shuttling in AD, as application of β -amyloid, the main component of senile plaques found in the brains of AD patients, to rat basal forebrain neurons triggered a cytosolic Ca²⁺ overload that could be partially rescued by CGP-37157. In PD, the genetic mutated markers Parkin and PINK1 are targeted to the mitochondria and regulate diverse aspects of mitochondrial function, including membrane potential and calcium homeostasis. PINK1-deficient neurons show excessive mitochondrial Ca²⁺ overload linked to profound inhibition of the mitochondrial NCX; however, how this kinase regulates the exchanger is still unresolved (46). Further studies are required to determine whether changes in the activity or expression pattern of the mitochondrial NCX are part of AD, PD, and HD etiology.

Mitochondrial Na⁺/Ca²⁺ Exchange in Heart

In cardiomyocytes, the ability of the mitochondria to modulate cytosolic Ca²⁺ transients is not fully resolved. An intriguing and controversial issue is whether the mitochondrial Ca²⁺ shuttling can manage to follow a beat-to-beat rate (reviewed in Refs. 96–98). Some of this controversy is probably related to differences in species and developmental stages of the cardiac preparations or the limited accessibility and specificity of the MCU and the NCX inhibitors in intact cardiomyocytes. In addition, ATP production during normal cardiac pacing does not seem to be strongly affected by mitochondrial Ca²⁺, consistent with multiple pathways involved in this process (99). Mitochondrial Ca²⁺ does seem to play a metabolic role during accelerated pacing or following ischemia. The latter syndrome may lead to a rise in cytosolic Na⁺ that triggers mitochondrial Ca²⁺ efflux, which in turn depletes mitochondrial Ca²⁺ content and causes oxidative phosphorylation inhibition and ATP shortage (59). Conversely, according to another study, hypoxia may lead to mitochondrial Ca²⁺ overload due to reversal of the mitochondrial exchanger (100). The molecular identification of both MCU and NCLX now provides potentially more selective genetic tools to assess the physiological and pathophysiological roles of mitochondrial Ca²⁺ shuttling in intact cardiac preparations.

Conclusions

Forty years of research led to the recognition of the major role played by the mitochondrial NCX in cellular biology. Numerous studies showed that, by releasing mitochondrial Ca²⁺, the mitochondrial NCX regulates the cells metabolic rate, shapes intracellular Ca²⁺ signaling, and contributes to cell death in normal or disease states. Other studies demonstrated that the mitochondrial NCX is regulated by the cellular ionic

milieu or by various proteins. A principal limitation in understanding the physiological roles of this exchanger was that its molecular identity was unknown, and studies had to rely on inhibitors (primarily CGP-37157) that could also modulate the activity of other Ca²⁺ transporters. Identification of the mitochondrial NCX/NCLX can facilitate molecular based biochemical and biophysical analysis aimed to identify its regulatory sites and partner proteins. NCLX knock-out or knockdown models may be used to study the role of the mitochondrial exchanger *in vivo* and under tight genetic control to allow a tissue-specific analysis of the exchanger's role in heart, brain, and endocrine pancreas and to determine whether a change in its expression pattern in animals and humans is linked to neuronal, cardiac, or endocrine disorders.

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