NME4/nucleoside diphosphate kinase D in cardiolipin signaling and mitophagy

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PATHOBIOLOGY IN FOCUS

NME4/nucleoside diphosphate kinase D in cardiolipin signaling and mitophagy

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Mitophagy is an emerging paradigm for mitochondrial quality control and cell homeostasis. Dysregulation of mitophagy can lead to human pathologies such as neurodegenerative disorders and contributes to the aging process. Complex protein signaling cascades have been described that regulate mitophagy. We have identified a novel lipid signaling pathway that involves the phospholipid cardiolipin (CL). CL is synthesized and normally confined at the inner mitochondrial membrane. However, upon a mitophagic trigger, ie, collapse of the inner membrane potential, CL is rapidly externalized to the mitochondrial surface with the assistance of the hexameric nucleoside diphosphate kinase D (NME4, NDPK-D, or NM23-H4). In addition to its NDP kinase activity, NME4/NDPK-D shows intermembrane phospholipid transfer activity *in vitro* and in cellular systems, which relies on NME4/NDPK-D interaction with CL, CL-dependent crosslinking of inner and outer mitochondrial membranes by symmetrical, hexameric NME4/NDPK-D, and a putative NME4/NDPK-D-based CL-transfer pathway. CL exposed at the mitochondrial surface then serves as an 'eat me' signal for the mitophagic machinery; it is recognized by the LC3 receptor of autophagosomes, targeting the dysfunctional mitochondrion to lysosomal degradation. Similar NME4-supported CL externalization is likely also involved in apoptosis and inflammatory reactions.

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The selective elimination of dysfunctional mitochondria, mitophagy, is emerging as a key player among cellular quality control mechanisms.[1,2] These mechanisms comprise signaling pathways acting on proteins and entire organelles, and are increasingly recognized as being causative in various pathological dysfunctions as well as for the aging process.[3] They are beneficial for health in the young, but become deregulated and thus detrimental in the old. Mitophagy is triggered when mitochondrial damage involving mutation, oxidation/nitration, and/or misfolding of mtDNA, proteins, or lipids exceeds the capacity of repair/detoxification systems (such as the mitochondrial unfolded protein response or ROS detoxification). It is well known that also apoptosis, the selective elimination of dysfunctional cells for tissue quality control, is sensed and induced by impaired mitochondrial function.[4] Whether mitophagy or apoptosis is triggered often depends on the dose or the duration of the stimulus, rather than its quality. Thus, below a certain threshold, only dysfunctional mitochondria are eliminated, while above this threshold the entire cell undergoes programmed death. Interestingly, both processes also share common features, including the collapse of the membrane potential, $\Delta\Psi$, across the mitochondrial inner membrane (MIM),[5] and both can use a similar lipid signaling pathway as outlined below.

PROTEIN AND LIPID SIGNALING IN MITOPHAGY AND BEYOND

The canonical pathways regulating mitophagy all involve protein signaling cascades, where $\Delta\Psi$ breakdown triggers exposure of specific proteins (or specific protein modifications) at the mitochondrial surface, recognized by the autophagic machinery via the microtubule-associated protein 1 light chain 3 (LC3). These canonical pathways mostly rely on the action of the mitochondrial kinase PINK1 and the

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cytosolic E3 ubiquitin ligase Parkin, and are relatively well studied.[6,7] Here, loss of $\Delta\Psi$ impedes mitochondrial import and degradation of PINK1, leading to a cascade of PINK1 exposition at the surface of the mitochondrial outer membrane (MOM), phosphorylation of ubiquitinated MOM proteins, recruitment of Parkin to MOM, further ubiqitination of MOM proteins, and recruitment of further receptor proteins that finally attract LC3-exposing autophagosomes. However, mitophagy can also occur in absence of the PINK/Parkin system. Alternative pathways, such as triggered by hypoxia, use recruitment of other proteins such as BNIP3L, NIX, or FUNDC1 to MOM to attract LC3.[8]

We have recently identified a novel pathway that describes for the first time a role of lipid signaling in mitophagy.[9–11] Different mitophageal stimuli such as rotenone, staurosporine, 6-hydroxydopamine, and others are able to trigger migration of the mitochondria-specific phospholipid cardiolipin (CL), from its site of synthesis, MIM, to the opposed MOM, and further to the mitochondrial surface.[9] This exposed CL directly serves as an 'eat me' signal, as it is recognized by two CL-binding pockets within the LC3 receptor.[9] Very much like phosphatidylserine externalized to the cellular surface serves for elimination of damaged cells via apoptosis, CL externalized to the mitochondrial surface acts as a signal for the elimination of damaged mitochondria via mitophagy.[11–14] Increased CL at MOM has been observed under different conditions that favor mitophagy like chemical models of Parkinson disease[10] and caloric restriction.[15]

Externalization of CL to the mitochondrial surface is also part of other damage-related signaling pathways, including early pro-apoptotic signaling[16–18] and inflammatory reactions in intracellular compartments and extracellular environments.[19] By providing a scaffold at the mitochondrial surface for recruiting and functionally altering different apoptotic proteins, including caspase 8, tBid, Bak, and Bax,[20–22] CL participates in launching the release of cytochrome c,[23–28] although this step may not be required for all forms of apoptosis.[29] In contrast to the CL that triggers mitophagy through recognition via LC3, the CL externalized during apoptosis has mostly peroxidized acyl chains, due to the CL oxidase function of cytochrome c,[30] and this oxidation step seems to be important for execution of the apoptotic program.[31,32] Externalized CL also participates in the complex activation the Nlrp3 inflammasomes trough interaction with the Nlrp3 LRR3 domain, leading to pyroptotic cell death.[33,34] Finally, when released into the extracellular space, CLs on the surface of mitochondria or mitochondrial fragments, again mostly in their oxidized form,[35] can act as pro-phagocytotic 'eat me' signals via CD36 scavenger receptor and/or as suppressors of cytokine production potentially leading to immunoparalysis.[36] All these findings add to an emerging paradigm, namely that the myriad of different cellular lipid species, and in particular CL, have not just a supportive structural role, but are very actively involved in regulatory and signaling functions.[19,37–41]

NME4/NDPK-D IN CL SIGNALING

The mechanism by which CL is able to move between MIM and MOM has long remained enigmatic. While phospholipid flip-flop between two membrane leaflets is probably assumed by nonspecific scramblases, and Bid may be involved in phospholipid transfer between MOM and other cellular membranes,[38,42] no enzyme supporting the transmembrane movement of CL between MIM and MOM had been identified. More recently, we could involve a known mitochondrial kinase in this process. NME4 (non-metastatic expressed isoform 4) or nucleoside diphosphate kinase D (NDPK-D, EC 2.7.4.6), also known as non-metastatic clone 23 human isoform 4 (NM23-H4), forms large homohexameric complexes localized in the mitochondrial intermembrane space. Besides its NDP kinase activity, it binds to both mitochondrial membranes via CL and other anionic phospholipids to induce contact sites. We have shown first for reconstituted *in vitro* systems,[43] and later for mitochondria and living cells[44,45] that NME4/NDPK-D can also support intermembrane phospholipid transfer. We found in different cellular model systems that a collapse of $\Delta\Psi$ (eg, using the protonophoric uncoupler CCCP) is triggering CL externalization and induction of mitophagy, and that this requires the presence of CL-binding competent NME4/NDPK-D that exposes triads of basic amino acids.[11] In fact, the CL movement requires a CL-transfer-competent NME4/NDPK-D topology, where the enzyme localized in the mitochondrial intermembrane space binds simultaneously to MIM and MOM, and is NDP kinase-inactive (Figure 1, right part). It has long been known that such MIM–MOM contact sites are enriched in NDPK and CL.[46–48] On the other hand, in healthy mitochondria, NME4/NDPK-D is mainly bound to MIM only and is NDP kinase-active (Figure 1, left part). Thus, $\Delta\Psi$-dependent regulation of CL-triggered mitophagy is mediated by a switch between these two different NME4/NDPK-D topologies (for details see legend to Figure 1 and recent reviews[12,13,38]). Interaction of NME4/NDPK-D with CL-containing membranes leads to clustering of CL, dehydration of lipid head groups, but also increased acyl chain mobility indicative for the protein splitting apart some CL molecules.[49] Further *in silico* evidence points to a CL pathway along the surface of the NME4/NDPK-D hexamer and/or a rotary transport mechanism involving detachment/reattachment of NME4/NDPK-D to two opposing membranes.[45] In any case, CL movement would be driven by the CL concentration gradient from MIM to MOM with the NME4/NDPK-D-supported intermembrane transfer as the rate-limiting step.

We have shown earlier that NDP kinase-active NME4/NDPK-D interacts also with OPA1,[45] a MIM-located dynamin-related GTPase responsible for MIM fusion and dynamics, and that it fuels OPA1 with GTP to maintain OPA1 functions (Figure 1, left part).[50] In fact, silencing of NME4/NDPK-D phenocopies OPA1 loss of function, both resulting in fusion defects that manifest in mitochondrial fragmentation.[50] This is consistent with a requirement of

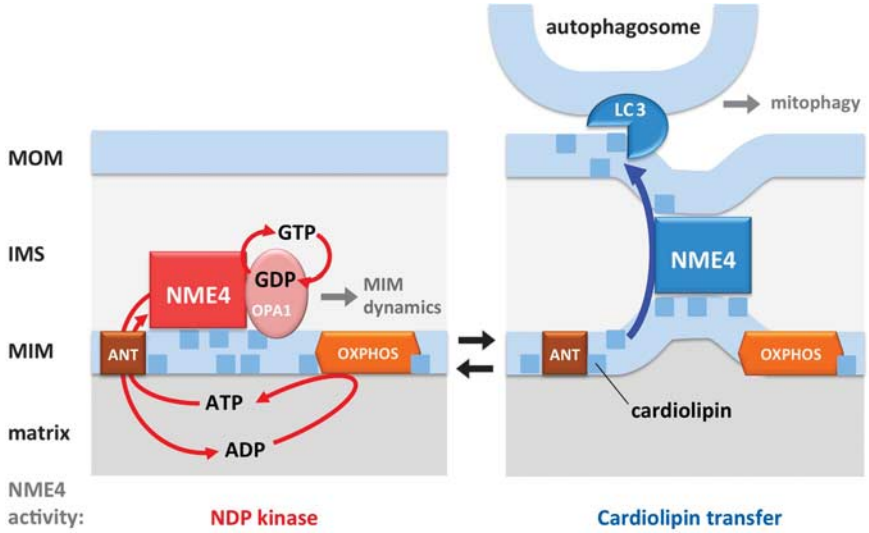

Figure 1 Dual function of NME4/NDPK-D in bioenergetics and mitophagy. Left: in healthy mitochondria, hexameric NME4 complexes interact with MIM (via CL binding) and OPA1, in close proximity of adenine nucleotide translocase (ANT). In this topology, NME4 has NDP kinase (phosphotransfer) activity. It uses mitochondrial ATP from oxidative phosphorylation (OXPHOS) to regenerate, eg, GTP for local fueling of the interacting OPA1 GTPase, while the produced ADP stimulates OXPHOS. GTP fueling will maintain OPA1 functions in MIM fusion, cristae remodeling, and MIM dynamics in general. There is no NME4-mediated transfer of phospholipids in this topology. Right: in mitochondria that received a mitophagy trigger, eg, collapsing the membrane potential across MIM, symmetrical hexameric NME4 complexes interact simultaneously with both MIM and MOM. In this topology, NME4 loses NDP kinase activity, but gains intermembrane phospholipid transfer activity. In particular, it moves CL from MIM to MOM, probably driven by the large gradient of CL concentration between these membranes. Full externalization of CL to the mitochondrial surface finally triggers mitophagic elimination of the damaged mitochondria. LC3 receptors at the surface of autophagosomes recognize and bind to exposed CL, thus targeting these mitochondria to lysosomal degradation. NDP kinase activity is inhibited in this topology, which would reduce MIM dynamics and mitochondrial fusion rates, thus leading to individual, small mitochondria as typical during mitophagy. The mechanism switching NME4 from phosphotransfer to lipid transfer function may involve OPA1, which undergoes proteolytic cleavage early during mitophagy, possibly weakening its interaction with NME4 (Figure modified from ref. 45).

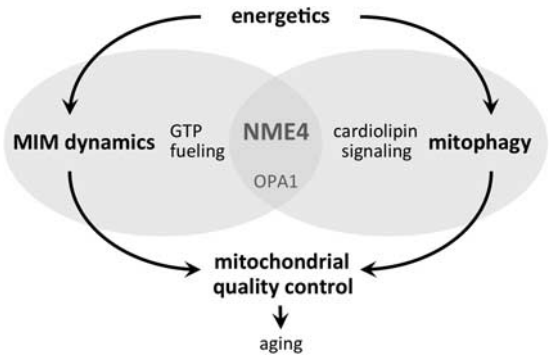

Figure 2 NME4/NDPK-D in mitochondrial quality control. NME4 is involved in two fundamental processes that are essential for the correct functioning of this organelle in bioenergetics: mitochondrial dynamics by MIM remodeling via OPA1 (including mitochondrial fusion); and mitochondrial turnover by execution of mitophagy via CL externalization and signaling. Both processes, by controlling mitochondrial quality, will potentially affect aging.

NME4/NDPK-D for full OPA1 function. Loss of NDP kinase activity in the CL-transfer-competent NME4/NDPK-D topology induced by mitophagic stimuli would thus also impair OPA1 function, and contribute to the mitochondrial fragmentation observed during mitophagy.

Interaction of NME4/NDPK-D with OPA1 may also regulate its switch from NDP kinase to CL-transfer activity. Some siRNA experiments indicate that OPA1 is a negative regulator of NME4/NDPK-D-supported CL transfer.[11] OPA1 in complex with NME4/NDPK-D may retain the latter in the NDP kinase topology (Figure 1, left part). The cleavage of OPA1 by mitochondrial proteases,[51] which occurs directly after $\Delta\Psi$ collapse and before execution of mitophagy,[52] may then weaken or even disrupt NME4/NDPK-D interaction with OPA1, thus releasing NME4/NDPK-D to crosslink MIM and MOM, and to engage into CL transfer (Figure 1, right part). These interpretations are still conjectural and need further experimental support. For example, it is tempting to speculate that the GTPase activity of OPA1 may be also engaged to energetically favor the rotary function of hexameric NME4/CL complexes thus facilitating CL transfer.

Unlike other mitochondrial quality control mechanisms, NME4-dependent, CL-triggered mitophagy relies on lipid signaling and provides a direct link between bioenergetics, mitophagy, and mitochondrial fragmentation (Figure 2). OPA1 is an important partner of NME4/NDPK-D in this respect. Their interaction allows direct fueling of OPA1 with GTP, and vice versa, OPA1 potentially regulates the dual function of NME4. As cellular quality control mechanisms are increasingly recognized as key factors in aging, we propose

that NME4/NDPK-D-dependent mitophagy also participates in this process. Indeed, NME4/NDPK-D is significantly downregulated in the hippocampus of 2-year-old mice,[53] and NME4/NDPK-D is part of a mortality-predicting signature identified in an age 90+ human cohort.[54] Finally, we provided initial evidence that NME4/NDPK-D-supported CL externalization is also important for apoptosis,[45] and the same may apply for inflammatory situations. This opens new perspectives for NME/NDPK biology that merit further research.

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DISCLOSURE/CONFLICT OF INTEREST

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