REVIEW

Mitochondria as intracellular signaling platforms in health and disease

Jay X. Tan[1,2] and Toren Finkel[1,3]

Mitochondria, long viewed solely in the context of bioenergetics, are increasingly emerging as critical hubs for intracellular signaling. Due to their bacterial origin, mitochondria possess their own genome and carry unique lipid components that endow these organelles with specialized properties to help orchestrate multiple signaling cascades. Mitochondrial signaling modulates diverse pathways ranging from metabolism to redox homeostasis to cell fate determination. Here, we review recent progress in our understanding of how mitochondria serve as intracellular signaling platforms with a particular emphasis on lipid-mediated signaling, innate immune activation, and retrograde signaling. We further discuss how these signaling properties might potentially be exploited to develop new therapeutic strategies for a range of age-related conditions.

Introduction

For those of us who survived the emotional trauma known as junior high school in America, the strategy for adolescent success appeared to be the unique ability to both blend in and stand out. While we will leave it to social scientists to describe the psychological ramifications of such approaches, we would argue that there is an important hidden biological lesson here as well. Mitochondria, present in hundreds to thousands of seemingly identical copies per cell, certainly can blend in. In doing so, they provide the cell with its bioenergetics requirements, supplying the chemical energy required to power essentially all cellular processes. Yet, to view these structures as indistinguishable and amorphous, organelles would be shortsighted. Endowed with a unique evolutionary history, mitochondria have retained a distinct set of lipid components, as well as their own genome, that under specific stress conditions, enables these organelles to also stand out. This singularity makes mitochondria uniquely situated to act as a signaling platform. Here, we review the evidence for how mitochondria participate in a wide range of cell fate decisions that exploit the unique qualities and properties of this organelle. In particular, we will focus on how specific unique mitochondrial lipids can modulate signaling events, how mitochondria participate in innate immune signaling, and how mitochondria can signal back to the nucleus to alter both transcription and the epigenome. These topics cover the multifaceted stress responses used by mitochondria, from signaling reactions within mitochondria, to the surface of mitochondria, to those pathways involving the nucleus and

the extracellular environment. Finally, we will describe the initial foray into leveraging these observations to develop new classes of therapies that may have the potential to treat a wide array of diseases, especially age-related diseases that are closely linked to mitochondrial dysfunction.

Mitochondrial lipid signaling

Involved in all aspects of cell biology and physiology, lipids are the major components of all cellular membranes, delineating the boundary between cells and subcellular organelles and supporting intercellular and intracellular communication. The most abundant lipid in mammalian cells is phosphatidylcholine (PC), whereas the amounts of other lipids such as phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), phosphatidic acid (PA), cholesterol, and sphingolipids vary depending on the type of cell and subcellular organelle. As an α-proteobacteria-derived organelle, mitochondria exhibit distinct lipid composition in both their inner and outer membranes, which establishes a biochemical basis for mitochondrial signaling. Mitochondrial lipid synthesis and transport have been the subject of several excellent recent reviews (Horvath and Daum, 2013; Tatsuta and Langer, 2017; Tatsuta et al., 2014), and as such, here, we will focus instead on mitochondrial signaling and metabolism regulated by two unique lipids, cardiolipin and PE (Fig. 1).

Cardiolipin-mediated signaling

Cardiolipin comprises nearly 20% of the inner mitochondrial membrane (IMM) and a much smaller fraction (up to 3%) of the

---

[1]Aging Institute, University of Pittsburgh School of Medicine/University of Pittsburgh Medical Center, Pittsburgh, PA; [2]Department of Cell Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA; [3]Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Correspondence to Toren Finkel: finkelt@pitt.edu; Jay X. Tan: jay.tan@pitt.edu.
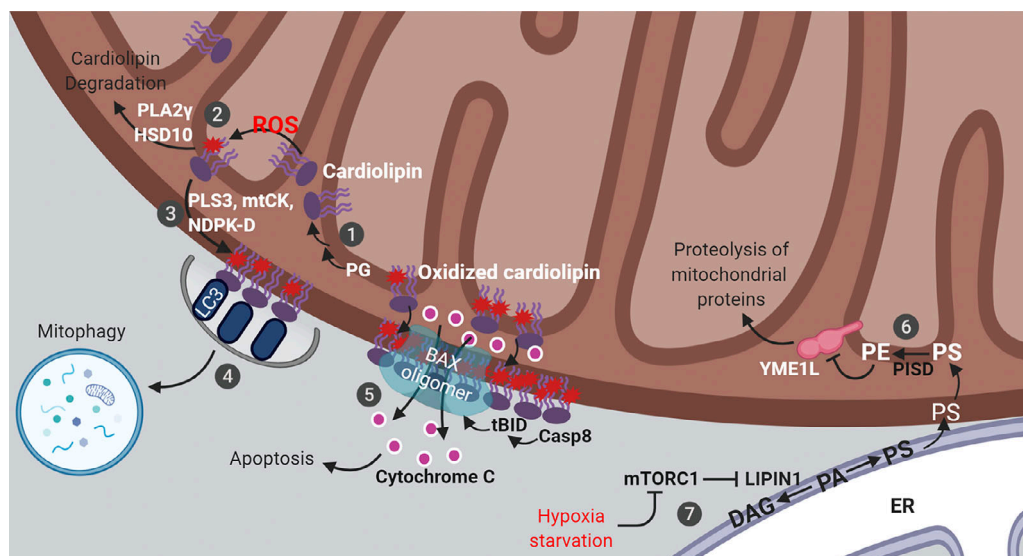
Figure 1. Lipid signaling in mitochondria. Mitochondrial cardiolipin and PE orchestrate multiple aspects of mitochondrial signaling and bioenergetics. These are summarized here diagrammatically. (1) Cardiolipin is synthesized from phosphatidylglycerol (PG) and remodeled at the IMM. (2) Cardiolipin acts as a ROS scavenger via oxidation and redox-mediated degradation. (3) Moderate levels of mitochondrial stress stimulates cardiolipin externalization. (4) Exposed cardiolipin can be recognized by LC3, stimulating mitophagic clearance. (5) Severe stress triggers cardiolipin-mediated cytochrome c release and apoptosis. (6) PE on the IMM is directly produced by the enzyme PISD, using PS synthesized on the ER and transported to mitochondria. (7) Stresses that inhibit mTORC1 rewires mitochondrial metabolism via a lipid signaling cascade, reducing PE levels on the IMM, leading to augmented YME1L activity, increased proteolysis and reduced mitochondrial biogenesis. DAG, diacylglycerol; mtCK, mitochondrial creatine kinase; tBID, truncated BH3-interacting domain death agonist. See text for additional details.

outer mitochondrial membrane (OMM), but it is not found in any other subcellular organelle (de Kroon et al., 1997; Gebert et al., 2009). Structurally, cardiolipin is composed of a dimeric phosphatidylglycerol lipid, with two PA molecules connected with a glycerol backbone. This cone-shaped structure makes cardiolipin ideal for the highly curved IMM, the structure of which is dramatically disrupted when cardiolipin is depleted (Dudek, 2017). Cardiolipin is also well known for its integration into all respiratory chain complexes in the IMM and several translocase of outer membrane protein complexes (Dudek, 2017; Schlame and Greenberg, 2017; Xu et al., 2006). In humans, cardiolipin is synthesized at the IMM by cardiolipin synthase, followed by acyl chain remodeling leading to the formation of mature cardiolipin with polyunsaturated fatty acyl chains (Schlame and Greenberg, 2017; Tatsuta et al., 2014). Perturbing cardiolipin synthesis or remodeling causes mitochondrial dysfunction including defective oxidative phosphorylation and severe oxidative stress, as seen in Barth syndrome, a devastating X-linked pediatric disease (Schlame and Greenberg, 2017). In mice, deletion of cardiolipin synthase is embryonic lethal, whereas neuronal specific depletion causes disrupted mitochondrial crista structure and respiration, altered mitochondrial calcium handling, and neurodegeneration (Kasahara et al., 2020), indicating an essential role for cardiolipin in mitochondrial structure and function.

Cardiolipin functions as a reactive oxygen species (ROS) scavenger that protects cells from oxidative stress via cardiolipin oxidation and degradation (Fig. 1). ROS in animal cells is largely produced by the respiratory chain complexes which are assembled in the cardiolipin-rich IMM. The proximity of

cardiolipin to the source of ROS and the presence of polyunsaturated fatty acyl chains in cardiolipin makes it an ideal sensor and scavenger of oxygen radicals. Oxidized cardiolipin is toxic (Paradies et al., 2001, 2002) and needs to be quickly degraded by enzymes including phospholipase A2γ (PLA2γ; Liu et al., 2017; Tyurina et al., 2014) and 17-β-hydroxysteroid dehydrogenase 10 (HSD10 [also known as amyloid β-peptide-binding alcohol dehydrogenase]; Boynton and Shimkets, 2015). PLA2γ, which can directly hydrolyze cardiolipin, appears to be the major enzyme for the degradation of oxidized cardiolipin, since either genetic depletion or pharmacological inhibition of PLA2γ results in robust accumulation of oxidized cardiolipin upon mitochondrial stress (Liu et al., 2017; Tyurina et al., 2014). HSD10 uses a different mechanism by in fact further oxidizing cardiolipin, causing subsequent spontaneous breakdown of the oxidized product into diacylglycerol, dihydroxyzcetone, and orthophosphate (Boynton and Shimkets, 2015). Thus, to ensure efficient removal of oxidized cardiolipin, the protein levels and enzymatic activity of PLA2γ and HSD10 must be tightly regulated. Indeed, increased levels of HSD10 have been observed in patients with neurological conditions, including multiple sclerosis and Alzheimer's disease (Krištofiková et al., 2009). In that regard, amyloid β peptide, which is mechanistically linked to Alzheimer's disease, directly binds and inhibits the enzymatic activity of HSD10 (Yan et al., 1997; He et al., 1998). Moreover, in a rotenone-induced rat model of Parkinson's disease, inhibiting PLA2γ activity causes increased oxidative stress, more mitochondrial lipid oxidation, augmented apoptosis, and a subsequent exacerbation of Parkinson's disease symptoms (Chao et al., 2018). From a signaling viewpoint,

metabolism of oxidized cardiolipin by PLA2γ and HSD10 generates a large group of second messengers, including oxidized fatty acid lipids and diacylglycerol (Boynton and Shimkets, 2015; Liu et al., 2017; Tyurina et al., 2014), which may be involved in mediating the cellular response to mitochondrial stress.

Cardiolipin externalization from the IMM to the OMM is another important lipid reorganization event that often occurs in response to various conditions involving moderate levels of mitochondrial damage (Fig. 1). In healthy cells, cardiolipin is maintained at very low levels on the OMM and almost exclusively accumulates within the inner membrane (~96.5 mol%; Kagan et al., 2016). In contrast, with mitochondrial damage or membrane depolarization, cardiolipin moves to the OMM. Three proteins have been reported to transport cardiolipin from the IMM to the OMM: phospholipid scramblase-3 (Liu et al., 2003), mitochondrial creatine kinase, and nucleoside diphosphate kinase D (Epand et al., 2007; Kagan et al., 2016; Schlattner et al., 2013). Although it is not clear whether these proteins work sequentially or in parallel, individual depletion of either phospholipid scramblase-3 or nucleoside diphosphate kinase D substantially reduces cardiolipin relocalization to the OMM (Chu et al., 2013; Kagan et al., 2016; Liu et al., 2008; Schlattner et al., 2013). Direct lipid transport through membrane contact sites between subcellular organelles have been described (Cockcroft and Raghu, 2018; Scorrano et al., 2019; Wu et al., 2018), but it is not clear exactly how these three cardiolipin transporters act, and whether they localize to IMM–OMM contact sites (Horvath et al., 2015).

Externalized, oxidized cardiolipin at the OMM establishes a signaling platform to orchestrate cellular response to mitochondrial damage that can include apoptosis or mitophagy (Fig. 1). In mitochondrial-dependent apoptosis, the recruitment and activation of the apoptotic initiator protease caspase-8, its substrate BH3-interacting domain death agonist (BID), and the downstream effector BCL-2–associated X (BAX) are all dependent on the presence of cardiolipin in the OMM (Gonzalvez et al., 2008; Kuwana et al., 2002; Lutter et al., 2000). The interactions among truncated BID (tBID), BAX, and cardiolipin drive the formation of supramolecular complexes containing BAX oligomers on the OMM surface that mediate membrane permeabilization and cytochrome c release (Kuwana et al., 2002; Lai et al., 2019; Lutter et al., 2000). The oxidation of cardiolipin reduces its interaction with cytochrome c, which further accelerates cytochrome c release to initiate apoptosis. In mitophagy-promoting conditions, increased cardiolipin at the OMM functions as an "eat-me" signal, recognized by the autophagy marker protein microtubule-associated-protein-1 light chain 3 (LC3) allowing for autophagic capture of the damaged mitochondria (Chu et al., 2013). As such, interfering with cardiolipin synthesis or maturation or its IMM-to-OMM transport potently affects mitophagic and apoptotic signaling (Chu et al., 2013; Garcia Fernandez et al., 2000; Hsu et al., 2015; Kagan et al., 2016).

Cardiolipin has been shown to interact with a litany of proteins linked to both protein aggregation and neurodegenerative diseases. Proteins such as α-synuclein, amyloid β, and Tau, known to form aggregates in neurodegenerative diseases, have

all been reported to bind to cardiolipin-containing membranes (Ahyayauch et al., 2012; Camilleri et al., 2013, 2020; Nakamura et al., 2011; Robotta et al., 2014). Such binding is believed to reduce cardiolipin levels and/or interfere with cardiolipin function in mitochondrial respiration and/or ROS sensing. However, cardiolipin may play a role in resolving these protein aggregates, including amyloid β (Ordóñez-Gutiérrez et al., 2015) and α-synuclein fibrils (Ryan et al., 2018). Thus, further work is needed to more precisely define the physiological relationship between cardiolipin and protein aggregates in neurodegeneration.

Sphingolipids, usually maintained at very low levels in mitochondria compared with other organelles (the Golgi complex, endolysosomes, and the plasma membrane), have been increasingly implicated in age-dependent mitochondrial dysfunction. Ceramide, which is at the center of sphingolipid metabolism, accumulates in response to almost all types of cellular stress, including chemotherapeutic stress, ionizing and ultraviolet radiation, serum starvation, injury, and infection (Ogretmen, 2018). Like cardiolipin, ceramide can also induce apoptosis by triggering BAX-dependent mitochondrial outer membrane permeabilization (Birbes et al., 2001; Ganesan et al., 2010). Mechanistically, this may involve the direct targeting of voltage-dependent anion channel 2 (VDAC2) by ceramide (Dadsena et al., 2019). In addition, during the initial phase of mitochondrial-mediated apoptosis, ceramide is further processed into two lipid messengers, sphingosine-1-phosphate and hexadecenal, which specifically activate Bcl-2 homologous antagonist killer (BAK) and BAX, respectively, for mitochondrial membrane permeabilization (Chipuk et al., 2012). Moreover, ceramide may also contribute to mitophagy, as the lethal autophagy induced by a natural bioactive sphingolipid, N-stearoyl-D-erythro-sphingosine (C18-ceramide), is dependent on the direct interaction between C18-ceramide and LC3 (Sentelle et al., 2012). As might be expected, crosstalk between cardiolipin and ceramide exists. For instance, cardiolipin has been shown to inhibit ceramide synthesis and promote its cleavage by ceramidase (El Bawab et al., 2001; Kim et al., 2016; Okino et al., 2003). On the other hand, manipulation of ceramide modulates in vivo cardiolipin levels (Babenko and Storozhenko, 2017), which is consistent with observations of an age-related accumulation of ceramide and a corresponding decline in cardiolipin (Helmy et al., 2003; Monette et al., 2011; Petrosillo et al., 2008; Sen et al., 2007). In addition, alterations in these sphingolipid-dependent pathways have been increasingly linked to conditions such as Alzheimer's disease (Pera et al., 2017).

Mitochondrial PE signaling
The IMM also contains the highest molar ratio of PE compared with other subcellular membranes, which is consistent with its bacterial origin, as PE is quite abundant in many bacteria (Kaval and Garsin, 2018; López-Lara and Geiger, 2017). This is mainly achieved by direct synthesis of PE from PS at the IMM (Fig. 1). Most phospholipids, including PC, PS, and PI, are synthesized in the ER and then transported to other organelles (Tatsuta and Langer, 2017). PS synthesized in the ER is transported to the mitochondrial outer membrane at ER–mitochondrial contact sites. PS is then further transferred from the mitochondrial

outer to the inner membrane after which it is converted by PS decarboxylase (PISD) into PE (Schuiki and Daum, 2009). PISD is an enzyme conserved from bacteria to humans that is critical for PE synthesis at the IMM, malfunction of which causes mitochondrial fragmentation and embryonic lethality in mice (Hartmann et al., 2016; Steenbergen et al., 2005), indicating that direct PE synthesis at the IMM cannot be compensated by other pathways.

PE synthesis at the IMM is dynamically controlled to balance cell growth and mitochondrial biogenesis. One key protein regulated by PISD-produced PE at the IMM is YME1L, a transmembrane ATP-dependent protease essential for mitochondrial proteostasis and dynamics, apoptotic resistance, and cell proliferation (Anand et al., 2014; Stiburek et al., 2012). Normal levels of PE suppresses the proteolytic activity of YME1L (Fig. 1). However, stresses such as hypoxia or nutrient deprivation induce a lipid signaling cascade upon inactivation of mechanistic target of rapamycin complex 1 (mTORC1) to reduce PE levels at the IMM, leading to activation of YME1L-mediated proteolysis of mitochondrial proteins and subsequent inhibition of mitochondrial biogenesis (MacVicar et al., 2019). This involves activation of LIPIN1, a substrate of mTORC1 that is phosphorylated and hence inhibited under basal conditions (e.g., available oxygen and nutrients). Inactivation of mTORC1 leads to dephosphorylation and activation of LIPIN1 that reduces the level of PA, resulting in a decline in the subsequent synthesis of PS, a substate of PE (MacVicar et al., 2019). Consistent with a key role for PE in suppressing YME1L, blocking PS transfer from the mitochondrial outer to inner membrane (hence reducing PE levels) activates YME1L (MacVicar et al., 2019). Thus, signals that modulate lipid transport and/or synthesis pathways can rewire mitochondrial metabolism in response to physiological changes. Interestingly, the proteolytic degradation of YME1L, as well as another mitochondrial protease, OMA1, is regulated by various cellular insults that perturb mitochondrial functions (Baker et al., 2014; Rainbolt et al., 2016). It would therefore be important to investigate whether this phenomenon is also modulated by changes in mitochondrial lipids.

While mitochondrial cardiolipin and PE have been extensively studied, other potential lipid signaling pathways are not yet well characterized. Although the mitochondrial outer membrane is very weakly charged, dynamic turnover of certain negatively charged phosphoinositides has been reported. For example, a low level of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P$_2$] can be detected on mitochondria (Watt et al., 2002). Removal or masking this mitochondrial pool of PI(4,5)P$_2$ leads to fragmentation and autophagic targeting of mitochondria (Rosivatz and Woscholski, 2011). However, the downstream effectors for these and related lipid signals are largely undefined.

In summary, the double-membrane structure of mitochondria and its unique lipid composition establish an unusual membrane environment that are essential for maintaining mitochondrial activity and homeostasis. In particular, mitochondrial lipid signaling, mediated through cardiolipin and PE, provides an immediate capacity to both sense and respond to mitochondrial stress. When this stress goes beyond the handling capacity of local lipid signaling, more mitochondrial damage is induced. This often leads to the activation of the next level of response, both on the surface mitochondria and in the cytosol, as we discuss below.

Mitochondrial innate immune signaling
Mitochondria are linked to various innate immune signaling pathways, with the best characterized being the cytosolic RNA-sensing pathway for which mitochondria function as an essential platform (Fig. 2). While endolysosomal microbial RNAs are sensed by Toll-like receptors (specifically Toll-like receptors 3/7/8), those in the cytosol are directly captured by cytosolic RNA sensors known as retinoic acid–inducible gene I (RIG-I)-like receptors (RLRs; Tan et al., 2018). These include RIG-I (Yoneyama et al., 2004), melanoma differentiation-associated gene 5 (MDA5; Kang et al., 2002), and laboratory of genetics and physiology 2 (Saito et al., 2007), the last of which has no known intrinsic signaling capacity. Once bound to RNA ligands, both RIG-I and MDA5 form oligomers that directly bind and activate mitochondrial antiviral-signaling protein (MAVS [also known as IPS-1, VISA, and CARDIF]), a signaling scaffold with a C-terminal transmembrane domain anchored on the OMM (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005). Activated MAVS forms aggregates that recruit signaling molecules (e.g., inhibitor of nuclear factor-κB kinase [IKK] and TANK-binding kinase [TBK1]), leading to the activation and nuclear translocation of NF-kB and IRF3/7, initiating transcriptional up-regulation of proinflammatory cytokines and type I IFNs (Liu et al., 2013). One key feature of MAVS signaling are its feedforward properties, whereby a small amount of MAVS aggregates can induce the formation of large MAVS aggregates by directly recruiting and activating other MAVS molecules on the mitochondria (Cai et al., 2014; Hou et al., 2011; Xu et al., 2015). The anchorage of MAVS on the mitochondrial surface is critical for its function, as removal of the MAVS transmembrane domain by a hepatitis C virus–encoded protease abrogates its signaling capacity (Li et al., 2005a; Li et al., 2005b; Meylan et al., 2005). Evidence suggests that MAVS may also localize to peroxisomes and mitochondrial-associated membranes (Dixit et al., 2010; Horner et al., 2011).

Cross-talk between MAVS signaling and cellular metabolism
There is extensive communication between MAVS signaling and mitochondrial metabolism (Fig. 2). It is well established that MAVS is C-terminally anchored on the OMM and that the formation of large MAVS aggregates causes substantial changes to mitochondrial morphology (Cai et al., 2014; Hou et al., 2011; Xu et al., 2015). Even endogenous levels of MAVS aggregates are sufficient to drive apoptosis, which is dependent on the MAVS transmembrane domain, likely linked to MAVS-driven mitochondrial fragmentation (Hwang et al., 2019). Driving such morphological changes are MAVS-dependent mitochondrial ROS generation, mitochondrial membrane hyperpolarization, inhibition of spare respiratory capacity, and modulation of ATP synthesis (see below; Buskiewicz et al., 2016; Lei et al., 2009).

An interesting link between MAVS signaling and glucose metabolism has recently been established (Fig. 2). The first step
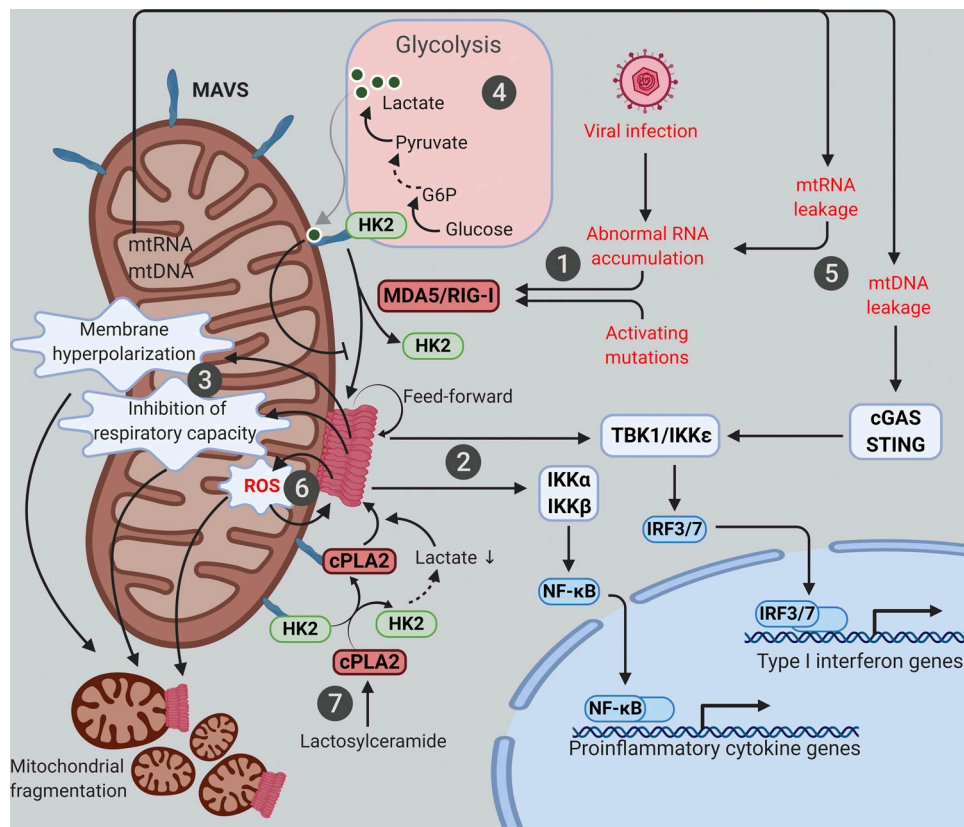
Figure 2. Mitochondria at the crossroads of innate immune signaling and metabolism. Mitochondria are platforms for MAVS innate immune signaling that modulates mitochondrial function and integrates immunity with cellular metabolism. The various known intersections between MAVS and mitochondrial function are diagramed. (1) Cytosolic RNAs perceived as foreign activate MAVS aggregation via the RNA sensors MDA5 and RIG-I. (2) Mitochondrial MAVS aggregates activate signaling cascades, leading to transcriptional induction of type I IFNs and proinflammatory cytokines. (3) MAVS aggregation causes marked alterations in a range of mitochondrial functions. (4) MAVS signaling inhibits glycolysis by releasing HK2 from mitochondria; lactate, a metabolite of anaerobic glycolysis, in turn suppresses MAVS aggregation via direct binding. (5) Mitochondrial leakage of RNA and DNA causes inflammation through MAVS and STING pathways, respectively. (6) Feedforward mechanism exists between MAVS aggregation and mitochondrial ROS. (7) Deregulated sphingolipid metabolism activates MAVS signaling independently of RNA ligands. cPLA2, cytosolic phospholipase A2; G6P, glucose 6-phosphate; HK2, hexokinase 2.

of glucose metabolism involves the phosphorylation of glucose into glucose-6 phosphate by hexokinase. Although glycolysis occurs in the cytosol, hexokinase 2 (HK2), the major enzyme that initiates glycolysis, requires mitochondrial localization for its glycolytic function (DeWaal et al., 2018; Roberts and Miyamoto, 2015; Wolf et al., 2016). The mitochondrial localization and activity of HK2 is dependent on the physical interactions of HK2 with both MAVS and VDAC situated on the OMM (Roberts and Miyamoto, 2015; Wolf et al., 2016; Zhang et al., 2019). Upon RLR activation, RIG-I binding to MAVS releases HK2 from mitochondria and thus inactivates glycolysis at its initial step (Zhang et al., 2019). On the other hand, up-regulation of glycolysis in turn suppresses MAVS signaling. Lactate, a key metabolite of anaerobic glycolysis, is a direct suppressor of RLR signaling, since it directly binds to the transmembrane domain of MAVS and prevents the formation of MAVS aggregates on the mitochondrial surface (Zhang et al., 2019).

Undesired MAVS signaling in diseases
While mitochondria play protective roles in innate immune signaling, abnormal activation of this system can nevertheless cause undesired inflammation and even cell death. For example,

the integrity of the mitochondrial genome and RNAs must be tightly controlled. In situations where membrane damage leads to the leakage of mitochondrial nucleic acids, immune responses and/or cell death can be triggered (Fig. 2). Due to bidirectional transcription, mitochondrial RNAs form extensive double-stranded RNAs that are efficiently digested by the mitochondrial RNA helicase SUV3 and the 3′–5′ RNA exonuclease PNPT1 (Aloni and Attardi, 1971; Dhir et al., 2018; Young and Attardi, 1975). Hypomorphic mutations of PNPT1 result in robust accumulation and cytosolic leakage of mitochondrial double-stranded RNAs that trigger MDA5- and MAVS-dependent inflammation (Dhir et al., 2018). Additionally, mitochondrial double-stranded RNA processed by RNase L in p53-deficient cells might also trigger MAVS signaling and transcription of proinflammatory factors (Wiatrek et al., 2019). Likewise, leakage of mitochondrial DNA (mtDNA) activates the cytosolic DNA sensor cyclic GMP-AMP synthase (cGAS), leading to the further activation of stimulator of IFN genes (STING) and type I IFN production (Kim et al., 2019; West et al., 2015). Such mtDNA-stimulated innate immune signaling appears to facilitate neurodegeneration in mouse models with mitochondrial stress but defective mitophagy (Sliter et al., 2018). It would be important

to further elucidate the role of inflammation mediated by mitochondrial-derived nucleic acid in other disease states.

Various RNA-independent mechanisms activating MAVS-mediated transcriptional up-regulation of proinflammatory cytokines have been described. One such mechanism involves the communication between oxidative stress and MAVS (Fig. 2). Oxidative stress is a common condition in infectious and inflammatory diseases, which can be sensed by MAVS to trigger RNA- and RLR-independent MAVS oligomerization and type I IFN production (Buskiewicz et al., 2016). Moreover, antioxidants can suppress ROS-induced MAVS oligomerization and IFN production (Buskiewicz et al., 2016), which may potentially benefit patients with autoimmune or inflammatory diseases. The fact that MAVS aggregates are resistant to detergent but sensitive to reducing agents is consistent with MAVS being an ROS sensor (Cai et al., 2014; Hou et al., 2011). In agreement with a possible role for ROS-driven disulfide bond formation in MAVS oligomerization, the MAVS-C79F variant does not oligomerize when exposed to ROS (Buskiewicz et al., 2016). Indeed, systemic lupus erythematosus patients carrying the MAVS-C79F variant show reduced plasma levels of MAVS oligomers, reduced type I IFN production, and milder disease symptoms (Buskiewicz et al., 2016; Pothlichet et al., 2011). Of note, while MAVS senses both cellular and mitochondrial ROS, MAVS aggregation can in turn promotes the generation of mitochondrial ROS and appears to be essential for infection-induced mitochondrial hyperpolarization, decrease in ATP synthesis, and drop in the spare respiratory capacity (Buskiewicz et al., 2016; Lei et al., 2009). It is still elusive how the prion-like aggregation of MAVS and the feed-forward cycle between ROS and MAVS aggregation are ultimately terminated and how mitochondrial function is restored in healthy patients after the clearance of an infection. Further mechanistic investigation of these critical deactivation steps could yield novel therapeutic strategies for autoimmune and inflammatory diseases.

Another unconventional mechanism that activates mitochondrial MAVS involves abnormal sphingolipid metabolism (Fig. 2). Sphingolipids are well known for their roles in promoting inflammation in both physiology and diseases. Expression changes of sphingolipid metabolizing enzymes have been documented upon induction of neuronal inflammation (Morita et al., 2020). In experimental autoimmune encephalomyelitis, a murine model of multiple sclerosis, the sphingolipid lactosylceramide promotes inflammation and subsequent neurodegeneration (Mayo et al., 2014). Recently, lactosylceramide has been found to trigger an interaction between MAVS and cytosolic phospholipase A2 (cPLA2) that activates MAVS aggregation and type I IFN production in astrocytes independently of RNA ligands and RLRs (Chao et al., 2019). Lactosylceramide directly binds to and activates cPLA2, a calcium-dependent phospholipase that cleaves phospholipids, releasing inflammatory lipid messengers (Chao et al., 2019; Leslie, 2015; Nakamura et al., 2013). Once activated, cPLA2 is recruited to mitochondria, where it binds and activates MAVS and changes mitochondrial metabolism by multiple mechanisms (Chao et al., 2019). First, cPLA2 binding directly triggers MAVS aggregation, leading to transcriptional production of pro-inflammatory cytokines and

type I IFN. Second, cPLA2 displaces HK2 from MAVS, which causes reduced HK2 activity in glycolysis (see above) and a subsequent decline in lactate production. Third, cPLA2 activation by lactosylceramide increases the expression of cPLA2 itself and the generation of ROS, both of which, in turn, further fuel MAVS aggregation (Chao et al., 2019). Given the reported associations of MAVS signaling, cPLA2, and sphingolipids with various neurodegeneration diseases, interventions targeting the cPLA2–MAVS signaling axis might prove beneficial.

Thus, MAVS is an integral part of the cellular stress response, which is supported by the extensive communications between MAVS signaling and cellular metabolism. While the MAVS pathway has likely evolved to protect cells from pathogens and damage, undesired, chronic activation of this RNA-sensing pathway, together with the cGAS–STING DNA-sensing pathway, is emerging as underlying contributor fueling inflammation-driven diseases.

Mitochondrial retrograde signaling

Besides lipid signaling within mitochondrial membrane and innate immune signaling on the mitochondrial surface and in the cytosol, mitochondria also send out stress signals via inter-organelle communication, adding another layer of potential regulation. Crosstalk between the mitochondria and nucleus is well defined in the regulation of cellular bioenergetics and stress responses. This includes anterograde signaling from the nucleus to the mitochondria, as most mitochondrial proteins are encoded by the nuclear genome, translated in the cytosol, and imported into mitochondria. On the other hand, metabolic, proteostatic or oxidative stress within mitochondria can be communicated in a retrograde fashion back to the nucleus (Fig. 3).

One such retrograde pathway is controlled by the lipid signaling we have already discussed. When mitochondrial function is compromised, cells activate a transcriptional response known as the mitochondrial unfolded protein response (UPR; Shpilka and Haynes, 2018). In yeasts, general cellular membrane stress caused by global lipid disequilibrium can be resolved by transcriptional activation of the mitochondrial UPR, which in turn maintains cellular membrane morphology (Thibault et al., 2012). Likewise, in worms, mitochondrial protein-folding stress can be decoded into local lipid signaling, which is further transmitted to the nucleus to transcriptionally turn on both the mitochondrial UPR and the cytosolic heat shock response. Specifically, disruption of mitochondrial proteostasis triggers an up-regulation of cardiolipin and down-regulation of ceramide to initiate a nuclear transcription program (Kim et al., 2016). Both cardiolipin accumulation and ceramide reduction are necessary and sufficient to trigger this program, which protects worm cells from cytosolic proteotoxicity when mitochondrial proteostasis is impaired (Kim et al., 2016).

An increasing number of proteins encoded by the nuclear genome have been shown to reside in, or on, mitochondria under basal conditions, whereas metabolic or other cellular stresses stimulate their translocation into the nucleus to activate transcriptional stress responses. For example, a well-conserved oxidative stress response factor, nuclear factor erythroid-2–related factor 2 (NRF2, known as SKN-1 in *Caenorhabditis*
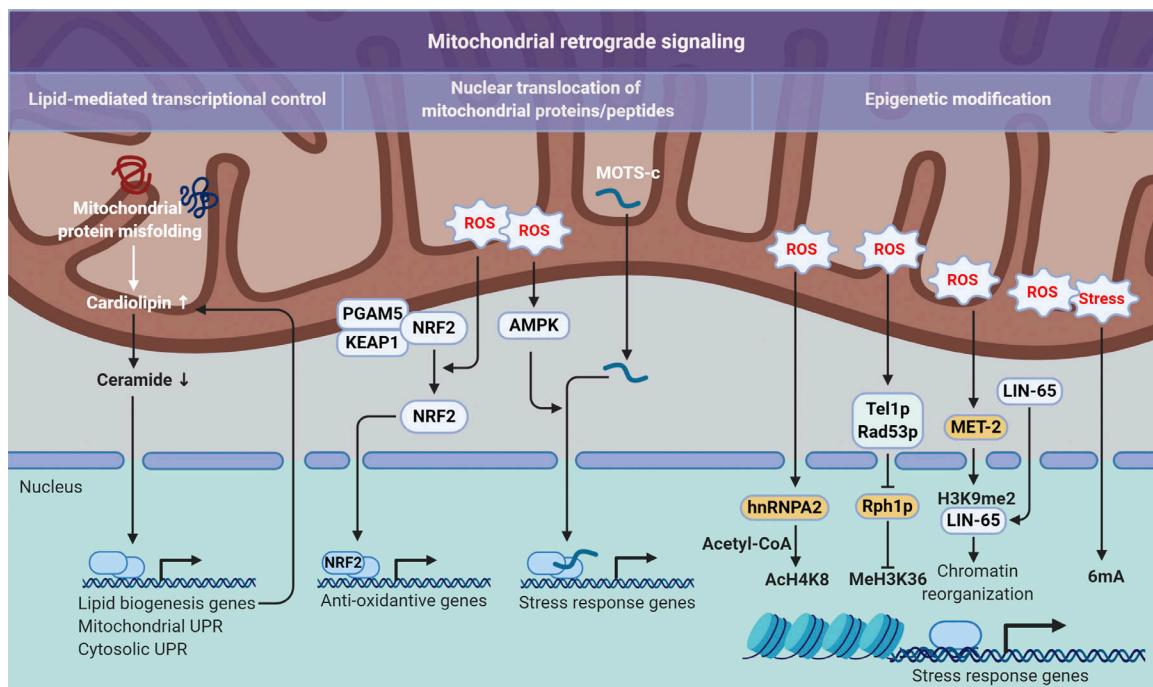
Figure 3. Mitochondrial retrograde signaling pathways. Three distinct modes of retrograde signaling that transform mitochondrial stress into nuclear programs are diagramed. On the left, mitochondrial protein folding stress is decoded into nuclear transcription via a lipid signaling-mediated UPR. In the center, mitochondrial stress provokes nuclear translocation of mitochondrial-tethered proteins or peptides that, in turn, initiate transcription of stress response genes. On the right, mitochondrial stresses, such as an increase in ROS levels, are translated into epigenetic modifications that facilitate transcriptional up-regulation of stress response genes. 6mA, N6-methyldeoxyadenine; AcH4K8, acetylation on histone H4 at lysine 8; H3K9me2, dimethylation at the ninth lysine residue of the histone H3 protein; MeH3K36, methylation on histone H3 at lysine 36.

elegans) is sequestered by interacting with Kelch-like ECH associated protein 1 (KEAP1) and the mitochondrial serine/threonine protein phosphatase PGAM5 on the surface of the OMM (An and Blackwell, 2003; Lo and Hannink, 2008). Oxidative stress disrupts this protein complex and triggers NRF2 translocation to the nucleus, where it activates the transcription of a large number of genes, including a set of antioxidant proteins, to help restore redox homeostasis (Lo and Hannink, 2008; Sekhar et al., 2002). Other nuclear-encoded mitochondrial proteins that translocate into the nuclei upon physiological or exogenous stresses have been recently reviewed (Monaghan and Whitmarsh, 2015). Interestingly, a 16-amino-acid peptide called MOTS-c, encoded by the mitochondrial genome of mammalian cells, also translocates from cytoplasmic structures to the nucleus upon metabolic stresses accompanied by ROS production (Kim et al., 2018). Activation of 5′-AMP–activated protein kinase (AMPK) downstream of ROS is required for MOTS-c nuclear translocation (Kim et al., 2018). In the nucleus, MOTS-c in turn initiates the transcription of a set of stress response genes that may protect against conditions characterized by metabolic dysfunction (Lee et al., 2015).

In additional to regulating translocation of transcription factors, mitochondria can also transmit stress signals that result in epigenetic modifications, such as histone acetylation and histone and DNA methylation. A mild increase in mitochondrial ROS, especially at the early stage of life, has been shown to benefit multiple organisms from yeast to humans. In *C. elegans*, early exposure to oxidants results in epigenetic change in the

adult organism that correlates with increased stress resistance and a longer lifespan (Bazopoulou et al., 2019). Although in many cases it remains unclear as to how mitochondrial stress is transmitted to the epigenome, some downstream mitochondrial ROS effectors have been identified in yeasts and worms with conserved homologues in mammals. For instance, in murine cells, the heterogeneous ribonucleoprotein A2 (hnRNPA2) acetylates lysine 8 of histone H4 at mitochondrial stress–responsive promoters in an acetyl-coenzyme A–dependent manner to activate gene transcription (Guha et al., 2016). In yeasts, two serine/threonine protein kinases, Tel1p and Rad53p, homologues of mammalian ataxia telangiectasia mutated (ATM) and Chk2 (Schroeder et al., 2013), promote yeast chronological lifespan in response to mitochondrial ROS by inactivating the histone H3K36 demethylase Rph1p, leading to methylation and transcriptional suppression of subtelomeric regions (Schroeder et al., 2013). The highly conserved histone lysine demethylases JMJD-1.2/PHF8 and JMJD-3.1/JMJD3 also play key roles in mitochondrial stress–induced lifespan extension in different species (Merkwirth et al., 2016). Another histone methyl transferase, MET-2, is involved in this process in worms by regulating chromatin reorganization as part of mitochondrial stress response in the nucleus, which is regulated by a nuclear cofactor LIN-65 that translocates from cytosol to the nucleus upon mitochondrial stress (Tian et al., 2016). Such chromatin reorganization causes gene silencing of many loci but often allows for transcriptional activation of stress response genes at other chromosome locations (Tian et al., 2016). Mitochondrial stress

also triggers global elevation of N6-methyldeoxyadenine in stress responsive genes, which can be inherited to modulate stress response signaling across generations of worms (Ma et al., 2019). Thus, in general, mitochondrial stress induces epigenetic reprograming of gene expression in order to promote stress adaptation.

It is clear that there are multiple retrograde signaling pathways deriving from the mitochondria. However, at present, these pathways do not always seem to be highly conserved among species. It is also conceivable that these observed species differences reflect the role mitochondrial communication plays in a largely postmitotic organism like *C. elegans* compared with more dynamic mammalian species. Whatever the explanation, it is fair to say that the molecular and biochemical mechanisms linking mitochondrial stress to the activation or inactivation of transcription factors and epigenetic enzymes are still, for the most part, poorly defined.

Therapeutic targeting of mitochondrial signaling

Most human diseases are age related. Aging is a complex process involving the impairment of mitochondrial respiratory activity, increase of oxidative stress, compromised stress response, and accumulation of damaged protein and organelles, leading to overall decline of cellular functions and induction of various age-related diseases. Since mitochondrial ROS contribute to much of the cellular damage in aging and disease, the most straightforward therapeutic approach would appear to be modulating mitochondrial ROS levels (Fig. 4). With that said, the history of broadly acting ROS scavengers is not particularly encouraging (Bjelakovic et al., 2007), although the antioxidant edaravone was recently approved for the treatment of amyotrophic lateral sclerosis (Jaiswal, 2019). One promising class of mitochondria-targeted antioxidants are plastoquinone derivatives that can accumulate within mitochondria and, once oxidized, can be reduced by accepting electrons from the mitochondrial respiratory chain (Feniouk and Skulachev, 2018b). One plastoquinone derivative, SkQ1 (a decyltriphenyl phosphonium cation conjugated to a quinone moiety), has been extensively characterized in vitro and in vivo and appears to have significant antioxidant activity at even nanomolar concentrations (Feniouk and Skulachev, 2018a). Long-term treatment with SkQ1 increased lifespan of a range of species, including mammals, and various clinical trials are currently testing clinical efficacy on various age-related diseases. An eyedrop form of SkQ1 (Visomitin) is currently in phase 3 trials for dry eye disease, a condition associated with oxidative stress (Feniouk and Skulachev, 2018a).

Another group of mitochondria-targeted antioxidants are peptides (Fig. 4). In 2004, Szeto and Schiller designed a series of cell-permeable antioxidant tetrapeptides (SS peptides) that specifically accumulate at the IMM (Zhao et al., 2004). Among these peptides, the most promising one, SS-31 (D-Arg-2′6′-dimethylTyr-Lys-Phe-NH2 [also known as MTP-131, elamipretide, or Bendavia]) is believed to directly bind to cardiolipin in the IMM and protect it from peroxidation, leading to restored mitochondrial bioenergetics (Birk et al., 2013). SS-31 has proven effective in protecting mitochondrial functions in different animal models, and clinical trials have also demonstrated promising effects in humans (Szeto, 2014). In an early small trial, 5 d of SS-31 treatment substantially increased exercise performance of patients with primary mitochondrial myopathy (Karaa et al., 2018). In another clinical trial for human atherosclerotic renal artery stenosis that required a revascularization procedure, SS-31 significantly suppressed procedure-associated ischemic renal injury and increased renal blood flow and renal function after the procedure, with overall improved outcome for revascularization (Saad et al., 2017). However, disappointingly, a recent randomized, phase 3 trial with this compound failed to demonstrate efficacy in patients with primary mitochondrial diseases.

When blocking ROS generation may be difficult or problematic, improving the removal of damaged mitochondria is an alternative therapeutic approach. Indeed, accumulation of damaged mitochondria is implicated in various degenerative diseases, and pharmacologically stimulating mitophagy has proven an effective way to prevent cell death (Fig. 4). A chemical screen identified the antibiotic actinonin as a potent activator of mitophagy (Sun et al., 2015). More recently, urolithin A and actinonin have been found to stimulate mitophagy and reverse memory impairment in various animal models of Alzheimer's disease (Fang et al., 2019), suggesting that mitophagy stimulation may be a promising therapeutic intervention for neurodegeneration.

Nucleic acid–stimulated innate immune signaling is emerging as a key mechanism for multiple age-related diseases. Downstream of mitochondrial oxidative stress and mitochondrial damage, RNA- and/or DNA-stimulated inflammation has been shown to be an essential contributing factor for tissue damage in various conditions (Dhir et al., 2018; Maekawa et al., 2019; Sliter et al., 2018). This usually involves genetic mutations or cellular damage that causes abnormal exposure of nucleic acids to innate immune sensors or gain-of-functions mutations directly activating nucleic acid sensors such as MDA5. Given the age-dependent increase of oxidative stress that could cross-activate MAVS signaling (Buskiewicz et al., 2016) and the age-dependent increase in circulating mtDNA (Pinti et al., 2014), inhibitors specifically targeting the MAVS pathway or the cGAS–STING pathway (Haag et al., 2018; Hall et al., 2017; Lama et al., 2019; Vincent et al., 2017) could be considered for inflammation-driven diseases (Fig. 4). This concept has been recently expanded on by the demonstration of mtDNA escape from the mitochondria via oxidative stress–induced macropores formed by VDAC oligomers on the OMM (Kim et al., 2019). Small molecules that inhibit VDAC oligomerization (Ben-Hail et al., 2016) appear to provide a mitochondrial-centric strategy to lessen the inflammatory symptoms seen in an animal model of lupus (Kim et al., 2019).

The ideal therapeutics for aging and age-related diseases may be a drug that can function through multiple mechanisms. Metformin, the most commonly used medication for type 2 diabetes, is one such drug that has drawn increasingly more attention due to its antiaging effects in many models (Barzilai et al., 2016). Metformin reduces insulin levels, mTORC1 signaling, ROS generation, DNA damage, and inflammation, whereas it appears to activate AMPK, autophagy, and removal of senescent
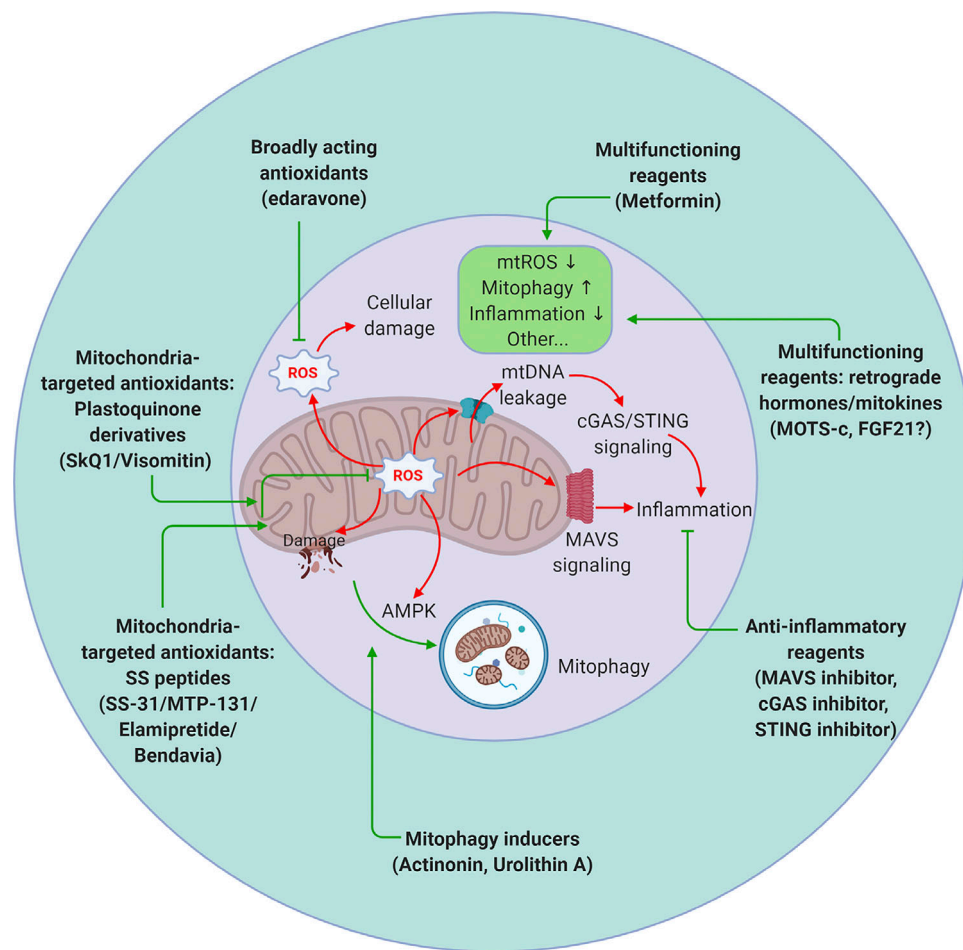
Figure 4. **Therapeutic targeting of mitochondrial signaling.** Strategies to target mitochondria include blocking mitochondrial damage by reducing mitochondrial ROS (antioxidants that are either broadly acting or mitochondrial specific), accelerating removal of damaged mitochondria by stimulating mitophagy (actinonin and urolithin A), and suppressing undesired inflammation caused by mitochondrial damage (inhibitors targeting the MAVS or cGAS–STING pathways). Metformin and the retrograde hormone MOTS-c appear to act through multiple mechanisms. mtROS, mitochondrial ROS.

cells (Barzilai et al., 2016). These effects may all be a result of the effects of metformin as a mild mitochondrial inhibitor. The Metformin in Longevity Study (MILES) was launched in 2014 with 14 patients and demonstrated the potential effects of metformin in modulating metabolic and nonmetabolic pathways linked to aging (Kulkarni et al., 2018). A much larger trial, Targeting Aging with Metformin (TAME), has been planned in order to test the effects of metformin on various age-related diseases (Barzilai et al., 2016). The mitochondrial genome-encoded retrograde hormone MOTS-c appears to act similarly to metformin by activating AMPK and increasing methionine turnover (Lee et al., 2015). Mice injected with this 16-amino-acid peptide show increased sensitivity to insulin and resistance to diet-induced obesity (Lee et al., 2015). Interestingly, the levels of MOTS-c decrease with age, suggesting that replenishing MOTS-c levels might have therapeutic potential in certain age-related diseases.

Conclusions and perspectives

Blending in and standing out, mitochondria are uniquely positioned to function as signaling hubs. Though expressed in hundreds of copies per cell, their unique composition and genome make them a complicated mix of the familiar and the distinct. Here, we have described how these properties can affect cell fate decisions, such as initiating cell death pathways, triggering innate immunity, or altering the epigenetic code. Many questions remain. These include a better understanding of how various other critical properties of mitochondrial function (e.g., fusion/fission and membrane potential) impact signaling. In that regard, it would be important to understand how age-dependent impairment of mitochondrial quality control alters, for instance, MAVS aggregation or epigenetic regulation. While we have focused on retrograde communication, namely the communication from mitochondria to the nucleus, other signaling connections are currently less understood. The tight physical connection between ER and mitochondria likely means these organelles can cross-regulate their biology, yet details are currently woefully incomplete. More distant connections might also exist (e.g., mitochondrial–lysosomal interactions). Of note, individual bacteria communicate with each other through mechanisms such as quorum sensing, and it would not be surprising if analogous mechanisms allowed the multiple mitochondria within a given cell to somehow talk to

each other. Finally, it remains likely that mitochondria can signal beyond the cell. We have touched briefly on mitochondrial-derived peptides such as MOTS-c, which circulate and are readily detectable in human serum. The notion of other circulating "mitokines" is attractive and represents a way to potentially coordinate organismal adaptation to mitochondrial stress. In simpler organisms, such as *C. elegans*, there is ample evidence for such pathways (Durieux et al., 2011; Zhang et al., 2018). In mammals, mitochondrial dysfunction in one tissue can trigger increased expression of circulating factors, such as FGF21, which may help to mitigate the initial stress (Kim et al., 2013). Interestingly, transgenic overexpression of FGF21 in mice appears to extend lifespan (Zhang et al., 2012). However, these animals exhibited supraphysiological levels of FGF21 (5–10 times), and in fact, in humans, levels of FGF21 actually appear to increase as we age (Conte et al., 2019). As such, questions remain as to whether FGF21 and related factors (e.g., GDF15) are sensitive or specific biomarkers of mitochondrial function and whether they play a protective or detrimental role.

While key questions remain unanswered, even with this very rudimentary understanding and significant knowledge gaps, the clinical applications of these insights are accelerating. Unlike the lessons of junior high school, these applications will likely have enduring consequences, as successful manipulation of mitochondrial signaling has the potential to impact a wide range of inflammatory and age-related conditions.

Acknowledgments

References

Ahyayauch, H., M. Raab, J.V. Busto, N. Andraka, J.-L.R. Arrondo, M. Masserini, I. Tvaroska, and F.M. Goñi. 2012. Binding of β-amyloid (1-42) peptide to negatively charged phospholipid membranes in the liquid-ordered state: modeling and experimental studies. *Biophys. J.* 103: 453–463. https://doi.org/10.1016/j.bpj.2012.06.043

Aloni, Y., and G. Attardi. 1971. Symmetrical in vivo transcription of mitochondrial DNA in HeLa cells. *Proc. Natl. Acad. Sci. USA.* 68:1757–1761. https://doi.org/10.1073/pnas.68.8.1757

An, J.H., and T.K. Blackwell. 2003. SKN-1 links C. elegans mesendodermal specification to a conserved oxidative stress response. *Genes Dev.* 17: 1882–1893. https://doi.org/10.1101/gad.1107803

Anand, R., T. Wai, M.J. Baker, N. Kladt, A.C. Schauss, E. Rugarli, and T. Langer. 2014. The i-AAA protease YME1L and OMA1 cleave OPA1 to balance mitochondrial fusion and fission. *J. Cell Biol.* 204:919–929. https://doi.org/10.1083/jcb.201308006

Babenko, N., and G. Storozhenko. 2017. Role of ceramide in the aging-related decrease of cardiolipin content in the rat heart. *Adv. Gerontol.* 7:195–200. https://doi.org/10.1134/S207905701703002X

Baker, M.J., P.A. Lampe, D. Stojanovski, A. Korwitz, R. Anand, T. Tatsuta, and T. Langer. 2014. Stress-induced OMA1 activation and autocatalytic

turnover regulate OPA1-dependent mitochondrial dynamics. *EMBO J.* 33:578–593. https://doi.org/10.1002/embj.201386474

Barzilai, N., J.P. Crandall, S.B. Kritchevsky, and M.A. Espeland. 2016. Metformin as a tool to target aging. *Cell Metab.* 23:1060–1065. https://doi.org/10.1016/j.cmet.2016.05.011

Bazopoulou, D., D. Knoefler, Y. Zheng, K. Ulrich, B.J. Oleson, L. Xie, M. Kim, A. Kaufmann, Y.T. Lee, Y. Dou, et al. 2019. Developmental ROS individualizes organismal stress resistance and lifespan. *Nature.* 576: 301–305. https://doi.org/10.1038/s41586-019-1814-y

Ben-Hail, D., R. Begas-Shvartz, M. Shalev, A. Shteinfer-Kuzmine, A. Gruzman, S. Reina, V. De Pinto, and V. Shoshan-Barmatz. 2016. Novel Compounds Targeting the Mitochondrial Protein VDAC1 Inhibit Apoptosis and Protect against Mitochondrial Dysfunction. *J. Biol. Chem.* 291: 24986–25003. https://doi.org/10.1074/jbc.M116.744284

Birbes, H., S. El Bawab, Y.A. Hannun, and L.M. Obeid. 2001. Selective hydrolysis of a mitochondrial pool of sphingomyelin induces apoptosis. *FASEB J.* 15:2669–2679. https://doi.org/10.1096/fj.01-0539com

Birk, A.V., S. Liu, Y. Soong, W. Mills, P. Singh, J.D. Warren, S.V. Seshan, J.D. Pardee, and H.H. Szeto. 2013. The mitochondrial-targeted compound SS-31 re-energizes ischemic mitochondria by interacting with cardiolipin. *J. Am. Soc. Nephrol.* 24:1250–1261. https://doi.org/10.1681/ASN.2012121216

Bjelakovic, G., D. Nikolova, L.L. Gluud, R.G. Simonetti, and C. Gluud. 2007. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA.* 297:842–857. https://doi.org/10.1001/jama.297.8.842

Boynton, T.O.H., and L.J. Shimkets. 2015. Myxococcus CsgA, Drosophila Sniffer, and human HSD10 are cardiolipin phospholipases. *Genes Dev.* 29:1903–1914. https://doi.org/10.1101/gad.268482.115

Buskiewicz, I.A., T. Montgomery, E.C. Yasewicz, S.A. Huber, M.P. Murphy, R.C. Hartley, R. Kelly, M.K. Crow, A. Perl, R.C. Budd, and A. Koenig. 2016. Reactive oxygen species induce virus-independent MAVS oligomerization in systemic lupus erythematosus. *Sci. Signal.* 9:ra115. https://doi.org/10.1126/scisignal.aaf1933

Cai, X., J. Chen, H. Xu, S. Liu, Q.-X. Jiang, R. Halfmann, and Z.J. Chen. 2014. Prion-like polymerization underlies signal transduction in antiviral immune defense and inflammasome activation. *Cell.* 156:1207–1222. https://doi.org/10.1016/j.cell.2014.01.063

Camilleri, A., C. Zarb, M. Caruana, U. Ostermeier, S. Ghio, T. Högen, F. Schmidt, A. Giese, and N. Vassallo. 2013. Mitochondrial membrane permeabilisation by amyloid aggregates and protection by polyphenols. *Biochim. Biophys. Acta.* 1828:2532–2543. https://doi.org/10.1016/j.bbamem.2013.06.026

Camilleri, A., S. Ghio, M. Caruana, D. Weckbecker, F. Schmidt, F. Kamp, A. Leonov, S. Ryazanov, C. Griesinger, A. Giese, et al. 2020. Tau-induced mitochondrial membrane perturbation is dependent upon cardiolipin. *Biochim. Biophys. Acta Biomembr.* 1862:183064. https://doi.org/10.1016/j.bbamem.2019.183064

Chao, H., Y. Liu, X. Fu, X. Xu, Z. Bao, C. Lin, Z. Li, Y. Liu, X. Wang, Y. You, et al. 2018. Lowered iPLA$_2$γ activity causes increased mitochondrial lipid peroxidation and mitochondrial dysfunction in a rotenone-induced model of Parkinson's disease. *Exp. Neurol.* 300:74–86. https://doi.org/10.1016/j.expneurol.2017.10.031

Chao, C.-C., C. Gutiérrez-Vázquez, V. Rothhammer, L. Mayo, M.A. Wheeler, E.C. Tjon, S.E. Zandee, M. Blain, K.A. de Lima, and M.C. Takenaka. 2019. Metabolic Control of Astrocyte Pathogenic Activity via cPLA2-MAVS. *Cell.* 179:1483–1498.e1422. https://doi.org/10.1016/j.cell.2019.11.016

Chipuk, J.E., G.P. McStay, A. Bharti, T. Kuwana, C.J. Clarke, L.J. Siskind, L.M. Obeid, and D.R. Green. 2012. Sphingolipid metabolism cooperates with BAK and BAX to promote the mitochondrial pathway of apoptosis. *Cell.* 148:988–1000. https://doi.org/10.1016/j.cell.2012.01.038

Chu, C.T., J. Ji, R.K. Dagda, J.F. Jiang, Y.Y. Tyurina, A.A. Kapralov, V.A. Tyurin, N. Yanamala, I.H. Shrivastava, D. Mohammadyani, et al. 2013. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat. Cell Biol.* 15: 1197–1205. https://doi.org/10.1038/ncb2837

Cockcroft, S., and P. Raghu. 2018. Phospholipid transport protein function at organelle contact sites. *Curr. Opin. Cell Biol.* 53:52–60. https://doi.org/10.1016/j.ceb.2018.04.011

Conte, M., R. Ostan, C. Fabbri, A. Santoro, G. Guidarelli, G. Vitale, D. Mari, F. Sevini, M. Capri, M. Sandri, et al. 2019. Human Aging and Longevity Are Characterized by High Levels of Mitokines. *J. Gerontol. A Biol. Sci. Med. Sci.* 74:600–607. https://doi.org/10.1093/gerona/gly153

Dadsena, S., S. Bockelmann, J.G.M. Mina, D.G. Hassan, S. Korneev, G. Razzera, H. Jahn, P. Niekamp, D. Müller, M. Schneider, et al. 2019.

Ceramides bind VDAC2 to trigger mitochondrial apoptosis. *Nat. Commun.* 10:1832. https://doi.org/10.1038/s41467-019-09654-4

de Kroon, A.I., D. Dolis, A. Mayer, R. Lill, and B. de Kruijff. 1997. Phospholipid composition of highly purified mitochondrial outer membranes of rat liver and Neurospora crassa. Is cardiolipin present in the mitochondrial outer membrane? *Biochim. Biophys. Acta.* 1325:108–116. https://doi.org/10.1016/S0005-2736(96)00240-4

DeWaal, D., V. Nogueira, A.R. Terry, K.C. Patra, S.-M. Jeon, G. Guzman, J. Au, C.P. Long, M.R. Antoniewicz, and N. Hay. 2018. Hexokinase-2 depletion inhibits glycolysis and induces oxidative phosphorylation in hepatocellular carcinoma and sensitizes to metformin. *Nat. Commun.* 9:446. https://doi.org/10.1038/s41467-017-02733-4

Dhir, A., S. Dhir, L.S. Borowski, L. Jimenez, M. Teitell, A. Rötig, Y.J. Crow, G.I. Rice, D. Duffy, C. Tamby, et al. 2018. Mitochondrial double-stranded RNA triggers antiviral signalling in humans. *Nature.* 560:238–242. https://doi.org/10.1038/s41586-018-0363-0

Dixit, E., S. Boulant, Y. Zhang, A.S. Lee, C. Odendall, B. Shum, N. Hacohen, Z.J. Chen, S.P. Whelan, M. Fransen, et al. 2010. Peroxisomes are signaling platforms for antiviral innate immunity. *Cell.* 141:668–681. https://doi.org/10.1016/j.cell.2010.04.018

Dudek, J. 2017. Role of cardiolipin in mitochondrial signaling pathways. *Front. Cell Dev. Biol.* 5:90. https://doi.org/10.3389/fcell.2017.00090

Durieux, J., S. Wolff, and A. Dillin. 2011. The cell-non-autonomous nature of electron transport chain-mediated longevity. *Cell.* 144:79–91. https://doi.org/10.1016/j.cell.2010.12.016

El Bawab, S., H. Birbes, P. Roddy, Z.M. Szulc, A. Bielawska, and Y.A. Hannun. 2001. Biochemical characterization of the reverse activity of rat brain ceramidase. A CoA-independent and fumonisin B1-insensitive ceramide synthase. *J. Biol. Chem.* 276:16758–16766. https://doi.org/10.1074/jbc.M009331200

Epand, R.F., U. Schlattner, T. Wallimann, M.-L. Lacombe, and R.M. Epand. 2007. Novel lipid transfer property of two mitochondrial proteins that bridge the inner and outer membranes. *Biophys. J.* 92:126–137. https://doi.org/10.1529/biophysj.106.092353

Fang, E.F., Y. Hou, K. Palikaras, B.A. Adriaanse, J.S. Kerr, B. Yang, S. Lautrup, M.M. Hasan-Olive, D. Caponio, X. Dan, et al. 2019. Mitophagy inhibits amyloid-β and tau pathology and reverses cognitive deficits in models of Alzheimer's disease. *Nat. Neurosci.* 22:401–412. https://doi.org/10.1038/s41593-018-0332-9

Feniouk, B.A., and M.V. Skulachev. 2018a. SkQ1: The Road from Laboratory Bench to the Market. In *Mitochondrial Biology and Experimental Therapeutics.* P.J. Oliveira, editor. Springer, Cham, Switzerland. 637–645. https://doi.org/10.1007/978-3-319-73344-9_28

Feniouk, B.A., and V.P. Skulachev. 2018b. Studies on mitochondria directed plastoquinones. In *Mitochondrial Biology and Experimental Therapeutics.* P.J. Oliveira, editor. Springer, Cham, Switzerland. 523–533. https://doi.org/10.1007/978-3-319-73344-9_23

Ganesan, V., M.N. Perera, D. Colombini, D. Datskovskiy, K. Chadha, and M. Colombini. 2010. Ceramide and activated Bax act synergistically to permeabilize the mitochondrial outer membrane. *Apoptosis.* 15:553–562. https://doi.org/10.1007/s10495-009-0449-0

Garcia Fernandez, M., L. Troiano, L. Moretti, J. Pedrazzi, S. Salvioli, I. Castilla-Cortazar, and A. Cossarizza. 2000. Changes in intramitochondrial cardiolipin distribution in apoptosis-resistant HCW-2 cells, derived from the human promyelocytic leukemia HL-60. *FEBS Lett.* 478:290–294. https://doi.org/10.1016/S0014-5793(00)01861-5

Gebert, N., A.S. Joshi, S. Kutik, T. Becker, M. McKenzie, X.L. Guan, V.P. Mooga, D.A. Stroud, G. Kulkarni, M.R. Wenk, et al. 2009. Mitochondrial cardiolipin involved in outer-membrane protein biogenesis: implications for Barth syndrome. *Curr. Biol.* 19:2133–2139. https://doi.org/10.1016/j.cub.2009.10.074

Gonzalvez, F., Z.T. Schug, R.H. Houtkooper, E.D. MacKenzie, D.G. Brooks, R.J. Wanders, P.X. Petit, F.M. Vaz, and E. Gottlieb. 2008. Cardiolipin provides an essential activating platform for caspase-8 on mitochondria. *J. Cell Biol.* 183:681–696. https://doi.org/10.1083/jcb.200803129

Guha, M., S. Srinivasan, K. Guja, E. Mejia, M. Garcia-Diaz, F.B. Johnson, G. Ruthel, B.A. Kaufman, E.F. Rappaport, M.R. Glineburg, et al. 2016. HnRNPA2 is a novel histone acetyltransferase that mediates mitochondrial stress-induced nuclear gene expression. *Cell Discov.* 2:16045. https://doi.org/10.1038/celldisc.2016.45

Haag, S.M., M.F. Gulen, L. Reymond, A. Gibelin, L. Abrami, A. Decout, M. Heymann, F.G. van der Goot, G. Turcatti, R. Behrendt, and A. Ablasser. 2018. Targeting STING with covalent small-molecule inhibitors. *Nature.* 559:269–273. https://doi.org/10.1038/s41586-018-0287-8

Hall, J., A. Brault, F. Vincent, S. Weng, H. Wang, D. Dumlao, A. Aulabaugh, D. Aivazian, D. Castro, M. Chen, et al. 2017. Discovery of PF-06928215 as a high affinity inhibitor of cGAS enabled by a novel fluorescence polarization assay. *PLoS One.* 12:e0184843. https://doi.org/10.1371/journal.pone.0184843

Hartmann, B., T. Wai, H. Hu, T. MacVicar, L. Musante, B. Fischer-Zirnsak, W. Stenzel, R. Gräf, L. van den Heuvel, H.-H. Ropers, et al. 2016. Homozygous YME1L1 mutation causes mitochondriopathy with optic atrophy and mitochondrial network fragmentation. *eLife.* 5:e16078. https://doi.org/10.7554/eLife.16078

He, X.-Y., H. Schulz, and S.-Y. Yang. 1998. A human brain L-3-hydroxyacyl-coenzyme A dehydrogenase is identical to an amyloid β-peptide-binding protein involved in Alzheimer's disease. *J. Biol. Chem.* 273:10741–10746. https://doi.org/10.1074/jbc.273.17.10741

Helmy, F.M., M.H. Hack, and A. Juracka. 2003. Age-related changes of the endogenous cardiolipin and plasmalogens of guinea pig kidney and their in vitro hydrolysis by endogenous phospholipases: a thin layer chromatographic analysis in conjunction with densitometric measurement. *Cell Biochem. Funct.* 21:337–344. https://doi.org/10.1002/cbf.1035

Horner, S.M., H.M. Liu, H.S. Park, J. Briley, and M. Gale Jr. 2011. Mitochondrial-associated endoplasmic reticulum membranes (MAM) form innate immune synapses and are targeted by hepatitis C virus. *Proc. Natl. Acad. Sci. USA.* 108:14590–14595. https://doi.org/10.1073/pnas.1110133108

Horvath, S.E., and G. Daum. 2013. Lipids of mitochondria. *Prog. Lipid Res.* 52:590–614. https://doi.org/10.1016/j.plipres.2013.07.002

Horvath, S.E., H. Rampelt, S. Oeljeklaus, B. Warscheid, M. van der Laan, and N. Pfanner. 2015. Role of membrane contact sites in protein import into mitochondria. *Protein Sci.* 24:277–297. https://doi.org/10.1002/pro.2625

Hou, F., L. Sun, H. Zheng, B. Skaug, Q.-X. Jiang, and Z.J. Chen. 2011. MAVS forms functional prion-like aggregates to activate and propagate antiviral innate immune response. *Cell.* 146:448–461. https://doi.org/10.1016/j.cell.2011.06.041

Hsu, P., X. Liu, J. Zhang, H.-G. Wang, J.-M. Ye, and Y. Shi. 2015. Cardiolipin remodeling by TAZ/tafazzin is selectively required for the initiation of mitophagy. *Autophagy.* 11:643–652. https://doi.org/10.1080/15548627.2015.1023984

Hwang, M.S., J. Boulanger, J.D. Howe, A. Albecka, M. Pasche, L. Mureşan, and Y. Modis. 2019. MAVS polymers smaller than 80 nm induce mitochondrial and membrane remodeling and interferon signaling. *FEBS J.* 286:1543–1560. https://doi.org/10.1111/febs.14772

Jaiswal, M.K. 2019. Riluzole and edaravone: A tale of two amyotrophic lateral sclerosis drugs. *Med. Res. Rev.* 39:733–748. https://doi.org/10.1002/med.21528

Kagan, V.E., J. Jiang, Z. Huang, Y.Y. Tyurina, C. Desbourdes, C. Cottet-Rousselle, H.H. Dar, M. Verma, V.A. Tyurin, A.A. Kapralov, et al. 2016. NDPK-D (NM23-H4)-mediated externalization of cardiolipin enables elimination of depolarized mitochondria by mitophagy. *Cell Death Differ.* 23:1140–1151. https://doi.org/10.1038/cdd.2015.160

Kang, D.C., R.V. Gopalkrishnan, Q. Wu, E. Jankowsky, A.M. Pyle, and P.B. Fisher. 2002. mda-5: An interferon-inducible putative RNA helicase with double-stranded RNA-dependent ATPase activity and melanoma growth-suppressive properties. *Proc. Natl. Acad. Sci. USA.* 99:637–642. https://doi.org/10.1073/pnas.022637199

Karaa, A., R. Haas, A. Goldstein, J. Vockley, W.D. Weaver, and B.H. Cohen. 2018. Randomized dose-escalation trial of elamipretide in adults with primary mitochondrial myopathy. *Neurology.* 90:e1212–e1221. https://doi.org/10.1212/WNL.0000000000005255

Kasahara, T., M. Kubota-Sakashita, Y. Nagatsuka, Y. Hirabayashi, T. Hanasaka, K. Tohyama, and T. Kato. 2020. Cardiolipin is essential for early embryonic viability and mitochondrial integrity of neurons in mammals. *FASEB J.* 34:1465–1480. https://doi.org/10.1096/fj.201901598R

Kaval, K.G., and D.A. Garsin. 2018. Ethanolamine utilization in bacteria. *MBio.* 9:e00066–e00018. https://doi.org/10.1128/mBio.00066-18

Kawai, T., K. Takahashi, S. Sato, C. Coban, H. Kumar, H. Kato, K.J. Ishii, O. Takeuchi, and S. Akira. 2005. IPS-1, an adaptor triggering RIG-I- and Mda5-mediated type I interferon induction. *Nat. Immunol.* 6:981–988. https://doi.org/10.1038/ni1243

Kim, K.H., Y.T. Jeong, H. Oh, S.H. Kim, J.M. Cho, Y.N. Kim, S.S. Kim, D.H. Kim, K.Y. Hur, H.K. Kim, et al. 2013. Autophagy deficiency leads to protection from obesity and insulin resistance by inducing Fgf21 as a mitokine. *Nat. Med.* 19:83–92. https://doi.org/10.1038/nm.3014

Kim, H.-E., A.R. Grant, M.S. Simic, R.A. Kohnz, D.K. Nomura, J. Durieux, C.E. Riera, M. Sanchez, E. Kapernick, and S. Wolff. 2016. Lipid biosynthesis coordinates a mitochondrial-to-cytosolic stress response. *Cell.* 166:1539–1552.

Kim, K.H., J.M. Son, B.A. Benayoun, and C. Lee. 2018. The mitochondrial-encoded peptide MOTS-c translocates to the nucleus to regulate nuclear gene expression in response to metabolic stress. *Cell Metab.* 28:516–524.

Kim, J., R. Gupta, L.P. Blanco, S. Yang, A. Shteinfer-Kuzmine, K. Wang, J. Zhu, H.E. Yoon, X. Wang, M. Kerkhofs, et al. 2019. VDAC oligomers form mitochondrial pores to release mtDNA fragments and promote lupus-like disease. *Science.* 366:1531–1536. https://doi.org/10.1126/science.aav4011

Krištofiková, Z., M. Bocková, K. Hegnerová, A. Bartoš, J. Klaschka, J. Rícný, D. Rípová, and J. Homola. 2009. Enhanced levels of mitochondrial enzyme 17β-hydroxysteroid dehydrogenase type 10 in patients with Alzheimer disease and multiple sclerosis. *Mol. Biosyst.* 5:1174–1179. https://doi.org/10.1039/b904799a

Kulkarni, A.S., E.F. Brutsaert, V. Anghel, K. Zhang, N. Bloomgarden, M. Pollak, J.C. Mar, M. Hawkins, J.P. Crandall, and N. Barzilai. 2018. Metformin regulates metabolic and nonmetabolic pathways in skeletal muscle and subcutaneous adipose tissues of older adults. *Aging Cell.* 17:e12723. https://doi.org/10.1111/acel.12723

Kuwana, T., M.R. Mackey, G. Perkins, M.H. Ellisman, M. Latterich, R. Schneiter, D.R. Green, and D.D. Newmeyer. 2002. Bid, Bax, and lipids cooperate to form supramolecular openings in the outer mitochondrial membrane. *Cell.* 111:331–342. https://doi.org/10.1016/S0092-8674(02)01036-X

Lai, Y.-C., C.-C. Li, T.-C. Sung, C.-W. Chang, Y.-J. Lan, and Y.-W. Chiang. 2019. The role of cardiolipin in promoting the membrane pore-forming activity of BAX oligomers. *Biochim. Biophys. Acta Biomembr.* 1861:268–280. https://doi.org/10.1016/j.bbamem.2018.06.014

Lama, L., C. Adura, W. Xie, D. Tomita, T. Kamei, V. Kuryavyi, T. Gogakos, J.I. Steinberg, M. Miller, L. Ramos-Espiritu, et al. 2019. Development of human cGAS-specific small-molecule inhibitors for repression of dsDNA-triggered interferon expression. *Nat. Commun.* 10:2261. https://doi.org/10.1038/s41467-019-08620-4

Lee, C., J. Zeng, B.G. Drew, T. Sallam, A. Martin-Montalvo, J. Wan, S.-J. Kim, H. Mehta, A.L. Hevener, R. de Cabo, and P. Cohen. 2015. The mitochondrial-derived peptide MOTS-c promotes metabolic homeostasis and reduces obesity and insulin resistance. *Cell Metab.* 21:443–454. https://doi.org/10.1016/j.cmet.2015.02.009

Lei, Y., C.B. Moore, R.M. Liesman, B.P. O'Connor, D.T. Bergstralh, Z.J. Chen, R.J. Pickles, and J.P.-Y. Ting. 2009. MAVS-mediated apoptosis and its inhibition by viral proteins. *PLoS One.* 4:e5466. https://doi.org/10.1371/journal.pone.0005466

Leslie, C.C. 2015. Cytosolic phospholipase A₂: physiological function and role in disease. *J. Lipid Res.* 56:1386–1402. https://doi.org/10.1194/jlr.R057588

Li, K., E. Foy, J.C. Ferreon, M. Nakamura, A.C. Ferreon, M. Ikeda, S.C. Ray, M. Gale Jr., and S.M. Lemon. 2005a. Immune evasion by hepatitis C virus NS3/4A protease-mediated cleavage of the Toll-like receptor 3 adaptor protein TRIF. *Proc. Natl. Acad. Sci. USA.* 102:2992–2997. https://doi.org/10.1073/pnas.0408824102

Li, X.-D., L. Sun, R.B. Seth, G. Pineda, and Z.J. Chen. 2005b. Hepatitis C virus protease NS3/4A cleaves mitochondrial antiviral signaling protein off the mitochondria to evade innate immunity. *Proc. Natl. Acad. Sci. USA.* 102:17717–17722. https://doi.org/10.1073/pnas.0508531102

Liu, J., Q. Dai, J. Chen, D. Durrant, A. Freeman, T. Liu, D. Grossman, and R.M. Lee. 2003. Phospholipid scramblase 3 controls mitochondrial structure, function, and apoptotic response. *Mol. Cancer Res.* 1:892–902.

Liu, J., R.F. Epand, D. Durrant, D. Grossman, N.W. Chi, R.M. Epand, and R.M. Lee. 2008. Role of phospholipid scramblase 3 in the regulation of tumor necrosis factor-α-induced apoptosis. *Biochemistry.* 47:4518–4529. https://doi.org/10.1021/bi701962c

Liu, S., J. Chen, X. Cai, J. Wu, X. Chen, Y.-T. Wu, L. Sun, and Z.J. Chen. 2013. MAVS recruits multiple ubiquitin E3 ligases to activate antiviral signaling cascades. *eLife.* 2:e00785. https://doi.org/10.7554/eLife.00785

Liu, G.-Y., S.H. Moon, C.M. Jenkins, M. Li, H.F. Sims, S. Guan, and R.W. Gross. 2017. The phospholipase iPLA₂γ is a major mediator releasing oxidized aliphatic chains from cardiolipin, integrating mitochondrial bioenergetics and signaling. *J. Biol. Chem.* 292:10672–10684. https://doi.org/10.1074/jbc.M117.783068

Lo, S.-C., and M. Hannink. 2008. PGAM5 tethers a ternary complex containing Keap1 and Nrf2 to mitochondria. *Exp. Cell Res.* 314:1789–1803. https://doi.org/10.1016/j.yexcr.2008.02.014

López-Lara, I.M., and O. Geiger. 2017. Bacterial lipid diversity. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids.* 1862:1287–1299. https://doi.org/10.1016/j.bbalip.2016.10.007

Lutter, M., M. Fang, X. Luo, M. Nishijima, X. Xie, and X. Wang. 2000. Cardiolipin provides specificity for targeting of tBid to mitochondria. *Nat. Cell Biol.* 2:754–756. https://doi.org/10.1038/35036395

Ma, C., R. Niu, T. Huang, L.-W. Shao, Y. Peng, W. Ding, Y. Wang, G. Jia, C. He, C.-Y. Li, et al. 2019. N6-methyldeoxyadenine is a transgenerational epigenetic signal for mitochondrial stress adaptation. *Nat. Cell Biol.* 21:319–327. https://doi.org/10.1038/s41556-018-0238-5

MacVicar, T., Y. Ohba, H. Nolte, F.C. Mayer, T. Tatsuta, H.-G. Sprenger, B. Lindner, Y. Zhao, J. Li, C. Bruns, et al. 2019. Lipid signalling drives proteolytic rewiring of mitochondria by YME1L. *Nature.* 575:361–365. https://doi.org/10.1038/s41586-019-1738-6

Maekawa, H., T. Inoue, H. Ouchi, T.-M. Jao, R. Inoue, H. Nishi, R. Fujii, F. Ishidate, T. Tanaka, and Y. Tanaka. 2019. Mitochondrial damage causes inflammation via cGAS-STING signaling in acute kidney injury. *Cell Rep.* 29:1261–1273.e1266. https://doi.org/10.1016/j.celrep.2019.09.050

Mayo, L., S.A. Trauger, M. Blain, M. Nadeau, B. Patel, J.I. Alvarez, I.D. Mascanfroni, A. Yeste, P. Kivisäkk, K. Kallas, et al. 2014. Regulation of astrocyte activation by glycolipids drives chronic CNS inflammation. *Nat. Med.* 20:1147–1156. https://doi.org/10.1038/nm.3681

Merkwirth, C., V. Jovaisaite, J. Durieux, O. Matilainen, S.D. Jordan, P.M. Quiros, K.K. Steffen, E.G. Williams, L. Mouchiroud, S.U. Tronnes, et al. 2016. Two conserved histone demethylases regulate mitochondrial stress-induced longevity. *Cell.* 165:1209–1223. https://doi.org/10.1016/j.cell.2016.04.012

Meylan, E., J. Curran, K. Hofmann, D. Moradpour, M. Binder, R. Bartenschlager, and J. Tschopp. 2005. Cardif is an adaptor protein in the RIG-I antiviral pathway and is targeted by hepatitis C virus. *Nature.* 437:1167–1172. https://doi.org/10.1038/nature04193

Monaghan, R.M., and A.J. Whitmarsh. 2015. Mitochondrial proteins moonlighting in the nucleus. *Trends Biochem. Sci.* 40:728–735. https://doi.org/10.1016/j.tibs.2015.10.003

Monette, J.S., L.A. Gómez, R.F. Moreau, K.C. Dunn, J.A. Butler, L.A. Finlay, A.J. Michels, K.P. Shay, E.J. Smith, and T.M. Hagen. 2011. (R)-α-Lipoic acid treatment restores ceramide balance in aging rat cardiac mitochondria. *Pharmacol. Res.* 63:23–29. https://doi.org/10.1016/j.phrs.2010.09.007

Morita, M., S. Watanabe, M. Oyama, T. Iwai, and M. Tanabe. 2020. Glycosphingolipid Biosynthesis Pathway in the Spinal Cord and Dorsal Root Ganglia During Inflammatory Pain: Early and Late Changes in Expression Patterns of Glycosyltransferase Genes. *Neuroscience.* 428:217–227. https://doi.org/10.1016/j.neuroscience.2019.12.029

Nakamura, K., V.M. Nemani, F. Azarbal, G. Skibinski, J.M. Levy, K. Egami, L. Munishkina, J. Zhang, B. Gardner, J. Wakabayashi, et al. 2011. Direct membrane association drives mitochondrial fission by the Parkinson disease-associated protein α-synuclein. *J. Biol. Chem.* 286:20710–20726. https://doi.org/10.1074/jbc.M110.213538

Nakamura, H., Y. Moriyama, T. Makiyama, S. Emori, H. Yamashita, R. Yamazaki, and T. Murayama. 2013. Lactosylceramide interacts with and activates cytosolic phospholipase A2α. *J. Biol. Chem.* 288:23264–23272. https://doi.org/10.1074/jbc.M113.491431

Ogretmen, B. 2018. Sphingolipid metabolism in cancer signalling and therapy. *Nat. Rev. Cancer.* 18:33–50. https://doi.org/10.1038/nrc.2017.96

Okino, N., X. He, S. Gatt, K. Sandhoff, M. Ito, and E.H. Schuchman. 2003. The reverse activity of human acid ceramidase. *J. Biol. Chem.* 278:29948–29953. https://doi.org/10.1074/jbc.M303310200

Ordóñez-Gutiérrez, L., F. Re, E. Bereczki, E. Ioja, M. Gregori, A.J. Andersen, M. Antón, S.M. Moghimi, J.-J. Pei, M. Masserini, and F. Wandosell. 2015. Repeated intraperitoneal injections of liposomes containing phosphatidic acid and cardiolipin reduce amyloid-β levels in APP/PS1 transgenic mice. *Nanomedicine (Lond.).* 11:421–430. https://doi.org/10.1016/j.nano.2014.09.015

Paradies, G., G. Petrosillo, M. Pistolese, and F.M. Ruggiero. 2001. Reactive oxygen species generated by the mitochondrial respiratory chain affect the complex III activity via cardiolipin peroxidation in beef-heart submitochondrial particles. *Mitochondrion.* 1:151–159. https://doi.org/10.1016/S1567-7249(01)00011-3

Paradies, G., G. Petrosillo, M. Pistolese, and F.M. Ruggiero. 2002. Reactive oxygen species affect mitochondrial electron transport complex I activity through oxidative cardiolipin damage. *Gene.* 286:135–141. https://doi.org/10.1016/S0378-1119(01)00814-9

Pera, M., D. Larrea, C. Guardia-Laguarta, J. Montesinos, K.R. Velasco, R.R. Agrawal, Y. Xu, R.B. Chan, G. Di Paolo, M.F. Mehler, et al. 2017. Increased localization of APP-C99 in mitochondria-associated ER membranes causes mitochondrial dysfunction in Alzheimer disease. *EMBO J.* 36:3356–3371. https://doi.org/10.15252/embj.201796797

Petrosillo, G., M. Matera, G. Casanova, F.M. Ruggiero, and G. Paradies. 2008. Mitochondrial dysfunction in rat brain with aging Involvement of complex I, reactive oxygen species and cardiolipin. *Neurochem. Int.* 53: 126–131. https://doi.org/10.1016/j.neuint.2008.07.001

Pinti, M., E. Cevenini, M. Nasi, S. De Biasi, S. Salvioli, D. Monti, S. Benatti, L. Gibellini, R. Cotichini, M.A. Stazi, et al. 2014. Circulating mitochondrial DNA increases with age and is a familiar trait: Implications for "inflamm-aging". *Eur. J. Immunol.* 44:1552–1562. https://doi.org/10.1002/eji.201343921

Pothlichet, J., T.B. Niewold, D. Vitour, B. Solhonne, M.K. Crow, and M. Si-Tahar. 2011. A loss-of-function variant of the antiviral molecule MAVS is associated with a subset of systemic lupus patients. *EMBO Mol. Med.* 3: 142–152. https://doi.org/10.1002/emmm.201000120

Rainbolt, T.K., J. Lebeau, C. Puchades, and R.L. Wiseman. 2016. Reciprocal degradation of YME1L and OMA1 adapts mitochondrial proteolytic activity during stress. *Cell Rep.* 14:2041–2049. https://doi.org/10.1016/j.celrep.2016.02.011

Roberts, D.J., and S. Miyamoto. 2015. Hexokinase II integrates energy metabolism and cellular protection: Akting on mitochondria and TORCing to autophagy. *Cell Death Differ.* 22:248–257. https://doi.org/10.1038/cdd.2014.173

Robotta, M., H.R. Gerding, A. Vogel, K. Hauser, S. Schildknecht, C. Karreman, M. Leist, V. Subramaniam, and M. Drescher. 2014. Alpha-synuclein binds to the inner membrane of mitochondria in an α-helical conformation. *ChemBioChem.* 15:2499–2502. https://doi.org/10.1002/cbic.201402281

Rosivatz, E., and R. Woscholski. 2011. Removal or masking of phosphatidylinositol(4,5)bisphosphate from the outer mitochondrial membrane causes mitochondrial fragmentation. *Cell. Signal.* 23:478–486. https://doi.org/10.1016/j.cellsig.2010.10.025

Ryan, T., V.V. Bamm, M.G. Stykel, C.L. Coackley, K.M. Humphries, R. Jamieson-Williams, R. Ambasudhan, D.D. Mosser, S.A. Lipton, G. Harauz, and S.D. Ryan. 2018. Cardiolipin exposure on the outer mitochondrial membrane modulates α-synuclein. *Nat. Commun.* 9:817. https://doi.org/10.1038/s41467-018-03241-9

Saad, A., S.M.S. Herrmann, A. Eirin, C.M. Ferguson, J.F. Glockner, H. Bjarnason, M.A. McKusick, S. Misra, L.O. Lerman, and S.C. Textor. 2017. Phase 2a clinical trial of mitochondrial protection (elamipretide) during stent revascularization in patients with atherosclerotic renal artery stenosis. *Circ. Cardiovasc. Interv.* 10:e005487. https://doi.org/10.1161/CIRCINTERVENTIONS.117.005487

Saito, T., R. Hirai, Y.-M. Loo, D. Owen, C.L. Johnson, S.C. Sinha, S. Akira, T. Fujita, and M. Gale Jr. 2007. Regulation of innate antiviral defenses through a shared repressor domain in RIG-I and LGP2. *Proc. Natl. Acad. Sci. USA.* 104:582–587. https://doi.org/10.1073/pnas.0606699104

Schlame, M., and M.L. Greenberg. 2017. Biosynthesis, remodeling and turnover of mitochondrial cardiolipin. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids.* 1862:3–7. https://doi.org/10.1016/j.bbalip.2016.08.010

Schlattner, U., M. Tokarska-Schlattner, S. Ramirez, Y.Y. Tyurina, A.A. Amoscato, D. Mohammadyani, Z. Huang, J. Jiang, N. Yanamala, A. Seffouh, et al. 2013. Dual function of mitochondrial Nm23-H4 protein in phosphotransfer and intermembrane lipid transfer: a cardiolipin-dependent switch. *J. Biol. Chem.* 288:111–121. https://doi.org/10.1074/jbc.M112.408633

Schroeder, E.A., N. Raimundo, and G.S. Shadel. 2013. Epigenetic silencing mediates mitochondria stress-induced longevity. *Cell Metab.* 17: 954–964. https://doi.org/10.1016/j.cmet.2013.04.003

Schuiki, I., and G. Daum. 2009. Phosphatidylserine decarboxylases, key enzymes of lipid metabolism. *IUBMB Life.* 61:151–162. https://doi.org/10.1002/iub.159

Scorrano, L., M.A. De Matteis, S. Emr, F. Giordano, G. Hajnóczky, B. Kornmann, L.L. Lackner, T.P. Levine, L. Pellegrini, K. Reinisch, et al. 2019. Coming together to define membrane contact sites. *Nat. Commun.* 10: 1287. https://doi.org/10.1038/s41467-019-09253-3

Sekhar, K.R., D.R. Spitz, S. Harris, T.T. Nguyen, M.J. Meredith, J.T. Holt, D. Gius, L.J. Marnett, M.L. Summar, and M.L. Freeman. 2002. Redox-sensitive interaction between KIAA0132 and Nrf2 mediates indomethacin-induced expression of γ-glutamylcysteine synthetase. *Free Radic. Biol. Med.* 32:650–662. https://doi.org/10.1016/S0891-5849(02)00755-4

Sen, T., N. Sen, S. Jana, F.H. Khan, U. Chatterjee, and S. Chakrabarti. 2007. Depolarization and cardiolipin depletion in aged rat brain mitochondria: relationship with oxidative stress and electron transport chain activity. *Neurochem. Int.* 50:719–725. https://doi.org/10.1016/j.neuint.2007.01.007

Sentelle, R.D., C.E. Senkal, W. Jiang, S. Ponnusamy, S. Gencer, S.P. Selvam, V.K. Ramshesh, Y.K. Peterson, J.J. Lemasters, Z.M. Szulc, et al. 2012. Ceramide targets autophagosomes to mitochondria and induces lethal mitophagy. *Nat. Chem. Biol.* 8:831–838. https://doi.org/10.1038/nchembio.1059

Seth, R.B., L. Sun, C.-K. Ea, and Z.J. Chen. 2005. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell.* 122:669–682. https://doi.org/10.1016/j.cell.2005.08.012

Shpilka, T., and C.M. Haynes. 2018. The mitochondrial UPR: mechanisms, physiological functions and implications in ageing. *Nat. Rev. Mol. Cell Biol.* 19:109–120. https://doi.org/10.1038/nrm.2017.110

Sliter, D.A., J. Martinez, L. Hao, X. Chen, N. Sun, T.D. Fischer, J.L. Burman, Y. Li, Z. Zhang, D.P. Narendra, et al. 2018. Parkin and PINK1 mitigate STING-induced inflammation. *Nature.* 561:258–262. https://doi.org/10.1038/s41586-018-0448-9

Steenbergen, R., T.S. Nanowski, A. Beigneux, A. Kulinski, S.G. Young, and J.E. Vance. 2005. Disruption of the phosphatidylserine decarboxylase gene in mice causes embryonic lethality and mitochondrial defects. *J. Biol. Chem.* 280:40032–40040. https://doi.org/10.1074/jbc.M506510200

Stiburek, L., J. Cesnekova, O. Kostkova, D. Fornuskova, K. Vinsova, L. Wenchich, J. Houstek, and J. Zeman. 2012. YME1L controls the accumulation of respiratory chain subunits and is required for apoptotic resistance, cristae morphogenesis, and cell proliferation. *Mol. Biol. Cell.* 23: 1010–1023. https://doi.org/10.1091/mbc.e11-08-0674

Sun, N., J. Yun, J. Liu, D. Malide, C. Liu, I.I. Rovira, K.M. Holmström, M.M. Fergusson, Y.H. Yoo, C.A. Combs, and T. Finkel. 2015. Measuring in vivo mitophagy. *Mol. Cell.* 60:685–696. https://doi.org/10.1016/j.molcel.2015.10.009

Szeto, H.H. 2014. First-in-class cardiolipin-protective compound as a therapeutic agent to restore mitochondrial bioenergetics. *Br. J. Pharmacol.* 171:2029–2050. https://doi.org/10.1111/bph.12461

Tan, X., L. Sun, J. Chen, and Z.J. Chen. 2018. Detection of microbial infections through innate immune sensing of nucleic acids. *Annu. Rev. Microbiol.* 72:447–478. https://doi.org/10.1146/annurev-micro-102215-095605

Tatsuta, T., and T. Langer. 2017. Intramitochondrial phospholipid trafficking. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids.* 1862:81–89. https://doi.org/10.1016/j.bbalip.2016.08.006

Tatsuta, T., M. Scharwey, and T. Langer. 2014. Mitochondrial lipid trafficking. *Trends Cell Biol.* 24:44–52. https://doi.org/10.1016/j.tcb.2013.07.011

Thibault, G., G. Shui, W. Kim, G.C. McAlister, N. Ismail, S.P. Gygi, M.R. Wenk, and D.T. Ng. 2012. The membrane stress response buffers lethal effects of lipid disequilibrium by reprogramming the protein homeostasis network. *Mol. Cell.* 48:16–27. https://doi.org/10.1016/j.molcel.2012.08.016

Tian, Y., G. Garcia, Q. Bian, K.K. Steffen, L. Joe, S. Wolff, B.J. Meyer, and A. Dillin. 2016. Mitochondrial stress induces chromatin reorganization to promote longevity and UPRmt. *Cell.* 165:1197–1208. https://doi.org/10.1016/j.cell.2016.04.011

Tyurina, Y.Y., S.M. Poloyac, V.A. Tyurin, A.A. Kapralov, J. Jiang, T.S. Anthonymuthu, V.I. Kapralova, A.S. Vikulina, M.-Y. Jung, M.W. Epperly, et al. 2014. A mitochondrial pathway for biosynthesis of lipid mediators. *Nat. Chem.* 6:542–552. https://doi.org/10.1038/nchem.1924

Vincent, J., C. Adura, P. Gao, A. Luz, L. Lama, Y. Asano, R. Okamoto, T. Imaeda, J. Aida, and K. Rothamel. 2017. Small molecule inhibition of cGAS reduces interferon expression in primary macrophages from autoimmune mice. *Nat. Commun.* 8:1–13.

Watt, S.A., G. Kular, I.N. Fleming, C.P. Downes, and J.M. Lucocq. 2002. Subcellular localization of phosphatidylinositol 4,5-bisphosphate using the pleckstrin homology domain of phospholipase C delta1. *Biochem. J.* 363:657–666. https://doi.org/10.1042/bj3630657

West, A.P., W. Khoury-Hanold, M. Staron, M.C. Tal, C.M. Pineda, S.M. Lang, M. Bestwick, B.A. Duguay, N. Raimundo, D.A. MacDuff, et al. 2015. Mitochondrial DNA stress primes the antiviral innate immune response. *Nature.* 520:553–557. https://doi.org/10.1038/nature14156

Wiatrek, D.M., M.E. Candela, J. Sedmík, J. Oppelt, L.P. Keegan, and M.A. O'Connell. 2019. Activation of innate immunity by mitochondrial dsRNA in mouse cells lacking p53 protein. *RNA.* 25:713–726. https://doi.org/10.1261/rna.069625.118

Wolf, A.J., C.N. Reyes, W. Liang, C. Becker, K. Shimada, M.L. Wheeler, H.C. Cho, N.I. Popescu, K.M. Coggeshall, M. Arditi, and D.M. Underhill. 2016. Hexokinase is an innate immune receptor for the detection of bacterial peptidoglycan. *Cell.* 166:624–636.

Wu, H., P. Carvalho, and G.K. Voeltz. 2018. Here, there, and everywhere: The importance of ER membrane contact sites. *Science.* 361:eaan5835.

Xu, L.-G., Y.-Y. Wang, K.-J. Han, L.-Y. Li, Z. Zhai, and H.-B. Shu. 2005. VISA is an adapter protein required for virus-triggered IFN-β signaling. *Mol. Cell.* 19:727–740. https://doi.org/10.1016/j.molcel.2005.08.014

Xu, Y., M. Condell, H. Plesken, I. Edelman-Novemsky, J. Ma, M. Ren, and M. Schlame. 2006. A Drosophila model of Barth syndrome. *Proc. Natl. Acad. Sci. USA.* 103:11584–11588. https://doi.org/10.1073/pnas.0603242103

Xu, H., X. He, H. Zheng, L.J. Huang, F. Hou, Z. Yu, M.J. de la Cruz, B. Borkowski, X. Zhang, Z.J. Chen, and Q.X. Jiang. 2015. Correction: Structural basis for the prion-like MAVS filaments in antiviral innate immunity. *eLife.* 4:e07546. https://doi.org/10.7554/eLife.07546

Yan, S.D., J. Fu, C. Soto, X. Chen, H. Zhu, F. Al-Mohanna, K. Collison, A. Zhu, E. Stern, T. Saido, et al. 1997. An intracellular protein that binds amyloid-β peptide and mediates neurotoxicity in Alzheimer's disease. *Nature.* 389:689–695. https://doi.org/10.1038/39522

Yoneyama, M., M. Kikuchi, T. Natsukawa, N. Shinobu, T. Imaizumi, M. Miyagishi, K. Taira, S. Akira, and T. Fujita. 2004. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat. Immunol.* 5:730–737. https://doi.org/10.1038/ni1087

Young, P.G., and G. Attardi. 1975. Characterization of double-stranded RNA from HeLa cell mitochondria. *Biochem. Biophys. Res. Commun.* 65:1201–1207. https://doi.org/10.1016/S0006-291X(75)80357-3

Zhang, Y., Y. Xie, E.D. Berglund, K.C. Coate, T.T. He, T. Katafuchi, G. Xiao, M.J. Potthoff, W. Wei, and Y. Wan. 2012. The starvation hormone, fibroblast growth factor-21, extends lifespan in mice. *eLife.* 1:e00065. https://doi.org/10.7554/eLife.00065

Zhang, Q., X. Wu, P. Chen, L. Liu, N. Xin, Y. Tian, and A. Dillin. 2018. The Mitochondrial Unfolded Protein Response Is Mediated Cell-Non-autonomously by Retromer-Dependent Wnt Signaling. *Cell.* 174:870–883.e17. https://doi.org/10.1016/j.cell.2018.06.029

Zhang, W., G. Wang, Z.-G. Xu, H. Tu, F. Hu, J. Dai, Y. Chang, Y. Chen, Y. Lu, H. Zeng, et al. 2019. Lactate Is a Natural Suppressor of RLR Signaling by Targeting MAVS. *Cell.* 178:176–189.e15. https://doi.org/10.1016/j.cell.2019.05.003

Zhao, K., G.-M. Zhao, D. Wu, Y. Soong, A.V. Birk, P.W. Schiller, and H.H. Szeto. 2004. Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *J. Biol. Chem.* 279:34682–34690. https://doi.org/10.1074/jbc.M402999200