Lipid-mediated signals that regulate mitochondrial biology

Jason R. Nielson¹² and Jared P. Rutter¹*  

From the ¹Department of Biochemistry, University of Utah, Salt Lake City, UT 84112; ²Department of Pharmacology and Toxicology, University of Utah, Salt Lake City, UT 84112

Running title: Mitochondrial lipid signaling

*To whom correspondence should be addressed: Jared Rutter; Rutter@biochem.utah.edu

Keywords: mitochondria, lipid signaling, lipid peroxidation, sphingolipids, proteostasis, mitophagy, stress response, cell signaling, lipid-protein interaction

Abstract

For decades, lipids were assumed to fulfill roles only in energy storage and membrane structure. Recent studies have discovered critical roles for phospholipids, sphingolipids, and sterols in many cellular pathways, including cell signaling and transcriptional regulation. Frequently, lipids from these various classes work together to achieve defined cellular outcomes. Specific mitochondrial lipids are critical for proper assembly of the electron transport chain complexes and for effective responses to mitochondrial damage, including maintenance of mitochondrial protein homeostasis, regulation of mitophagy, and induction of apoptosis. In this review, we will primarily focus on mitochondrial lipid signaling mediated by lipid-protein interactions.

Introduction

Lipids are a class of non-genetically encoded hydrophobic or amphipathic molecules essential for life (1). Historically, lipids were ascribed roles exclusive to energy storage and membrane structure. However, studies conducted over the last quarter of a century have demonstrated active roles for lipids in information transfer through cell signaling and transcriptional regulation. Bioactive lipids fulfill these roles through lipid-lipid and lipid-protein interactions. Lipid-lipid interactions alter the biophysical properties of a membrane, thereby affecting membrane architecture and the ability of proteins and other molecules to interact with the membrane and each other (2). Lipid-protein interactions can regulate the function of target proteins by modulating their activity or by targeting proteins to distinct regions of the cell to carry out specific activities or act on specific substrates (3).

The abundance of individual lipids within the cell strongly influences the mechanisms whereby a given lipid can participate in cell signaling (3). Changes in the abundance of a rare lipid, such as sphingosine-1-phosphate, are unlikely to alter the biophysical properties of a membrane. Rather, such lipids are more likely to interact with high-affinity receptors that can detect changes in their abundance, even at low absolute concentrations. On the other hand, highly abundant lipids have the ability to perturb the organization and biophysical properties of a membrane as their concentration changes. Because of the more substantial progress in our understanding of this area, this review will primarily focus primarily on lipid signaling that is mediated by lipid-protein interactions.

Pioneering discoveries in lipid signaling

One of the pioneering discoveries of how lipid-protein interactions affect signaling pathways centers around the different fates of phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂). PI(4,5)P₂ can be hydrolyzed by phospholipase C to produce the second messengers, inositol trisphosphate (IP₃) and diacylglycerol (DAG) (4). IP₃ bind to IP₃ receptors at the endoplasmic reticulum membrane to promote Ca²⁺-dependent signaling and DAG, in combination with Ca²⁺, aids in the activation of protein kinase C (5-7). Alternatively, PI(4,5)P₂ can be phosphorylated by a phosphoinositide 3-kinase (PI3K) to generate phosphatidylinositol 3,4,5-trisphosphate (PIP₃). PIP₃ then recruits PH domain-containing proteins, such as the AKT protein kinase, which in turn...
activate pathways required for cell growth and survival (5). In the remainder of this review, we will examine how other lipid species, similar to the phosphoinositides described above, affect cellular processes through specific protein interactions, with a specific focus on signaling pathways that regulate mitochondrial function and quality.

**Cardiolipin trafficking and mitophagy**

Cardiolipin is a specialized phospholipid, comprising almost 20% of the lipid content in the mitochondrial inner membrane (MIM). In healthy mitochondria, cardiolipin is found almost exclusively in the MIM, where it helps to stabilize cristae and support the assembly and function of electron transport chain complexes (8,9). However, recent studies have shown that mitochondrial injury, depolarization, and other damage signals cause externalization of cardiolipin to the mitochondrial outer membrane (MOM) where it is exposed to the cytosol (8,10). This MOM-localized cardiolipin appears to have very important signaling functions. Decreased delivery of mitochondria to autophagosomes was observed upon genetic deletion or RNAi knockdown of cardiolipin synthase or upon knockdown of two enzymes that translocate cardiolipin to the MOM, phospholipid scramblase-3 or nucleoside diphosphate kinase D (NDPK-D) (8,10,11). Through a combination of molecular docking analyses, in vitro binding assays, and fluorescence microscopy, Chu et al. identified sites on LC3, a component of the autophagy machinery, required for direct interaction with MOM cardiolipin and for mitophagy (Figure 1) (8). Additional in vitro binding studies suggest that a conserved domain of Beclin 1, another component of the autophagy machinery, exhibits enhanced affinity towards cardiolipin-enriched membranes (12). These data suggest that the translocation of cardiolipin from the MIM to the MOM acts as a signal, perhaps indicative of mitochondrial dysfunction, to eliminate these organelles through the process of mitophagy (8).

**Sphingosine-1-phosphate and the electron transport chain**

One of the most studied bioactive sphingolipids, sphingosine-1-phosphate (S1P), is a critical regulator of various physiological processes and has been implicated in multiple human diseases, including cancer, diabetes, and atherosclerosis. Secreted S1P can bind to one of five GPCRs and regulate cell proliferation, angiogenesis, migration, cytoskeleton organization, endothelial cell chemotaxis, immune cell trafficking, mitogenesis, and other processes (13). Alternatively, S1P can regulate processes intracellularly, through interaction with various proteins, including the histone deacetylases, HDAC1 and HDAC2 (14), PKCδ (15), and TRAF2 (16).

Through a series of lipid pull down and immunoprecipitation assays, Strub et al., show that S1P directly binds the prohibitin PHB2, a highly conserved chaperone regulating mitochondrial assembly and function (17). Knockout of the SphK2 sphingosine kinase, the principal source of mitochondrial S1P, disrupts interactions between PHB2 and cytochrome c oxidase, accompanied by aberrant assembly of cytochrome c oxidase and reduced mitochondrial respiration. Another study demonstrated that inhibition of SphK2 caused decreased expression of mitochondrial transcription factor A (TFAM) and superoxide dismutase 2 (SOD2) as well as a reduction in cellular ATP (18). These data support the hypothesis that S1P is important for regulating mitochondrial function, at least partly through cytochrome c oxidase assembly and mitochondrial respiration (Figure 1) (17).

**Ceramide, mitochondrial stress, and apoptosis**

As discussed earlier, lipids confer bioactivity through specific lipid-protein interactions or through lipid-lipid interactions that modulate membrane structure and dynamics. The lipids presented thus far, phosphoinositides, cardiolipin, and S1P, all appear to confer bioactivity primarily through specific lipid-protein interactions. On the other hand, both lipid-lipid interactions that affect membrane structure as well as specific lipid-protein interactions have been implicated in the mitochondrial effects conferred by ceramide.

First, ceramide fulfills a role in regulating mitophagy. Sentelle, et al. show that overexpression of CerS1, which generates C_{18} ceramides, induces mitophagy. They demonstrate that C_{18}-ceramide directly binds LC3B-II, thereby recruiting the autophagosomal machinery to
mitochondria and inducing mitophagy (19). Accordingly, amino acid substitutions that disrupt the interaction between LC3B-II and ceramide prevents LC3B-II-containing autophagosomes from being targeted to mitochondria (19). In this manner, C18-ceramide serves as a receptor or anchor for autophagosomes, selectively recruiting them to mitochondria and promoting mitophagy (Figure 1). How ceramide activities in recruiting the mitophagy machinery relate to those of cardiolipin remains to be determined.

Ceramide has also been implicated in promoting apoptosis. Ceramide is generated within the cell via de novo synthesis or via sphingomyelin hydrolysis and its levels increase in response to various stress stimuli. Mitochondrial targeting of a bacterial sphingomyelinase (20) or stress-induced activation of neutral sphingomyelinase 1 (nSmase1) (21) promotes sphingomyelin hydrolysis to produce ceramide and promote apoptosis. Ectopically targeting the ceramide transfer protein (CERT) to mitochondria causes ceramide import into mitochondria and apoptosis (22). Exogenous addition of ceramides to cultured cells or isolated mitochondria has been shown to induce cytochrome c release from mitochondria, a step that sets the apoptotic process in motion. It is possible that this apoptotic process is mediated by the formation of ceramide channels in the MOM, which is supported by observations that ceramides form stable pores in membranes in vitro (23). Furthermore, exogenous ceramide addition to cells promotes translocation of the pro-apoptotic BAX protein to mitochondria. In experiments using purified mitochondria, activated BAX and ceramide work synergistically to create proteolipid pores through which cytochrome c can exit mitochondria and activate the apoptotic pathway (Figure 1) (24).

Additional studies demonstrate roles opposing ceramide-mediated apoptosis for anti-apoptotic members of the Bcl-2 protein family. Overexpression of the pro-survival Bcl-2 attenuates nSmase1 activation, ceramide accumulation, and subsequent apoptosis in response to etoposide treatment in glioma cells (25). Addition of purified mammalian Bcl-xL or CED-9, its C. elegans homolog, to rat mitochondria prevents ceramide-induced MOM permeability, possibly by disrupting the formation of ceramide channels (26).

Proteotoxic stress in the mitochondria is detected by a surveillance system called the mitochondrial unfolded protein response (UPR mt) (27). In C. elegans, activation of the UPR mt causes the induction of several mitochondrial chaperone genes, including hsp-6 (28). One study demonstrated that inhibition of sphingolipid and ceramide synthesis, either genetically or pharmacologically, prevents nematodes from inducing hsp-6 in response to antimycin treatment (29). Conversely, these treatments have no effect on the induction of ER stress reporters, suggesting a specific role for ceramides in the response to mitochondrial stress. Furthermore, exogenous addition of ceramides to these nematodes, particularly C24-ceramide, restores hsp-6 induction in the presence of antimycin, but does not induce hsp-6 in the absence of stress (29), suggesting that ceramide is necessary, but not sufficient, for the cellular response to this mitochondrial stress.

Several studies have also implicated ceramides in affecting electron transport chain (ETC) activity. Hepatocytes from mice heterozygous for CerS2, the ceramide synthase that preferentially produces very long-chain ceramides in the liver, exhibit decreased activity of ETC complex II and IV. These effects on ETC activity are largely phenocopied by overexpression of CerS6, which produces shorter ceramides (30). Another study demonstrated increased mitochondrial biogenesis and beta-oxidative capacity in brown adipocytes upon CerS6 deletion (31). Increased ETC activity has also been observed in adipocytes lacking serine palmitoyltransferase, an enzyme required for the synthesis of ceramide precursors (32). Although these experiments provide compelling evidence for a role for ceramides in modulating ETC activity, the mechanisms through which this occurs are unclear.

**Ergosterol peroxide and protein quality control at mitochondria**

Recently, we discovered a distinct mechanism activated in response to mitochondrial stress that is dependent on another type of lipid, oxidized sterols (33,34). In S. cerevisiae, there are low levels of the oxidized sterol, ergosterol peroxide (EP), in the MOM in unstressed conditions. We hypothesize that stressors induce mitochondrial damage (35) and increased
production of damaging ROS (36), which can disrupt mitochondrial protein import and damage existing macromolecules. Furthermore, these ROS oxidize ergosterol in the MOM to EP (37), which acts as a signal to recruit Vms1 to damaged mitochondria. We show that EP directly binds to Vms1, a protein adaptor for the AAA-ATPase, Cdc48, and that increased EP abundance enhances Vms1 binding to membranes (Figure 1) (34). Upon recruitment of the Vms1-Cdc48 complex by EP, Cdc48 is thought to extract ubiquitylated polypeptides from the MOM for their subsequent degradation by the proteasome (33). Recent data also suggest that Vms1 might be recruited as part of the Ribosome Quality Control (RQC) system to assist in the ubiquitin-mediated degradation of nascent polypeptides whose ribosomal synthesis and concurrent import into mitochondria have been stalled (38). This combination of structural, genetic, and biochemical analyses suggests how mitochondrial stress causes a non-enzymatic increase in mitochondrial EP abundance that can recruit the Vms1-Cdc48 complex and/or the RQC machinery to aid in maintaining mitochondrial protein homeostasis.

The discovery that mitochondrial sterol oxidation manifests an “SOS” signal to the rest of the cell represents a novel mechanism whereby a direct byproduct of oxidative stress/damage acts as a direct signal. It is also unique in that it couples damage to the recruitment or engagement of the degradative functions of the mitochondrial ubiquitin-proteasome system. Through this mechanism, the cell can initiate a rapid response to aid the damaged mitochondria before mitophagy and/or apoptosis become necessary.

Oxidized sterols contribute to myriad cell signaling pathways

The very recent discovery that sterol oxidation contributes to mitochondrial quality control is quite distinct from roles previously described for oxidized sterols. To provide a more complete backdrop of the diverse contributions made by oxidized sterols to signaling pathways throughout the cell, we will outline the wide range of non-mitochondrial signaling pathways influenced by oxidized sterols, beginning with Hedgehog (Hh) signaling. In medulloblastoma (MB) cells, Hh signaling is inhibited upon treatment with zaragozic acid, which disrupts sterol biosynthesis without inhibiting isoprenoid biosynthesis. However, the addition of exogenous cholesterol or oxysterols restores Hh signaling in MB cells, likely by directly associating with and activating Smoothened (39,40).

Cholesterols and oxysterols also regulate Sterol Regulatory Element Binding Protein (SREBP) transcription factor trafficking and activation by promoting interactions between SREBP cleavage activating protein (SCAP) and Insig proteins (41,42). Cholesterol binds to SCAP triggering an association with Insigs, while oxysterols bind Insigs triggering Insig association with SCAP. Insig dissociation from SCAP is required for proper SREBP trafficking and induction of expression of the enzymes for fatty acid and cholesterol biosynthesis (43). Through this mechanism, elevated cholesterol or oxysterol abundance acts as a negative feedback inhibitor of the expression of cholesterol biosynthetic genes.

In addition to influencing Hh signaling and SREBP processing, oxysterols modulate several other pathways. Liver X receptors (LXRs) are nuclear receptors that regulate cholesterol metabolism and homeostasis and are activated upon binding to oxysterols (44). In a potentially deleterious manner, several oxysterols inhibit estrogen receptor activity (45). 27-hydroxycholesterol (27HC) inhibits estrogen-dependent production of nitric oxide leading to reduced vasorelaxation of rat aorta. Furthermore, dietary, pharmacologic, or genetic perturbations that cause increased 27HC levels, cause repression of carotid artery re-endothelialization (45). Lastly, several proteins conserved throughout eukarya, belonging to the oxysterol-binding protein-related family, bind to oxysterol ligands to regulate various processes, including lipid metabolism, vesicle transport, cell adhesion, and JAK/STAT signaling (46,47).

Conclusion

Lipids actively contribute to both intracellular and extracellular signaling pathways regulating diverse cellular processes. In many instances, lipids from various classes appear to cooperate to achieve a specific cellular outcome. For example, the phospholipid cardiolipin and the sphingolipid ceramide, combine to induce mitophagy. Cardiolipin binds to LC3 (8) and ceramide binds to LC3B-II (19), leading to
autophagosome recruitment and formation. The diverse combinations of lipid species that might cooperate to achieve a specific outcome illustrates the diversity of lipid signaling possibilities (48).

Advances in the technology we use to measure lipid abundance, localization, and interactions with proteins will undoubtedly increase our ability to discover and define new signaling mechanisms mediated by lipids. However, the greatest advancements in our understanding of how lipids mediate cell signaling will come as the manner in which we think about lipids continues to evolve. As our view of possible lipid functions expands, we will uncover novel contributions from lipids to signaling pathways of importance in human health and disease.

**The authors declare that they have no conflicts of interest with the contents of this article. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References
1. Fahy, E., Subramaniam, S., Murphy, R. C., Nishijima, M., Raetz, C. R., Shimizu, T., Spener, F., van Meer, G., Wakelam, M. J., and Dennis, E. A. (2009) Update of the LIPID MAPS comprehensive classification system for lipids. *J Lipid Res* **50 Suppl**, S9-14
2. Edidin, M. (1997) Lipid microdomains in cell surface membranes. *Curr Opin Struct Biol* **7**, 528-532
3. Hannun, Y. A., and Obeid, L. M. (2008) Principles of bioactive lipid signalling: lessons from sphingolipids. *Nat Rev Mol Cell Biol* **9**, 139-150
4. Nishizuka, Y. (1992) Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **258**, 607-614
5. Czech, M. P. (2000) PIP2 and PIP3: complex roles at the cell surface. *Cell* **100**, 603-606
6. Lappano, R., and Maggiolini, M. (2011) G protein-coupled receptors: novel targets for drug discovery in cancer. *Nat Rev Drug Discov* **10**, 47-60
7. van Rheenen, J., Achame, E. M., Janssen, H., Calafat, J., and Jalink, K. (2005) PIP2 signaling in lipid domains: a critical re-evaluation. *EMBO J* **24**, 1664-1673
8. Chu, C. T., Ji, J., Dagda, R. K., Jiang, J. F., Tyurina, Y. Y., Kapralov, A. A., Tyurin, V. A., Yanamala, N., Shrivastava, I. H., Mohammadyani, D., Qiang Wang, K. Z., Zhu, J., Klein-Seetharaman, J., Balasubramanian, K., Amoscato, A. A., Borisenko, G., Huang, Z., Gusdon, A. M., Cheikhi, A., Steer, E. K., Wang, R., Baty, C., Watkins, S., Bahar, I., Bayir, H., and Kagan, V. E. (2013) Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. *Nat Cell Biol* **15**, 1197-1205
9. Paradies, G., Paradies, V., De Benedictis, V., Ruggiero, F. M., and Petrosillo, G. (2014) Functional role of cardiolipin in mitochondrial bioenergetics. *Biochim Biophys Acta* **1837**, 408-417
10. Kagan, V. E., Jiang, J., Huang, Z., Tyurina, Y. Y., Desbourdes, C., Cottet-Rousselle, C., Dar, H. H., Verma, M., Tyurin, V. A., Kapralov, A. A., Cheikhi, A., Mao, G., Stolz, D., St Croix, C. M., Watkins, S., Shen, Z., Li, Y., Greenberg, M. L., Tokarska-Schlattner, M., Boissan, M., Lacombe, M. L., Epand, R. M., Chu, C. T., Mallampalli, R. K., Bayir, H., and Schlattner, U. (2016) NDPK-D (NM23-H4)-mediated externalization of cardiolipin enables elimination of depolarized mitochondria by mitophagy. *Cell Death Differ* **23**, 1140-1151
11. Shen, Z., Li, Y., Gasparski, A. N., Abeliovich, H., and Greenberg, M. L. (2017) Cardiolipin Regulates Mitophagy through the Protein Kinase C Pathway. *J Biol Chem* **292**, 2916-2923
12. Huang, W., Choi, W., Hu, W., Mi, N., Guo, Q., Ma, M., Liu, M., Tian, Y., Lu, P., Wang, F. L., Deng, H., Liu, L., Gao, N., Yu, L., and Shi, Y. (2012) Crystal structure and biochemical analyses reveal Beclin 1 as a novel membrane binding protein. *Cell Res* **22**, 473-489
13. Maceyk, M., Harikumar, K. B., Milstien, S., and Spiegel, S. (2012) Sphingosine-1-phosphate signaling and its role in disease. *Trends Cell Biol* **22**, 50-60
14. Hait, N. C., Allegood, J., Maceyka, M., Strub, G. M., Harikumar, K. B., Singh, S. K., Luo, C., Marmorstein, R., Kordula, T., Milstien, S., and Spiegel, S. (2009) Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. *Science* **325**, 1254-1257

15. Puneet, P., Yap, C. T., Wong, L., Lam, Y., Koh, D. R., Moochhala, S., Pfeilschifter, J., Huwiler, A., and Melendez, A. J. (2010) SphK1 regulates proinflammatory responses associated with endotoxin and polymicrobial sepsis. *Science* **328**, 1290-1294

16. Alvarez, S. E., Harikumar, K. B., Hait, N. C., Allegood, J., Strub, G. M., Kim, E. Y., Maceyka, M., Jiang, H., Luo, C., Kordula, T., Milstien, S., and Spiegel, S. (2010) Sphingosine-1-phosphate is a missing cofactor for the E3 ubiquitin ligase TRAF2. *Nature* **465**, 1084-1088

17. Strub, G. M., Paillard, M., Liang, J., Gomez, L., Allegood, J. C., Hait, N. C., Maceyka, M., Price, M. M., Chen, Q., Simpson, D. C., Kordula, T., Milstien, S., Lesnfsky, E. J., and Spiegel, S. (2011) Sphingosine-1-phosphate produced by sphingosine kinase 2 in mitochondria interacts with prohibitin 2 to regulate complex IV assembly and respiration. *FASEB J* **25**, 600-612

18. Sivasubramanian, M., Kanagaraj, N., Dheen, S. T., and Tay, S. S. (2015) Sphingosine kinase 2 and sphingosine-1-phosphate promotes mitochondrial function in dopaminergic neurons of mouse model of Parkinson's disease and in MPP+ -treated MN9D cells in vitro. *Neuroscience* **290**, 636-648

19. Sentelle, R. D., Senkal, C. E., Jiang, W., Ponnusamy, S., Gencer, S., Selvam, S. P., Ramshesh, V. K., Peterson, Y. K., Lemasters, J. J., Szulc, Z. M., Bielawski, J., and Ogretmen, B. (2012) Ceramide targets autophagosomes to mitochondria and induces lethal mitophagy. *Nat Chem Biol* **8**, 831-838

20. Birbes, H., El Bawab, S., Hannun, Y. A., and Obeid, L. M. (2001) Selective hydrolysis of a mitochondrial pool of sphingomyelin induces apoptosis. *FASEB J* **15**, 2669-2679

21. Yabu, T., Shiba, H., Shibasaki, Y., Nakanishi, T., Imamura, S., Touhata, K., and Yamashita, M. (2015) Stress-induced ceramide generation and apoptosis via the phosphorylation and activation of nSMase1 by JNK signaling. *Cell Death Differ* **22**, 258-273

22. Jain, A., Beutel, O., Ebell, K., Korneev, S., and Holthuis, J. C. (2017) Diverting CERT-mediated ceramide transport to mitochondria triggers Bax-dependent apoptosis. *J Cell Sci* **130**, 360-371

23. Siskind, L. J., and Colombini, M. (2000) The lipids C2- and C16-ceramide form large stable channels. Implications for apoptosis. *J Biol Chem* **275**, 38640-38644

24. Ganesan, V., Perera, M. N., Colombini, D., Datskovskiy, D., Chadha, K., and Colombini, M. (2010) Ceramide and activated Bax act synergistically to permeabilize the mitochondrial outer membrane. *Apoptosis* **15**, 553-562

25. Sawada, M., Nakashima, S., Banno, Y., Yamakawa, H., Takenaka, K., Shinoda, J., Nishimura, Y., Sakai, N., and Nozawa, Y. (2000) Influence of Bax or Bcl-2 overexpression on the ceramide-dependent apoptotic pathway in glioma cells. *Oncogene* **19**, 3508-3520

26. Siskind, L. J., Feinstein, L., Yu, T., Davis, J. S., Jones, D., Choi, J., Zuckerman, J. E., Tan, W., Hill, R. B., Hardwick, J. M., and Colombini, M. (2008) Anti-apoptotic Bcl-2 Family Proteins Disassemble Ceramide Channels. *J Biol Chem* **283**, 6622-6630

27. Jovaisaite, V., Mouchiroud, L., and Auwerx, J. (2014) The mitochondrial unfolded protein response, a conserved stress response pathway with implications in health and disease. *J Exp Biol* **217**, 137-143

28. Kimura, K., Tanaka, N., Nakamura, N., Takano, A., and Okuma, S. (2007) Knockdown of mitochondrial heat shock protein 70 promotes progeria-like phenotypes in caenorhabditis elegans. *J Biol Chem* **282**, 5910-5918

29. Liu, Y., Samuel, B. S., Breen, P. C., and Ruvkun, G. (2014) Caenorhabditis elegans...
pathways that surveil and defend mitochondria. *Nature* 508, 406-410

30. Raichur, S., Wang, S. T., Chan, P. W., Li, Y., Ching, J., Chaurasia, B., Dogra, S., Ohman, M. K., Takeda, K., Sugit, S., Pewzner-Jung, Y., Futerman, A. H., and Summers, S. A. (2014) CerS2 haploinsufficiency inhibits beta-oxidation and confers susceptibility to diet-induced steatohepatitis and insulin resistance. *Cell Metab* 20, 687-695

31. Turpin, S. M., Nicholls, H. T., Willmes, D. M., Mourier, A., Brodesser, S., Wunderlich, C. M., Mauer, J., Xu, E., Hammerschmidt, P., Bronneke, H. S., Trifunovic, A., LoSasso, G., Wunderlich, F. T., Kornfeld, J. W., Bluher, M., Kronke, M., and Bruning, J. C. (2014) Obesity-induced CerS6-dependent C16:0 ceramide production promotes weight gain and glucose intolerance. *Cell Metab* 20, 678-686

32. Chaurasia, B., Kaddai, V. A., Lancaster, G. I., Henstridge, D. C., Sriram, S., Galam, D. L., Gopalan, V., Prakash, K. N., Velan, S. S., Bulchand, S., Tsong, T. J., Wang, M., Siddique, M. M., Yuguang, G., Sigmundsson, K., Mellet, N. A., Weir, J. M., Meikle, P. J., Bin, M. Y. M. S., Shabbir, A., Shayman, J. A., Hirabayashi, Y., Shiow, S. T., Sugii, S., and Summers, S. A. (2016) Adipocyte Ceramides Regulate Subcutaneous Adipose Browning, Inflammation, and Metabolism. *Cell Metab* 24, 820-834

33. Heo, J. M., Livnat-Levanon, N., Taylor, E. B., Jones, K. T., Dephoure, N., Ring, J., Xie, J., Brodsky, J. L., Madeo, F., Gygi, S. P., Ashrafii, K., Glickman, M. H., and Rutter, J. (2010) A stress-responsive system for mitochondrial protein degradation. *Mol Cell* 40, 465-480

34. Nielson, J. R., Fredrickson, E. K., Waller, T. C., Rendon, O. Z., Schubert, H. L., Lin, Z., Hill, C. P., and Rutter, J. (2017) Sterol Oxidation Mediates Stress-Responsive Vms1 Translocation to Mitochondria. *Mol Cell* 68, 673-685 e676

35. Kurihara, Y., Kanki, T., Aoki, Y., Hirola, Y., Saigusa, T., Uchiimi, T., and Kang, D. (2012) Mitophagy plays an essential role in reducing mitochondrial production of reactive oxygen species and mutation of mitochondrial DNA by maintaining mitochondrial quantity and quality in yeast. *J Biol Chem* 287, 3265-3272

36. Guo, C., Sun, L., Chen, X., and Zhang, D. (2013) Oxidative stress, mitochondrial damage and neurodegenerative diseases. *Neural Regen Res* 8, 2003-2014

37. Bocking, T., Barrow, K. D., Netting, A. G., Chilcott, T. C., Coster, H. G., and Hofer, M. (2000) Effects of singlet oxygen on membrane sterols in the yeast Saccharomyces cerevisiae. *Eur J Biochem* 267, 1607-1618

38. Izawa, T., Park, S. H., Zhao, L., Hartl, F. U., and Neupert, W. (2017) Cytosolic Protein Vms1 Links Ribosome Quality Control to Mitochondrial and Cellular Homeostasis. *Cell* 171, 890-903 e818

39. Corcoran, R. B., and Scott, M. P. (2006) Oxysterols stimulate Sonic hedgehog signal transduction and proliferation of medulloblastoma cells. *Proc Natl Acad Sci U S A* 103, 8408-8413

40. Myers, B. R., Neahring, L., Zhang, Y., Roberts, K. J., and Beachy, P. A. (2017) Rapid, direct activity assays for Smoothened reveal Hedgehog pathway regulation by membrane cholesterol and extracellular sodium. *Proc Natl Acad Sci U S A*

41. Radhakrishnan, A., Ikeda, Y., Kwon, H. J., Brown, M. S., and Goldstein, J. L. (2007) Sterol-regulated transport of SREBP5 from endoplasmic reticulum to Golgi: oxysterols block transport by binding to Insig. *Proc Natl Acad Sci U S A* 104, 6511-6518

42. Sun, L. P., Seemann, J., Goldstein, J. L., and Brown, M. S. (2006) Sterol-regulated transport of SREBPs from endoplasmic reticulum to Golgi: Insig renders sorting signal in Scap inaccessible to COPII proteins. *Proc Natl Acad Sci U S A* 104, 6519-6526

43. Goldstein, J. L., DeBose-Boyd, R. A., and Brown, M. S. (2006) Protein sensors for membrane sterols. *Cell* 124, 35-46

44. Peet, D. J., Janowski, B. A., and Mangelsdorf, D. J. (1998) The LXRs: a...
new class of oxysterol receptors. *Curr Opin Genet Dev* **8**, 571-575

45. Umetani, M., Domoto, H., Gormley, A. K., Yuhanna, I. S., Cummins, C. L., Javitt, N. B., Korach, K. S., Shaul, P. W., and Mangelsdorf, D. J. (2007) 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. *Nat Med* **13**, 1185-1192

46. Ngo, M. H., Colbourne, T. R., and Ridgway, N. D. (2010) Functional implications of sterol transport by the oxysterol-binding protein gene family. *Biochem J* **429**, 13-24

47. Raychaudhuri, S., and Prinz, W. A. (2010) The diverse functions of oxysterol-binding proteins. *Annu Rev Cell Dev Biol* **26**, 157-177

48. Schultz, C. (2010) Challenges in studying phospholipid signaling. *Nat Chem Biol* **6**, 473-475
Figure 1. Mitochondrial lipids contribute to several mitochondrial functions.

A. Under mitochondrial stress conditions, ergosterol (erg) oxidation to ergosterol peroxide (EP recruits the Vms1-Cdc48 complex to mitochondria to aid in maintaining mitochondrial protein homeostasis. B. In response to mitochondrial damage, cardiolipin (CL) translocates from the mitochondrial inner membrane to the outer membrane where cardiolipin and ceramide (Cer) bind LC3 and LC3B-II to recruit autophagosomes to damaged organelles. C. Mitochondrial damage can also lead to accumulation of mitochondrial ceramide where BAX and ceramide work synergistically to create proteolipid pores through which cytochrome c can exit and activate the apoptotic pathway. D. Sphingosine-1-phosphate (S1P) binds the chaperone prohibitin (PHB2) which facilitates PHB2 interactions with cytochrome c oxidase and its subsequent assembly.
