Mitochondria-associated ER membranes (MAMs) and lysosomal storage diseases

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Abstract
Lysosomal storage diseases (LSDs) comprise a large group of disorders of catabolism, mostly due to deficiency of a single glycan-cleaving hydrolase. The consequent endo-lysosomal accumulation of undigested or partially digested substrates in cells of virtually all organs, including the nervous system, is diagnostic of these diseases and underlies pathogenesis. A subgroup of LSDs, the glycosphingolipidoses, are caused by deficiency of glycosidases that process/degrade sphingolipids and glycosphingolipids (GSLs). GSLs are among the lipid constituents of mammalian membranes, where they orderly distribute and, together with a plethora of membrane proteins, contribute to the formation of discrete membrane microdomains or lipid rafts. The composition of intracellular membranes enclosing organelles reflects that at the plasma membrane (PM). Organelles have the tendencies to tether to one another and to the PM at specific membrane contact sites that, owing to their lipid and protein content, resemble PM lipid rafts. The focus of this review is on the MAMs, mitochondria associated ER membranes, sites of juxtaposition between ER and mitochondria that function as biological hubs for the exchange of molecules and ions, and control the functional status of the reciprocal organelles. We will focus on the lipid components of the MAMs, and highlight how failure to digest or process the sialylated GSL, GM1 ganglioside, in lysosomes alters the lipid conformation and functional properties of the MAMs and leads to neuronal cell death and neurodegeneration.

Facts
- MAMs are discrete tethering sites between the ER and mitochondria membranes with a defined composition of lipids and proteins that resemble the PM lipid rafts.
- MAMs serve as functional hubs for many physiological processes that are influenced by the specific distribution and local concentration of the lipid components (i.e. cholesterol and gangliosides).
- Lipid contents limit the recruitment and clustering of specific proteins at the MAMs, and influence their biochemical properties and functions.
- Impaired lysosomal turnover and degradation of GM1 in the LSD GM1-gangliosidosis alter the molecular composition of the MAMs and Ca2+ signaling, resulting in ER-mitochondria-mediated neuronal apoptosis.

Open questions
- Are there other genetic defects of lysosomal catabolism that influence the function and structural characteristics of the MAMs?
- Does deregulation of signaling pathways at the MAMs, caused by their altered lipid composition, underlie aspects of pathophysiology of lipid storage diseases, especially with regard to neurodegeneration?
- Are there differences in the lipid and protein makeup of the MAMs depending on the cell types or physiological state of the cells?
- How can we monitor and assess lipid redistribution to the MAMs?
**Lysosomes and lysosomal storage diseases**

The lysosome and the ubiquitin-proteasome systems converge the bulk of cellular catabolism in virtually all mammalian cells. The dynamic interplay between these catabolic machineries counterbalances the metabolic needs of cells and ensures the homeostatic maintenance of cell physiology. Lysosomes are delegated to the compartmentalized processing/degradation/recycling of long-lived macromolecules that reach the organelles through the biosynthetic, endocytic, phagocytic or autophagic routes. In addition, lysosomes have recently emerged as central platforms for specialized cellular functions, such as nutrient sensing, Ca²⁺ signaling, PM repair, cholesterol trafficking, viral/bacterial infections and cell death. The constituents of these organelles include soluble and membrane-bound hydrolytic enzymes, lysosomal integral membrane proteins, ion channels and transporters, whose expression is regulated in turn by transcriptional and post-translational modifications that adapt their activities and functions to the cell types and the metabolic status of the cells. Activation of the lysosomal system may occur under both physiologic and pathologic stimuli; the result is multiform, and displays the involvement of lysosomes in such fundamental processes as metabolic regulation, nutrition, differentiation and cell defense. A failure of the lysosomal system to perform its functions results in severe clinical conditions known as LSDs.

The majority of LSDs are caused by deficient or defective activity of glycosidases. The natural substrates of these enzymes comprise a wide array of glycoconjugates, many of which are still unidentified, that are themselves differentially expressed and distributed in different cell types. Genetic mutations that impair their hydrolytic capacity provoke a cascade of pathologic events that are mostly triggered by the relentless accumulation of partially unprocessed/degraded substrates in these organelles, leading to the characteristic appearance of swollen lysosomes, the hallmark of these diseases. The heterogeneous composition of lysosomes and their ubiquitous distribution account for the systemic clinical manifestations of LSDs.

With a combined incidence of 1:5000 live births, this group of over 70, individually rare, monogenic disorders affect multiple organs, including the nervous system. While the genetic bases of the LSDs are relatively easy to define, the age of onset and complexity of the symptoms may depend on the type of LSD, the amount of residual enzymatic activity, the biochemical properties of the storage material, and the cell types where the storage mostly occurs. Clinical signs may include coarse face, myoclonus, macular cherry red spot, hepatosplenomegaly, cardiac and kidney involvement and dysostosis multiplex. In addition, more than 70% of LSDs display an array of neurological abnormalities, associated with severe developmental delay, and psychiatric problems. The complexity of the phenotypes has rendered the understanding of the pathogenesis particularly challenging but also created a means for dissecting the biology of the lysosomal system and more broadly for deciphering the molecular effectors downstream of a single enzyme deficiency and accumulated metabolites.

A subgroup of LSDs linked to defective catabolism of lipids comprises the glycosphingolipidoses, NPC, and Wolman disease. Sphingolipids and their glycosylated and sialylated derivatives, the GSLs and gangliosides, are ubiquitous and abundant components of cellular membranes in mammalian cells. Their amphipathic structure includes a basic hydrophobic membrane moiety, ceramide, composed of sphingosine and fatty acid, and a hydrophilic oligosaccharide chain that may contain one or more sialic acid residues. They are synthesized in the ER, Golgi and trans Golgi network by the sequential addition of carbohydrate residues to their backbone ceramide; they are then transferred via vesicular and non-vesicular mechanisms to the outer leaflet of the PM. Degradation of these compounds occurs along the endocytic route by the stepwise removal of individual sugars by lysosomal exoglycosidases or membrane sialoglycoproteins in concert with activator proteins.
Membrane microdomains: MAMs and their lipid raft-like characteristics

Mammalian membranes represent highly dynamic structures that maintain cell integrity and govern a myriad of cellular processes indispensable for the proper physiology of cells and tissues. PM and intracellular membranes are organized in discrete subdomains/microdomains, sharing features of lipid rafts, that are enriched in GSLs, cholesterol and ceramide. These microdomains are also known as DRMs or GEMs.

The type of lipids in these membrane microdomains limits the recruitment and clustering of specific protein components, creating functional cellular hubs for efficient (lipid) shuttling, trafficking of ions and signaling molecules.

It is now well established that all intracellular organelles are in a state of constant communication achieved at MCS. These membrane structures create a transitory but efficient way of transferring or exchanging lipids and other macromolecules between organelar membranes. MCS are established by the tethering of membrane protein complexes and lipids, which maintain the two organelles in close proximity without undergoing fusion. Considering the widespread ER network within cells, the membranes of the ER cisternae can engage in multiple contact sites with other organelar membranes, including those of the mitochondria, the lysosomes, the Golgi apparatus, the endosomes and the PM. This configuration allows for the compartmentalization of specific functions/activities in a controlled milieu, and explains why MCS between intracellular organelles have raft-like characteristics.

So far, the best studied of these contact sites are the ones formed between the membranes of the ER and the mitochondria. These membrane microdomains, known as mitochondria associated ER-membranes or MAMs, are now established as reversible tethers that co-regulate and influence a variety of cellular processes, i.e., synthesis/transport of lipids and lipid intermediates, Ca^{2+} dynamics/signaling, autophagy, mitochondrial shape and size, apoptosis and energy metabolism. These functions of the MAMs are tightly interconnected, given that changes in the topology and concentration of their constituents can dramatically alter their homeostatic control of specific ER and mitochondrial functions with pathological effects that can lead to cell demise.

To date, there is still little understanding of the way the turnover of membrane lipids, particularly GSLs and...
| Protein | Localization | Function | Known interactors | References |
|---------|--------------|----------|-------------------|------------|
| β-galactosidase | Lysosome | Catalyzes the hydrolysis of a terminal β-linked galactose residue from ganglioside substrates and other glycoconjugates | Protective protein cathepsin A (PPCA), neuraminidase 1 (NEU1) | 126, 127 |
| Cathepsin D | Lysosome | Aspartic-type endopeptidase activity | 80 |
| Palmitoyl-protein thioesterase 1, PPT1 | Lysosome | Removes thioester-linked fatty acyl groups such as palmitate from cysteine residues | CLNS | 80 |
| Tripeptidyl peptidase 1, TPP1 | Lysosome | Cleaves N-terminal tripeptides from substrates, and has weaker endopeptidase activity | CLNS | 80 |
| PSS1 | ER face of the MAMs | Phosphatidylserine synthase 1 | 128 |
| PSS2 | ER face of the MAMs | Phosphatidylserine synthase 2 | 128 |
| PS-decarboxylase | Mitochondrial side of the MAMs | Phosphatidylserine-decarboxylase | 59 |
| PE-methyltransferase PEMT | MAMs | Phosphatidylethanolamine-methyltransferase | 62 |
| Mdm10 | Mitochondria/MAMs | ERMES complex | Mdm34, Mmm1, Mdm12, Gem1 | 45, 71 |
| Mdm34 | Mitochondria/MAMs | ERMES complex | Mdm10, Mmm1, Mdm12, Gem1 | 45, 73 |
| Mmm1 | ER/MAMs | ERMES complex | Mdm34, Mmm10, Mdm12, Gem1 | 45, 72 |
| Mdm12 | Cytosol/MAMs | ERMES complex | Mdm34, Mmm10, Mdm1, Gem1 | 45, 73 |
| Lam6 | MAMs, vacuole-mitochondria patches, nuclear-vacuole contact sites | ERMES complex, vCLAMP (vacuole and mitochondria patch), and NVJ (nuclear vacuolar junction) | Mdm10, Mmm1, Mdm12, Mdm34, Vps39, Nvj1, Vac8 | 74, 75 |
| Gem1 | MAMs | ERMES complex | Mdm10, Mmm1, Mdm12, Mdm34 | 70, 72 |
| EMC1 | MAMs | EMC, PS transfer, MAMs architecture | TOM5 | 78 |
| EMC2 | MAMs | EMC, PS transfer, MAMs architecture | TOM5 | 78 |
| EMC3 | MAMs | EMC, PS transfer, MAMs architecture | TOM5 | 78 |
| EMC4 | MAMs | EMC, PS transfer, MAMs architecture | TOM5 | 78 |
| EMC5 | MAMs | EMC, PS transfer, MAMs architecture | TOM5 | 78 |
| EMC6 | MAMs | EMC, PS transfer, MAMs architecture | TOM5 | 78 |
| Protein                     | Localization                           | Function                                                                 | Known interactors                                                                 | References |
|----------------------------|----------------------------------------|--------------------------------------------------------------------------|----------------------------------------------------------------------------------|------------|
| CLN8                       | ER integral membrane protein           | Transmembrane protein belonging to a family of proteins containing TLC domains, which are postulated to function in lipid synthesis, transport, or sensing. | CLN5                                                              | 80         |
| Caspase 12                 | ER                                     | Member of the cysteine-aspartic acid protease family responsible for ER-stress-induced apoptosis. |                                                                                  | 102, 129   |
| PC-cytidylyltransferase, CTP| Cytosol, MAMs                          | Involved in the regulation of phosphatidylcholine biosynthesis.           |                                                                                  | 86         |
| Acetyl-CoA:cholesterol acyltransferase, ACAT | MAMs | Catalyzes the reversible formation of acetoacetyl-CoA from two molecules of acetyl-CoA. |                                                                                  | 35, 90     |
| Steroidogenic acute regulatory protein, StaR | MAMs, mitochondria | Plays a key role in the acute regulation of steroid hormone synthesis by enhancing the conversion of cholesterol into pregnenolone. | TOMM22, VDAC2                                                                    | 97         |
| Sigma-1 receptor           | ER face of the MAMs                    | Interacts with a variety of psychotomimetic drugs, including cocaine and amphetamines. The receptor is believed to play an important role in the cellular functions of various tissues associated with the endocrine, immune, and nervous systems. | BIP, IP3R3                                                                  | 100        |
| VDAC-1                     | Mitochondrial side of the MAMs         | Facilitates the exchange of metabolites and ions across the outer mitochondrial membrane |                                                                                  | 130        |
| IP3R-1                     | ER, MAMs                               | Mediates calcium release from the endoplasmic reticulum following stimulation by inositol 1,4,5-trisphosphate. | GRP75                                                                           | 52, 130, 131 |
| GRP75                      | ER, mitochondria, MAMs                 | Member of the heat shock protein 70 gene family, functions as scaffold between IP3R-1 and VDAC1 |                                                                                  | 52, 130    |
| BIP                        | ER, MAMs                               | Involved in the folding and assembly of proteins in the ER.               | Interacts with many ER proteins                                                  | 108, 132   |
| Calnexin                   | ER, MAMs                               | Ca²⁺-binding protein that interacts transiently with newly synthesized N-linked glycoproteins, facilitating protein folding and assembly | PACS2, AMBRA1, WIPI1                                                             | 116, 133   |
| hFis1                      | Mitochondria                           | Promotes mitochondrial fission                                            |                                                                                  | 134, 135   |
| t-Bid                      | Mitochondria                           | Member of the BCL-2 family of cell death regulators                      | Eterodimerizes with either agonist BAX or antagonist BCL2, VDAC1                | 134, 136   |
| Bax                        | Mitochondria                           | Member of the BCL-2 family of cell death regulators                      | BCL2 family members form hetero- or homodimers and act as anti- or pro-apoptotic regulators | 134, 137   |
cholesterol, by lysosomal hydrolases can modulate the local concentration of these constituents at the MAMs and, in turn, their function. However, the prediction is that defective lysosomal catabolism of lipids, causing the buildup of these compounds at the PM and in lysosomes, may provoke their redistribution to the limiting membranes of other organelles and eventually alter the lipid composites of MCS. In this review, we will give a specific example of how impaired degradation of the sialylated GSL, GM1, due to deficiency of the lysosomal enzyme β-Gal (Table 1) in the neurodegenerative LSD GM1-gangliosidosis, provokes the redistribution of this ganglioside to the ER membranes, which, in turn, changes its local concentration within MAMs and alters MAM’s functions (Fig. 2).

**Lipid synthesis at the MAMs**

**Phospholipids**

Since the discovery of the MAMs and the identification of their role in maintaining intracellular Ca^{2+} homeostasis, a large body of experimental work has implicated these specific microdomains in both physiological and pathological processes. One of the fundamental functions of the MAMs is the coordinated biosynthesis of phospholipids and their transport. This is because, beside the ER, other intracellular organelles, including the mitochondria, lack the ability to synthesize de novo their phospholipids and rely on the ER as their only source. On these premises, the close proximity of the ER and the mitochondria at the MAMs (~10–30 nm) provides an insulated environment against the hydrophilicity of the cytosol, and favors the bi-directional, non-vesicular lipid transfer at these microdomains. This exchange in trans is evidenced in the metabolism of PS and PC, that has been shown to occur in part at the MAMs. In mammalian cells two PS synthases, PSS1 and PSS2 (Table 1), are present at high concentrations in the MAMs, where they produce PS by base-exchange of the head groups of PC or PE with serine in a Ca^{2+} dependent manner. These reactions promote the release of Ca^{2+} into the ER, an ATP dependent process. PSS1 and PSS2 localize at the ER face of the MAMs, opposite to the PS-decarboxylase (Table 1), which is located at the mitochondrial side. Their strategic position creates a gradient of PS concentration that facilitates the transfer of PS from the MAMs to the mitochondrial inner membrane. The latter is a rate-limiting step for PS conversion into PE by the decarboxylase. Alternatively, PE is generated by acylation of lyso-PE, a reaction that also occurs in the MAMs. Within these contact sites PE can then undergo conversion into PC by three consecutive methylation reactions catalyzed by the methyltransferase PEMT (Table 1), also localized at the MAMs. This set of enzymatic reactions ensures the controlled supply of...
phospholipids to the mitochondria, which is necessary for maintaining mitochondrial membrane integrity, especially following fusion and fission of the organelles64–66. Notably, the ablation of either PSS1 or PSS2, but not both enzymes, in mice is compatible with life, despite the reduction of serine exchange activity and overall tissue reduction of PS67; instead, silencing of PS-decarboxylase reduces mitochondrial PE content, and results in mitochondrial dysfunction and lethality in embryos68.

The active transfer of phospholipids by multiprotein complexes has also been shown to occur at the MAMs, although so far only in the budding yeast and not in mammalian cells. In yeast, genetic screenings have identified macromolecular protein complexes that tether the ER and mitochondrial membranes at contact sites, and possibly coordinate the trafficking of phospholipids and phospholipid intermediates between the two organelles. One of these complexes, named ERMES45,65,69, (Table 1) was found by screening for yeast mutants that failed to grow unless they expressed a synthetic fusion protein that artificially created contact sites between the two organelar membranes. ERMES comprises the two mitochondrial outer membrane proteins Mdm10 and Mdm34, the integral ER membrane protein Mmm1 and the cytosolic protein Mdm1245,70–72. In addition, Gem1, the yeast ortholog of the mitochondrial Rho GTPases 1 and 2, Miro1 and Miro270, and more recently Lam674,75 (Table 1), have been shown to be part of and to regulate the ERMES complex. It is noteworthy that Lam6 contains a START domain (see below) and has the ability to bind and transfer sterol across intracellular contact sites76; hence, Lam6 may exert a similar task within the ERMES complex. However, while the role of this complex in tethering ER and mitochondria has been demonstrated, its function in phospholipid transport remains controversial because of apparently contradictory results reported in the literature. In one study, ERMES was shown to mediate lipid exchange between ER and mitochondria45, while in another study the transport of PS between these organelles was shown to be ERMES- and Gem1-independent77. These authors argued that the altered lipid profiles of cells lacking ERMES45 were caused by defects in mitochondrial morphology, rather than in phospholipid metabolism77. Furthermore, all components of the ERMES complex, with the exception of Gem1 and Lam6, lack obvious homologs in higher eukaryotes; for another example of the latter group is CLN8, a transmembrane protein that is localized in the ER membranes or shuttles between the ER and Golgi compartments, and is thought to take part in lipid synthesis and transport83. Deficiency of CLN8 is linked to one of the late infantile variants of NCLs, and is associated with increase in glycerophospholipids containing polyunsaturated fatty acyl chains, such as PS and PE, and reduced concentrations of ceramide, galactosyl- and lactosylceramide, sulfatides and fatty acyl chain-containing molecular species84. A homozygous mutation in the orthologous mouse gene (Cln8) was identified in a naturally occurring NCL model, the motor neuron degeneration (mnd) mouse85. Using MAMs isolated from the liver of these animals, Vance et al79, demonstrated that the levels and activity of PEMT2 and other two key enzymes for the synthesis of phospholipids (CTP and PS synthase)86 (Table 1) were greatly reduced. Although these reduced activities did not hamper the import of phospholipids into the mitochondria nor the PS metabolism, other proteins normally present in mitochondria partially redistributed to microsomes, suggesting intrinsic defects of the MAMs in the mitochondrial function. The EMC complex contains six conserved proteins, EMC1–6, and interacts with TOM578. This large multiprotein complex represents another tethering structure between ER and mitochondrial membranes that transfers phospholipids independently of ERMES79. However, while Lam6 and the EMC proteins do have metazoan orthologs, it is still unclear whether these orthologs effectively function as regulators of ER-mitochondria contact sites in mammalian cells, and whether they facilitate lipid shuttling. In addition, we cannot exclude that physical flipping of the lipids may occur between the juxtaposed membranes if they are in sufficiently close proximity.

**Defective phospholipid synthesis in the MAMs of Cln8 deficient mice**

Interestingly, already two decades ago Vance et al79, examined the role of the MAMs in the metabolism of phospholipids, particularly PS, using the mouse model of the motor neuron disorder, neuronal ceroid lipofuscinosis or NCL. NCLs, also referred to as CLNs80, are a group of at least 14 congenital neurodegenerative disorders with onset in infancy, adolescence, or young adulthood, depending on the gene involved and the severity of the clinical symptoms81. These diseases are characterized by progressive psychomotor deterioration, epilepsy, and blindness81. Their histopathology hallmark is the accumulation in neurons and other cell types of the autofluorescent lipopigment, lipofuscin82. NCLs are caused by mutations in different genes, encoding soluble lysosomal enzymes (i.e., cathepsin D, PPT1, TPP1) or (non-lysosomal) integral membrane proteins (Table 1)80. One example of the latter group is CLN8, a transmembrane protein that is localized in the ER membranes or shuttles between the ER and Golgi compartments, and is thought to take part in lipid synthesis and transport83. Deficiency of CLN8 is linked to one of the late infantile variants of NCLs, and is associated with increase in glycerophospholipids containing polyunsaturated fatty acyl chains, such as PS and PE, and reduced concentrations of ceramide, galactosyl- and lactosylceramide, sulfatides and fatty acyl chain-containing molecular species84. A homozygous mutation in the orthologous mouse gene (Cln8) was identified in a naturally occurring NCL model, the motor neuron degeneration (mnd) mouse85. Using MAMs isolated from the liver of these animals, Vance et al79, demonstrated that the levels and activity of PEMT2 and other two key enzymes for the synthesis of phospholipids (CTP and PS synthase)86 (Table 1) were greatly reduced. Although these reduced activities did not hamper the import of phospholipids into the mitochondria nor the PS metabolism, other proteins normally present in mitochondria partially redistributed to microsomes, suggesting intrinsic defects of the MAMs in the
mutant mice. Nearly 10 years later, by analyzing the neurodegenerative events occurring in specific brain regions of the same mouse model at different stages of disease progression, two groups of investigators reported variable activation of effectors of ER-stress-mediated apoptosis and inflammation, as well as defective mitochondrial buffering capacities of neurons. Together these studies support the idea that deregulated activity/function of the MAMs relative to lipid synthesis and transport could be a major contributor of neurodegeneration and disease progression in CNL deficiency.

**Cholesterol and ceramide**

Other essential lipids at the mitochondrial membranes are cholesterol and ceramide (Fig. 2). As it is the case for the phospholipids, cholesterol and ceramide are imported into the mitochondria where they serve as structural components of the membranes or as precursors for the synthesis of steroid hormones. These lipids are produced at least in part in the MAMs, and this accounts for the activities of the cholesterol synthetic enzymes and ceramide synthase being higher in the MAMs than in the ER or mitochondria, respectively. Basal storage of cholesterol in the form of cholesteryl esters is maintained predominantly through the activity of ACAT (Table 1). This enzyme catalyzes the esterification of insoluble, membrane-bound cholesterol into cholesteryl ester, which is subsequently incorporated into lipid droplets, thereby preventing the buildup of toxic cholesterol at the membranes. ACAT and its enzymatic activity are enriched in MAMs, and in fact, this enzyme has been used as a reliable marker of these microdomains. Under stress or other pathological conditions, the flux of cholesterol may change dramatically due to either augmented hydrolysis of stored cholesteryl esters, increased uptake of plasma cholesterol, or transport of free cholesterol into the mitochondria. The ensuing accumulation of cholesterol within the MAMs is at the basis of severe disease conditions including Alzheimer’s disease, steatohepatitis, and cancer, each of whom have been linked to altered signaling pathways occurring at the MAMs.

Similarly, changes in ceramide levels in the MAMs may occur under pathological stimuli. For instance, upon radiation both ceramide synthesis and ceramide levels increase exclusively in the MAMs, but not in the ER and the mitochondria, albeit the implications of this phenomenon are unknown. We can postulate, however, that the increased pool of ceramide in the MAMs creates a reservoir of this lipid, which can be readily transferred into the mitochondria for the initiation of an apoptotic process, if the pathogenic insult persists. Together these observations suggest that enrichment of lipid synthetic enzymes at the MAMs may serve the purpose of compartmentalizing the transfer of lipid pools into the mitochondria, thereby limiting the toxic effects of lipid overload in this organelle.

Cholesterol is not only synthesized in the MAMs, but also uses these microdomains for its transport into the mitochondria. This process occurs under acute stress condition or when there is the need for increased cholesterol transfer into the mitochondria, for instance during the synthesis of steroid hormones. The latter process is catalyzed by the sequential action of a set of steroidogenic enzymes, one of which, StAR (Table 1), rate limiting for the subsequent biosynthetic reactions. As mentioned earlier, StAR contains a START domain that is required for the binding to and transport of cholesterol. The mode of activation of this enzyme is unusual because the 37 kDa StAR precursor is first translocated into the MAMs, where it binds cholesterol and interacts with VDAC2, a prerequisite for its processing into a 32 kDa intermediate. This form is then imported into the mitochondrial matrix as a fully active 30 kDa cholesterol-bound protein by a complex formed by TOMM22 and VDAC2 (Table 1). VDAC2 appears to be a key player in this process, because, in its absence, the amount of mitochondrial StAR is reduced and the protein cannot be efficiently loaded onto the MAMs. Consequently, a reduced amount of cholesterol is imported into the mitochondria, which eventually affects the synthesis of steroids. Also in this example, the MAMs act as functional hubs for the pathways of cholesterol transport and steroidogenesis.

The actual lipid composition of the MAMs has gained special attention in recent years. Several studies have revealed that specific types of lipids are embedded and concentrate in these microdomains, rendering the composition of the tethering membranes topologically different from that of the reciprocal ER and mitochondrial membranes. The gathering of lipids at the MAMs, and the modulation of their local concentration, change the structure of these contact sites and their ability to recruit specialized protein assemblies, ultimately altering the MAMs’ physiological functions and activities. For example, sigma-1 receptor, a structural component of the MAMs that participates in Ca2+ signaling, associates within the raft/GEM fraction of the MAMs with ceramide and cholesterol. The high levels of cholesterol within the MAMs, which is 5–7 times higher than that in the ER, and the accumulation of ceramide at the same sites have been shown to cause partitioning of sigma-1 receptor to the raft/GEM subdomain of the MAMs. Instead, depletion of either lipid from the MAMs relocates the sigma-1 receptor from the MAMs to the ER membrane, likely altering Ca2+ signaling. It was also demonstrated that reducing cholesterol concentration within the MAMs with MBCD, a circular oligosaccharide used to extract cholesterol
from membranes, significantly increases MAMs copurification with mitochondria, suggesting that cholesterol may also act as a negative regulator of MAMs formation.

**Sialylated glycosingolipids: gangliosides**

Other quantitatively minor, but important lipids for the function of the MAMs, are the gangliosides, the major class of acidic GSLs, containing one or more sialic acid residues. Gangliosides are structural components of the outer leaflet of PM and the nuclear envelop, where they segregate, together with cholesterol and other sphingolipids, into raft microdomains. They are particularly abundant in the nervous system, where they account for approximately 10–12% of the total lipid contents of neuronal membranes, whereas they occur at relatively low levels in other tissues. Their diversity and structural complexity suggest that gangliosides are not biologically redundant, but have unique functions. Beside exhibiting receptor or co-receptor function for cytokines, toxins, viruses and bacteria, gangliosides are key signaling molecules that take part in biological processes as pivotal as cellular recognition and adhesion, receptor signal transduction, 

![Fig. 3 Schematic representation of a single MAM, depicted as a functional hub for the aberrant transfer of Ca^{2+} between ER and mitochondria, leading to ER- and mitochondria-mediated neuronal cell death in GM1-gangliosidosis. The figure also lists the principal effectors of the apoptotic process described in Tessitore et al., 2004 and Sano et al., 2009](image)
Once formed, this multimolecular complex triggers mitochondria depolarization and apoptosis. In support of these findings is the observation that disruption of lipid microdomains in isolated mitochondria by MBCD rescues the mitochondria depolarization induced by GD3113. Besides its role in apoptosis, recently GD3 has also been implicated in the formation of autophagosomes, because it was shown to physically interact with MAP1LC3/LC3 (Table 1)114,115. In a follow-up study, the same group has demonstrated that specifically in the raft/GEM fraction of the MAMs, GD3 is associated with two important effectors of autophagosome’s formation, AMBRA1 and WIPI1 (Table 1)116. These authors further showed that under starvation-induced autophagy the concentration of GD3 increases within the microdomains, which favors the interaction of this ganglioside with the chaperon calnexin, another resident protein of the MAMs that interacts with AMBRA1 and WIPI1. Thus, following autophagic induction, an increased amount of GD3 clusters together with AMBRA1, WIPI1 and calnexin in the lipid-enriched fraction of the MAMs; these results point to an active role for this ganglioside in the early phases of the autophagic process, specifically at these membrane microdomains. In contrast, reducing the levels of GD3 by inhibition of ganglioside synthesis hinders the initiation of autophagy.116 Thus, as it is the case for other lipids, modulation of the gangliosides’ concentration in the MAMs greatly influences the coordinated regulation of autophagy116.

**GM1-gangliosidosis: GM1 at the MAMs and neurodegeneration**

Another sialylated GSL identified as a structural component of the MAMs, albeit present at very low concentration under physiological conditions, is GM1. GM1 is one of the most abundant gangliosides present in the adult neuronal membranes, and is the only ganglioside shown to bind Ca2+ and to modulate Ca2+ flux across membranes (Figs. 2 and 3)52,102,108,117,118. Accumulation of GM1 above its physiological threshold occurs in GM1-gangliosidosis, a generalized, neurodegenerative LSD caused by deficiency of the GM1-cleaving enzyme β–Gal119. This very severe condition affects primarily infants, but milder cases, with a longer survival, occur in adolescents and adults119. The early onset form
of GM1-gangliosidosis presents with growth retardation, progressive neurologic deterioration, due to extensive brain atrophy, visceromegaly, and skeletal dysplasia. Abnormal amounts of GM1 and, to a lesser extent its asialo-derivative GA1, accumulate in the brain, and oligosaccharides derived from glycoproteins and keratan sulfate are excreted in the urine. Beta-Gal−/− mice, an authentic model of GM1-gangliosidosis, develop a severe, generalized nervous system disease, resulting in tremors, ataxia and abnormal gait, which culminates with rigidity and paralysis of the hind limbs.

Neurons, the primary affected cells of the disease, undergo massive, age-dependent accumulation of both GM1 and GA1. This phenotype is accompanied by profound histopathological changes throughout the CNS, associated with progressive expansion of the lysosomal compartment as the animals age. Degenerating neurons filled with lysosomes containing membranous material and remnants of the ER and ribosomes are emblematic of the disease. Neuronal cell death is followed by micro- and astrogliosis, a sign of an elicited widespread CNS inflammation. In β-Gal−/− mice impaired lysosomal degradation of GM1 in neurons is accompanied by the abnormal redistribution of this ganglioside from the PM and lysosomes to the ER membranes (Fig. 2). Studies seeking for a pathogenic mechanism that would link the neuronal cell death to the primary storage product, revealed that the abnormal GM1 levels at the ER membranes led to depletion of the ER Ca2+ store, followed by activation of an UPR. Initially elicited as survival mechanism via upregulation of ER-resident chaperones and folding catalysts, such as BiP, and inhibition of protein synthesis, prolonged UPR activation under a persistent pathologic stimulus causes permanent damage and triggers an ER stress-mediated apoptotic program. In β-Gal−/− brain and spinal cord, upregulation and activation of the UPR molecular effectors responsible for cell survival (e.g., BiP) is associated with increased levels of the transcription factor ATF6, the proapoptotic mediator CHOP, the phosphorylated form of the kinase JNK2, and the cleaved, active form of caspase-12, and culminates with apoptosis (Fig. 3 and Table 1). Activation of the UPR appeared to be directly downstream of GM1 accumulation in the ER membranes, because reducing the levels of de novo synthesized GM1 reverted UPR activation.
The intrinsic ability of GM1 to bind Ca\(^{2+}\) and its redistribution in the ER membranes suggested that this ganglioside could reside also in the MAMs. At this location, GM1 accumulation could perturb the topology of the microdomains and their Ca\(^{2+}\) buffering capacity. Transmission electron microscopy analyses of crude mitochondria isolated from \(\beta\text{-Gal}^{-/-}\) mouse brains indeed identified an increased number of ER remnants juxtaposed to mitochondrial membranes, compared to those from the WT samples (Fig. 5). This indicated that GM1 accumulation in the ER membranes fostered the formation of more contact sites between ER and mitochondria. Moreover, MAMs isolated from \(\beta\text{-Gal}^{-/-}\) brains\(^{36,52}\) contained substantially higher levels of GM1 than WT MAMs\(^{52}\), and the amount was even greater in the purified raft/GEM fractions of the MAMs (Fig. 5). These findings unequivocally proved that GM1 is a normal constituent of these lipid microdomains, albeit present in minuscule amounts, and accumulates in these microdomains from \(\beta\text{-Gal}^{-/-}\) MAMs. These studies have established the existence of rafts/GEMs microdomains within the MAMs and demonstrated that under disease conditions the increased concentration of GM1 at the GEMs facilitates the formation and constitutive activation of a Ca\(^{2+}\) pore composed of the IP3R1, VDAC1 and GRP75 (Figs. 2 and 5; Table 1)\(^{52}\). Remarkably, the observation that the phosphorylated and active form of the IP3R1 (P-IP3R1) is enriched in the \(\beta\text{-Gal}^{-/-}\) GEMs, and co-precipitates with GM1 (Fig. 5) suggests that GM1 accumulation alters the dynamics of the lipid microenvironment, and favors the partitioning of IP3R1 in an active conformation into these microdomains. Concomitantly, the high levels of VDAC1 and GRP75 observed in the \(\beta\text{-Gal}^{-/-}\) GEMs (Fig. 5), further potentiates the activity of the Ca\(^{2+}\) megachannel composed by these proteins. These pathological changes in the composition of the \(\beta\text{-Gal}^{-/-}\) MAMs promotes the continuous Ca\(^{2+}\) flux into the mitochondria, which ultimately leads to mitochondria Ca\(^{2+}\) overload (Figs. 2 and 3). The net result of these events affects the bioenergetic activities of the mitochondria, and activates the mitochondrial intrinsic apoptotic pathway (Fig. 3)\(^{52}\). Signs of mitochondrial dysfunction include mitochondrial depolarization, opening of the PTP and mitochondrial membrane permeabilization, which together contribute to mitochondria-mediated apoptosis downstream of GM1 accumulation in the MAMs/GEMs (Fig. 3). Treatment of \(\beta\text{-Gal}^{-/-}\) cells with MBCD, that efficiently extracts GM1 from the MAMs, rescues the opening of the PTP, the dissipation of the potential, and the apoptotic process (Fig. 6). Taken together these studies underscore the significance of the MAMs/GEMs microdomains in preserving physiological ER homeostasis and mitochondrial bioenergetics activity.

**Conclusions and perspectives**

In eukaryotic cells membrane-enclosed organelles, albeit individually entrusted to different functions, are highly dynamic and in constant communication with one another (Fig. 1). Their regulated interplay occurs at membrane tethering sites embedded in an active cytoskeletal network, whose formation creates the most efficient setting for the cell to respond quickly to certain
physiological or pathological cues. The contact sites between the ER and mitochondria or MAMs are a good example of these functional hubs that control fundamental processes of the corresponding organelles by limiting or enhancing the bidirectional transfer of molecules and ions. The fact that the MAMs embed subdomains enriched in cholesterol and GM1, similar to the lipid rafts of the PM, emphasizes the direct involvement of these constituents in the regulated trafficking of molecules and ions between the two organelles, which can tilt the cell fate towards survival or apoptosis. Thus, it is not surprising that changes in the MAM’s lipid composition/concentration in response to pathological conditions, as in GM1-gangliosidosis, lead to cell death. Many fundamental questions on the biology of the MAMs remain unanswered. For instance, it is still a matter of debate whether variations in the lipid and/or protein signature of the MAMs exist in different cell types or in response to physiologic/pathologic stimuli. What is clear is that specific lipid combinations within the MAMs dictate the recruitment and activity of distinct sets of proteins. It will be of great interest to understand if in other LSDs, like NPC, the failed egression of cholesterol from lysosomal membranes and its accumulation in intracellular membranes also affect the raft/GEM subdomain of the MAMs. If this would be the case, we could anticipate that impaired activity of the MAMs could represent a major contributing factor to disease pathogenesis, especially in lipid storage diseases.

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Competing interest
The authors declare no competing financial interest.

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References
1. A. C. H. & Kwon, Y. T. Crosstalk and Interplay between the ubiquitin-proteasome system and autophagy. Mol. Cell 40, 441–449 (2017).
2. Saftig. Physiology of the lysosome. In: Mehta, A., Beck, M., Sunder-Plasmann, G. (eds) Fabry Disease: Perspectives from 5 Years of FOS Oxford (PharmaGenesis, Oxford, 2006) Chapter 3.
3. Saftig, P. & Klumperman, J. Lysosome biogenesis and lysosomal membrane proteins: trafficking meets function. Nat. Rev. Mol. Cell. Biol. 10, 623–635 (2009).
4. Carette, J. E., Raaben, M., Wong, A. C., Herbert, A. S., Obemester, G. & Mulherkar, N. et al. Ebola virus entry requires the cholesterol transporter Niemann-Pick C1. Nature 477, 340–343 (2011).
5. Ndongou, E., Herbert, A. S., Raaben, M., Obemester, G., Biswas, R., Miller, E. H. et al. A single residue in Ebola virus receptor npc1 influences cellular host range in reptiles. mSphere 1 e00070-16 (2016).
6. Weis, N. A. M., Herbert, A. S., Klumperman, J., Kuemmerle, A., Witt, H. T., et al. Virus entry. Lassa virus entry requires a trigger-induced receptor switch. Science 2014;344:1506–1510.
7. Finean, P., Lloyd-Evans, E., Lack, N. A., Platt, N., Davis, L. C. & Morgan, A. J. et al. Pathogenic mycobacteria achieve cellular persistence by inhibiting the Niemann-Pick Type C disease cellular pathway. Wellcome Open Rep. 1, 18 (2016).
8. Verhey, M., Fruin, J., Männer, A. & Callabio, S. from the lysosome: a control centre for cellular clearance and energy metabolism. Nat. Rev. Mol. Cell. Biol. 14, 283–296 (2013).
9. Lloyd-Evans, E. & Haslett, L. J. The lysosomal storage disease continuum with ageing-related neurodegenerative disease. Ageing Res. Rev. 32, 104–121 (2016).
10. Ferguson, S. M. Beyond indigestion: emerging roles for lysosome-based signaling in human disease. Curr. Opin. Cell. Biol. 35, 59–68 (2015).
11. Bellavita, C. M. & Scarpa, M. Pathophysiology of neuropathic lysosomal storage disorders. J. Inherit. Metabol. Dis. 33, 347–362 (2010).
12. Platt, F. M., Boland, B. & van der Speel, A. C. The cell biology of disease: lysosomal storage disorders: the cellular impact of lysosomal dysfunction. J. Cell Biol. 199, 723–734 (2012).
13. Rigante, D., Cipolla, C., Basile, U., Gulli, F. & Savastano, M. C. Overview of immune abnormalities in lysosomal storage disorders. Immunol. Lett. 188, 79–85 (2017).
14. Cox, T. M. & Cachon-Gonzalez, M. B. The cellular pathology of lysosomal diseases. J. Pathol. 226, 241–254 (2012).
15. Regier, D. S., Proia, R. L., D’Azzo, A. & Tiff, C. J. The GM1 and GM2 gangliosidoses: natural history and progress toward therapy. Pediatr. Endocrinol. Rev. 13(Suppl. 1), 663–673 (2016).
16. Razi-Rothschild, A., Panikova-Kholmyansky, I., Kachra, Y. & Puterman, A. H. Glycosphingolipidoses: beyond the enzymatic defect. Glycoconj. J. 21, 295–304 (2004).
17. Vanier, M. T. Niemann-Pick diseases. Handb. Clin. Neurol. 113, 1717–1721 (2013).
18. Burton, B. K., Deegian, P. B., Enni, G. M., Guardamagna, O., Horslen, S. & Hovingh, G. K. et al. Clinical features of lysosomal acid lipase deficiency. J. Pediatr. Gastroenterol. Nutr. 61, 619–625 (2015).
19. Schnaar, R. L. Gangliosides of the vertebrate nervous system. J. Mol. Biol. 428, 3325–3336 (2016).
20. Hakomori, S. I. Structure and function of glycosphingolipids and sphingolipids: recollections and future trends. Biochim. Biophys. Acta 1780, 325–346 (2008).
21. Schnaar, R. L., Suzuki, A., Stanley, P. Glycosphingolipids in Essentials of Glycobiology, 2nd edn (eds Varli, A; Cummings, R. D., Esko, J. D., Freeze, H. H., Stanley, P., Bertozzi, C. R., et al.) (Cold Spring Harbor, NY, 2009).
22. Sandhoff, K. & Kolter, T. Biosynthesis and degradation of mammalian glycosphingolipids. Philos. Trans. R. Soc. Lond. B Biol. Sci. 358, 847–861 (2003).
23. Seigirin, E., Levental, I., Mayor, S. & Eggeling, C. The mystery of membrane organization: composition, regulation and roles of lipid rafts. Nat. Rev. Mol. Cell. Biol. 18, 361–374 (2017).
24. Hooper, N. M. Detergent-insoluble glycosphingolipid/cholesterol-rich membrane domains, lipid rafts and caveolae (review). Mol. Membr. Biol. 16, 145–156 (1999).
25. Ikemoto, E. Roles of lipid rafts in membrane transport. Curr Opin. Cell. Biol. 13, 470–477 (2001).
26. Simons, K. & Toomre, D. Lipid rafts and signal transduction. Nat. Rev. Mol. Cell. Biol. 1, 31–39 (2000).
27. Helle, S. C., Kaner, G., Kolar, K., Lang, A., Michel, A. H., Kommann, B. Organization and function of membrane contact sites. Biochim. Biophys. Acta 2526–2541 (2013).
28. Kvam, E. & Goldfarb, D. S. Nucleus-vacuole junctions in yeast: anatomy of a membrane contact site. Biochem. Soc. Trans. 34 (Pt. 3), 340–342 (2006).
29. Levine, T. P. & Patel, S. Signalling at membrane contact sites two membranes come together to handle second messengers. Curr. Opin. Cell. Biol. 39, 77–83 (2016).
30. Pinto, W. A. Bridging the gap membrane contact sites in signaling, metabolism, and organelle dynamics. J. Cell Biol. 205, 759–769 (2014).
31. Wong, L. H. & Levine, T. P. Lipid transfer proteins do their thing anchored at membrane contact sites… but what is their thing? Biochim. Soc. Trans. 44, 517–527 (2016).
32. Levine, T. & Loewen, C. Inter-organellar membrane contact sites: through a glass, darkly. Curr. Opin. Cell Biol. 18, 371–378 (2006).
33. Gatta, A. T. & Levine, T. P. Piecing together the patchwork of contact sites. Trends Cell Biol. 27, 214–219 (2017).
34. Rizzuto, R. P. P., Carnagino, W., Fay, F. S., Fogarty, K. E., Llhiftnz, L. M., Tuft, A. & Pozzan, T. Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+-responses. Science 280, 1763–1766 (1998).
35. Vance, J. E. Phospholipid synthesis in a membrane fraction associated with mitochondria. J. Biol. Chem. 265, 7248–7256 (1990).
36. Annunziata, I., Patterson, A., d’Azzo, A. Mitochondria-associated ER membranes (MAMs) and glycosphingolipid enriched microdomains (GEMs): isolation from mouse brain. J. Vis. Exp. 75, e50215 (2013).
37. Baumann, O. & Walz, B. Endoplasmic reticulum of animal cells and its organization into structural and functional domains. Int. Rev. Cytol. 205, 149–201 (2011).
38. Bionda, C. P. J., Schmitt, D., Rodriguez-Lafraisse, C. & Ardail, D. Subcellular compartmentalisation of ceramide metabolism: MAM (mitochondria-associated membrane) and/or mitochondria? Biochem. J. 382, 527–533 (2004).
39. Copeland, D. E. & Dalton, A. J. An association between mitochondria and the endoplasmic reticulum in cells of the pseudobranch gland of a teleost. J. Biophys. Biochem. Cytol. 5, 399–396 (1959).
40. Csordes, G., Remken, C., Varnai, P., Walter, L., Weaver, D. & Buttke, K. F. et al. Structural and functional features and significance of the physical linkage between ER and mitochondria. J. Cell Biol. 174, 915–922 (2006).
41. Csordes, G., Varnai, P., Golenter, T., Roy, S., Parks, G. & Schneider, T. G. et al. Imaging interorganellar contacts and local calcium dynamics at the ER-mitochondrial interface. Mol. Cell 39, 121–132 (2010).
42. de Brito, O. M. & Scorrano, L. Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456, 605–610 (2008).
43. Grimm, S. The ER-mitochondria interface: the social network of cell death. Biochim. Biophys. Acta 1823, 327–334 (2011).
44. Iishi, K. H. K. & Ino, M., Ca2+–shuttling between endoplasmic reticulum and mitochondria underlying Ca2+–oscillations. EMBO Rep. 7, 390–396 (2006).
45. Kornmann, B., Curie, E., Collins, S. R., Schuldiner, M., Nunnari, J. & Weissman, J. S. et al. An ER-mitochondria tethering complex revealed by a synthetic biology screen. Science 325, 477–481 (2009).
46. Liu, X., Weaver, D., Shihrai, O. & Hajnoczy, G. Mitochondrial kiss-and-run: interplay between mitochondrial motility and fusion-fission dynamics. EMBO J. 28, 3074–3089 (2009).
47. Csordes, G. & Hajnoczy, G. SR/ER-mitochondrial local communication: calcium and ROS. Biochim. Biophys. Acta 1787, 1532–1562 (2009).
48. Hayashi, T., Rizzuto, R., Hajnoczy, G. & Su, T. P. MAM: more than just a housekeeper. Trends Cell Biol. 19, 81–88 (2009).
49. Rizzuto, R. B. M., Murgia, M. & Pozzan, T. Microdomains with high Ca2+ close to IP3-sensitive channels that are sensed by neighboring mitochondria. Science 262, 744–747 (1993).
50. Wang, H. J., Gauy, G., Pogan, L., Sauve, R. & Nabi, I. R. Calcium regulates the association between mitochondria and a smooth subdomain of the endoplasmic reticulum. J. Cell Biol. 150, 1485–1489 (2000).
51. Szabadkai, G. B. K., Vamai, P., De Stefani, D., Wiedemann, N. et al. Lam6 regulates the extent of contacts between ER and mitochondria. J. Mol. Biol. 405, 901–911 (2011).
52. Sano, R., Annunziata, I., Patteson, A., Moschiah, S., Genore, E. & Opferman, J. et al. GM1-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to Ca2(2+)-dependent mitochondrial apoptosis. Mol. Cell 36, 500–507 (2010).
53. Missiroi, S., Bonora, M., Pateregiani, S., Poletti, F., Perrone, M. & Gafa, R. et al. PML at Mitochondria-Associated Membranes Is Critical for the Repression of Autophagy and Cancer Development. Cell Rep. 16, 2415–2427 (2016).
54. Theurey, P. & Reusset, J. Mitochondria-associated membranes response to nutrient availability and role in metabolic diseases. Trends Endocrinol. Metabol. 28, 32–45 (2017).
55. Pinton, P., Gorgi, C., Siviero, R., Zecchin, E. & Rizzuto, R. Calcium and apoptosis. ER-mitochondria Ca2+ transfer in the control of apoptosis. Oncogene 27, 6407–6418 (2008).
56. Voeltz, G. K., Rolls, M. M. & Rapport, T. A. Structural organization of the endoplasmic reticulum. EMBO Rep. 3, 944–950 (2002).
57. Ruisin, A. E., Cui, Z., Chen, M. H. & Vance, J. E. A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipid-proteins. J. Biol. Chem. 269, 27494–27502 (1994).
58. Vance, J. E. & Steenbergen, R. Metabolism and functions of phosphatidylethanolamine in mammalian cells. Biochim. Biophys. Acta 1831, 543–554 (2013).
59. Schülein, I. & Daum, G. Phosphatidylethanolamine deacylases, key enzymes of lipid metabolism. JLBMB life 61, 151–162 (2009).
60. Vance, J. E. & Tasseva, G. Formation and function of phosphatidylserine and phosphatidylglycerol in mammalian cells. Biochim. Biophys. Acta 1831, 543–554 (2013).
facilitates phospholipid transfer from the ER to mitochondria. PLoS Biol. 12, e1001969 (2014).

79. Vance, J. E., S. S. J. & Faust, J. R. Abnormalities in mitochondria-associated membranes and phospholipid biosynthetic enzymes in the mmd/mmd mouse model of neuronal ceroid lipofuscinosis. Biochim. Biophys. Acta 1344, 286–290 (1997).

80. Carcel-Tullol, J., Kovacs, A. D. & Pearce, D. A. Cell biology of the NCL proteins: what they do and don’t do. Biochim. Biophys. Acta 1852, 2242–2255 (2015).

81. Nita, D. A., Mole, S. E. & Minassian, B. A. Neuronal ceroid lipofuscinoses. Adv. Exp. Med. Biol. 688, 165–184 (2010).

82. d’Azzo, A. T. A. & Sano, R. Gangliosides as apoptotic signals in ER stress response. Cell Death Differ. 13, 404–414 (2006).

83. Lenti, L., Malagoli, A. M., Mesiani, R., Tinari, A. & Gambardella, L. et al. Evidence for the involvement of lipid rafts localized at the ER-mitochondria-associated membranes in autophagosome formation. Autophagy 12, 917–935 (2016).

84. Sonnino, S. & Prinetti, A. Gangliosides as regulators of cell membrane organization and functions. Adv. Exp. Med. Biol. 688, 165–184 (2010).

85. Yoshii, S. R., Muzumura, N. Monitoring and measuring autophagy. Int. J. Mol. Sci. 18, pii: E1865 (2017).

86. Maternese, P., Garofalo, T., Manganelli, V., Gambardella, L., Marconi, M. & Grasso, M. et al. Evidence for the involvement of GD3 ganglioside in autophagosomal formation and maturation. Autophagy 10, 750–765 (2014).

87. Hahn, C. N., d’Pina, M., Schneider, M., Vanier, M. T., Hara, Y., Suzuki, K., Suzuki, K., d’Azzo, A. Generalized CND disease and massive GM1-ganglioside accumulation in mice defective in lysosomal acid beta-galactosidase. Hum. Mol. Genet. 6, 205–211 (1997).

88. Itoha, M., Matuda, J., Suzuki, O., Ogura, A., Oshima, A. & Tai, T. et al. Development of lysosomal storage in mice with targeted disruption of the beta-galactosidase gene: a model of human GM1-gangliosidosis. Brain Dev. 23, 379–384 (2001).

89. Jeyakumar, M., Thomas, R., Elliott-Smith, E., Smith, D. A., van der Speel, A. C. & d’Azzo, A. et al. Central nervous system inflammation is a hallmark of pathogenesis in mouse models of GM1 and GM2 gangliosidoses. Brain 126, 974–987 (2003). (Pt. 4).

90. Ron, D. & Walter, P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat. Rev. Mol. Cell Biol. 8, 519–529 (2007).

91. Walter, P. & Ron, D. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat. Rev. Mol. Cell Biol. 13, 89–102 (2012).
d’Azzo, A. & Bonten, E. Molecular mechanisms of pathogenesis in a glyco-
sphingolipid and a glycoprotein storage disease. Biochem. Soc. Trans. 38,
1453–1457 (2010).

Bonten, E. J., Annunziata, I. & d’Azzo, A. Lysosomal multienzyme complex
pros and cons of working together. Cell. Mol. Life Sci. 71, 2017–2032 (2014).

Stone, S. J. Vps34-atgm-am-Bic5. Phosphatidylycerine synthase-1 and -2 are
localized to mitochondria-associated membranes. J. Biol. Chem. 3, 34534–34540
(2000).

Nakagawa, T., Zhu, H., Morishima, N., Li, E., Xu, J. & Yankner, B. A. et al. Caspase-
12 mediates endoplasmic-reticulum-specific apoptosis and cytotoxicity by
amyloid-beta. Nature 403, 98–103 (2000).

Szabadkai, G., Bianchi, K., Yamai, P., De Stefani, D., Wieckowski, M. R. &
Cavagna, D. et al. Chaperone-mediated coupling of endoplasmic reticulum
and mitochondrial Ca2+ channels. J. Cell Biol. 175, 901–911 (2006).

Mikoshiba, K. The IP3 receptor/Ca2+ channel and its cellular function. Bio-
chem. Soc. Symp. 74, 9–22 (2007).

Wang, M., Wey, S., Zhang, Y., Ye, R. & Lee, A. S. Role of the unfolded protein
response regulator GRP78/BiP in development, cancer, and neurological
disorders. Antioxid. Redox. Signal. 11, 2307–2316 (2009).

Myhill, N., Lynes, E. M., Nanji, J. A., Blagoveshchenskaya, A. D., Fei, H. & Carmine
Simmen, K. et al. The subcellular distribution of calnexin is mediated by
PACS-2. Mol. Biol. Cell 19, 2777–2788 (2008).

Garofalo, T. G. A., Misasi, R., Tinari, A., Manganelli, V., Gambardella, L., Pavan,
A., Maloni, W. & Sorice, M. Lipid microdomains contribute to apoptosis-
associated modifications of mitochondria in T cells. Cell Death Differ. 12,
1378–1389 (2005).

James, D. I., Parone, P. A., Mattenberger, Y. & Martinou, J. C. Hfis1, a novel
component of the mammalian mitochondrial fission machinery. J. Biol.
Chem. 328, 3633–36379 (2003).

Kantari, C. & Walczak, H. Caspase-8 and bid: caught in the act between death
receptors and mitochondria. Biochim. Biophys. Acta 1813, 538–563
(2011).

Birkinshaw, R. W., Czabotar, P. E. The BCL-2 family of proteins and mito-
chondrial outer membrane permeabilisation. Semin. Cell Dev. Biol. pii:
S1084-9521(17)30189-1, 2017.

Behrends, C., Sovia, M. E., Gygi, S. P. & Harper, J. W. Network organization of
the human autophagy system. Nature 466, 66–76 (2010).

Colecchia, D., Strambi, A., Sanzone, S., Iavarone, C., Rossi, M. & Dall’Armi, C.
et al. MAPK15/ERK8 stimulates autophagy by interacting with LC3 and
GARAPar proteins. Autophagy 8, 1724–1740 (2012).

Itoh, T., Kanno, E., Uemura, T., Wagon, S. & Fukuda, M. OATL1, a novel
autophagosome-resident Rab33B-GAP, regulates autophagosomal matura-
tion. J. Cell Biol. 192, 839–853 (2011).

Khamnet, A., Heinrich, T., Mari, M., Grumati, P., Huebner, A. K. & Akutsu, M.
et al. Regulation of endoplasmic reticulum turnover by selective autophagy.
Nature 522, 354–358 (2015).

Lee, J. E., Jeon, I. S., Han, N. E., Song, H. J., Kim, E. G. & Choi, J. W. et al. Ubiquilin
1 interacts with Orail to regulate calcium mobilization. Mol. Cell 35,
41–46 (2013).

Popovic, D., Akutsu, M., Novak, I., Harper, J. W., Behrends, C. & Dikic, I. Rab
GTPase-activating proteins in autophagy: regulation of endocytic and
autophagy pathways by direct binding to human ATG8 modifier. Mol. Cell.
Biol. 32, 1753–1744 (2012).

Rothenberg, C., Srenivasan, D., Mah, L., Kaushik, S., Peterhoff, C. M. & Ugolino, J.
et al. Ubiquitin functions in autophagy and is degraded by chaperone-
mediated autophagy. Hum. Mol. Genet. 19, 3219–3232 (2010).

Sancho, A. Duran J, Garcia-Espana A, Mauvezin C, Alemu E A, Lamark T, et al.
DOR/Tip33mp2 and Tip33mp1 constitute a metazoan gene family encoding
dual regulators of autophagy and transcription. PloS ONE 2012. e34034

Seidenbecher, C. I., Landwehr, M., Smalla, K. H., Kreutz, M., Dietrich, D. C.
& Zuschatter, W. et al. Calendrin but not calmodulin binds to light chain 3
of MAP1A/B; an association with the microtubule cytoskeleton highlighting
exclusive binding partners for neuronal Cal2(+)sensor proteins. J. Mol.
Cell. Biol. 336, 957–970 (2004).

Suzuki, H., Tabata, K., Mosta, E., Kawasaki, M., Kato, R. & Dobson, R. C. et al.
Structural basis of the autophagy-related LC3/Atg13 LIR complex: recogni-
tion and interaction mechanism. Structure 22, 47–58 (2014).

Tang, Z., Lin, M. G., Stowell, T. R., Chen, S., Zhu, M. & Stearns, T. et al. Autophagy
promotes primary cilogenesis by removing ODF1 from centriolar satellites.
Nature 502, 254–257 (2013).

Zhu, Y., Massen, S., Teerenstra, M., Lang, V., Chen-Lindner, S. & Els, R. et al.
Modulation of serines 17 and 24 in the LC3-interacting region of Bnip3
determines prosurvival mitophagy versus apoptosis. J. Biol. Chem. 288,
1099–1113 (2013).

Di Bartolomeo, S., Corazzari, M., Nazio, F., Olierico, S., Lisi, G. & Antonioli, M.
et al. The dynamic interaction of AMBRA1 with the dynein motor complex
regulates mammalian autophagy. J. Cell Biol. 191, 155–168 (2010).

Di Rita, A. & Strappazzon, F. AMBRA1, a novel BH3-like protein: new insights
into the AMBRA1-BCL2 family proteins relationship. Int. Rev. Cell Mol.
Biol. 330, 85–113 (2017).

Prokas-Cezanne, T., Waddell, S., Gauget, A., Frickey, T., Lupas, A. & Nordheim,
A. WIPi-1alpha (WIPiHPI), a member of the novel 7-bladed WIP protein
family, is aberrantly expressed in human cancer and is linked to starvation-induced
autophagy. Oncogene 23, 9314–9325 (2004).

Schorer, M. Endoplasmic reticulum stress responses. Cell. Mol. Life Sci. 65,
862–894 (2008).