Life in the lumen: The multivesicular endosome

Jean Gruenberg

Correspondence
Jean Gruenberg, Biochemistry Department, University of Geneva, 30 quai E. Ansermet, 1211, Geneva 4, Switzerland. Email: jean.gruenberg@unige.ch

Abstract

The late endosomes/endo-lysosomes of vertebrates contain an atypical phospholipid, lysobisphosphatidic acid (LBPA) (also termed bis[monoacylglycerol]phosphate [BMP]), which is not detected elsewhere in the cell. LBPA is abundant in the membrane system present in the lumen of this compartment, including intralumenal vesicles (ILVs). In this review, the current knowledge on LBPA and LBPA-containing membranes will be summarized, and their role in the control of endosomal cholesterol will be outlined. Some speculations will also be made on how this system may be overwhelmed in the cholesterol storage disorder Niemann-Pick C. Then, the roles of intralumenal membranes in endo-lysosomal dynamics and functions will be discussed in broader terms. Likewise, the mechanisms that drive the biogenesis of intralumenal membranes, including ESCRTs, will also be discussed, as well as their diverse composition and fate, including degradation in lysosomes and secretion as exosomes. This review will also discuss how intralumenal membranes are hijacked by pathogenic agents during intoxication and infection, and what is the biochemical composition and function of the intra-endosomal luminal milieu. Finally, this review will allude to the size limitations imposed on intralumenal vesicle functions and speculate on the possible role of LBPA as calcium chelator in the acidic calcium stores of endo-lysosomes.

KEYWORDS
ALIX, anthrax, bis(monoacylglycerol)phosphate BMP, calcium store, cholesterol, enveloped virus, ESCRTs, exosome, intralumenal vesicle ILV, lipidomics, lysobisphosphatidic acid, lysosome, lysosome storage disease, multivesicular endosome, Niemann-pick C, pathogen, penetration, toxin

1 | SETTING THE STAGE: THE ENDOSONAL SYSTEM

The endosomes of eukaryotic cells are at center stage in controlling the reutilization vs degradation of membrane components, and thus regulate fundamental cellular processes in nutrient uptake, immunity, signaling, adhesion, membrane turnover and development. Components that have been endocytosed by several pathways are delivered to a common early endosome, from where some lipids and proteins, including housekeeping receptors, are recycled back to the plasma membrane (Figure 1), and others are routed by retrograde transport to the trans-Golgi network (TGN).1-3 By contrast, molecules that are destined for late endosomes and lysosomes, including activated signaling receptors, are selectively sorted into lumenal invaginations, which are pinched off as free cargo-containing intralumenal vesicles (ILVs). These multivesicular regions detach or mature from early endosomes and become multivesicular endosomes (or multivesicular bodies) that transport cargoes toward late endosomes and lysosomes.1,2,4

Late endosomes and lysosomes rapidly exchange membrane components and solutes in vivo leading to the prevailing notion that, upon fusion, they form a transient hybrid endo-lysosome, which is then re-converted into secondary lysosomes, where hydrolases are stored1,2,5,6 (Figure 1). As
a result, in this network, the net distinction between late endosomes, endo-lysosomes and lysosomes is often blurred. Late endosomes also function at a crossroad with the autophagy pathway, which, in addition to endocytosis and TGN-derived traffic, provides an additional entry point in the endocytic pathway for the degradation of cytoplasmic material, including organelles. In addition, endosomes engage in physical contacts with other organelles, including in particular the endoplasmic reticulum, via membrane contact sites that play a key role in lipid movement, calcium exchange and endosome dynamics.

Endosomes and lysosomes can also acquire the capacity to fuse with the plasma membrane as secretory endo-lysosomes—process reminiscent of the regulated exocytosis of lysosome-related organelles in specialized cell types. As a consequence, ILVs not only mediate protein and lipid transport to lysosomes for degradation, but can also be released extracellularly as exosomes, which package cellular molecules that, upon delivery to target cells, regulate a wide range of functions at a distance from the exosome-secreting cell. ILVs may also meet additional fates in specialized cell types and contribute to the biogenesis of melanosomes in melanocytes, or harbor MHC class II molecules loaded with peptides for presentation at the plasma membrane in antigen-presenting cells. They may also undergo back-fusion with the endosome limiting membrane as do exosomes after endocytosis by the target cell. Well-integrated with the above functions, late endosomes serve as key sensing/signaling platforms that inform the cell about the cell nutrient situation.

2 | AN ATYPICAL LIPID WITH TWO NAMES

Lysobisphosphatidic acid (LBPA) was discovered as a structural isomer of phosphatidyl glycerol (PG) in 1967 by Body and Gray (Figure 2), close to a decade after PG and a century after the description of the first phospholipid (lecithin or phosphatidyl choline). Soon after its discovery, it was found that LBPA accumulates in the lysosomal
storage disease Niemann-Pick at a time when a precise diagnosis of this lipidosis was uncertain, \(^{38}\) and later that the lipid is enriched in rat liver lysosomes. \(^{39,40}\) In the early 70s, LBPA was re-named bis(monoacylglycero)phosphate (bis[MAG]P or today BMP)\(^{39}\) — a name unfortunately easily confused with the cognate bone morphogenetic factor (\(\approx 17,000\) citations in PubMed).
LBPA seems to be ubiquitously distributed in all mammalian cells and tissues of high eukaryotic cells. However, with the possible exception of Dictyostelium, the lipid has not been detected in lower eukaryotes, including yeast. Prokaryotes and perhaps plants, however, contain the related lipid, acyl-PG. Immunofluorescence and immunogold labeling of cryosections using a monoclonal antibody against LBPA revealed that the lipid is present exclusively in multivesicular regions of late endosomes and abundant in intraluminal membranes (Figure 3), a distribution further confirmed by subcellular fractionation. This distribution is consistent with the original finding—before endosomes had been characterized—that LBPA is present in lysosomes. While LBPA is a minor cellular lipid, it is abundant in these late endocytic compartments (late endosomes or endo-lysosomes), where it may account for 15-20 mol% of total phospholipids. This distribution is unique, because other phospholipids, in contrast to phosphoinositides, are not restricted to a subset of membranes of endocytic and secretory organelles, even though their relative abundance varies between organelles or plasma membrane domains.

### 2.1 Stereo-configuration and biosynthesis

LBPA is an unconventional phospholipid not only because of its restricted distribution, but also because it exhibits a unique sn-1,glycerophosphatetan-1'-glycerol (sn-1:sn-1') stereo-configuration (Figure 2). LBPA is thus a poor substrate for most phospholipases, and a perfect candidate to reside in the degradative environment of late endocytic compartments. However, despite its unusual headgroup and acyl chain organization, LBPA does not act like a detergent and has properties similar to other phospholipids.

Both the unconventional stereo-configuration and sub-cellular distribution raise the issue of LBPA metabolism. In contrast to other phospholipids of the vacuolar apparatus that are synthesized in the early secretory pathway, LBPA is believed to be synthesized in late endocytic compartments from a phospholipid precursor. In vitroope and in vivo observations have led to the notion that LBPA may be synthesized from phosphatidyl glycerol (PG), and that sn-3-PG could be converted into sn-1:sn-1'-LBPA through a complex series of enzymatic reactions. Since then, it has been shown that PG, but not cardiolipin, is indeed an LBPA precursor, but the biosynthetic pathway remains unclear. PG is synthesized in and confined to mitochondria—like cardiolipin that is synthesized from PG. This raises the interesting possibility that mitochondrial PG may become available as LBPA precursor in late endocytic compartments through mitophagy.

### 2.2 Trans-bilayer distribution

The enzymes that mediate LBPA biosynthesis—conversion from PG—should be present in the endosome lumen, a situation that may contribute to explain the restricted distribution of LBPA to late endocytic compartments. Newly synthesized LBPA is thus expected to be asymmetrically inserted into the exoplasmic leaflet of late endosomal membranes, including presumably ILV and limiting membranes. The presence of LBPA in the exoplasmic leaflet of the bilayer is consistent with observations that it binds endocytosed function-blocking anti-LBPA antibodies. Similarly, LBPA-rich membranes may also serve as antigen for endocytosed antibodies associated with the antiphospholipid syndrome perhaps via beta(2)glycoprotein 1.

While LBPA is present in the exoplasmic leaflet of the bilayer, translocation across the bilayer to the cytoplasmic leaflet must occur because the lipid also interacts with the cytosolic ESCRT-protein ALIX. So far, no LBPA flippase has been identified. However, like other negatively-charged phospholipids, LBPA may rapidly flip across the membrane if the charge were neutralized at low pH. The close proximity of the headgroups because of LBPA self-assembly or clustering may cause partial protonation of proximal LBPA phosphate groups and transbilayer redistribution of the protonated form. In turn, this may drive membrane shape changes, consistent with the capacity of LBPA to deform the bilayer in a pH-dependent fashion—keeping in mind that the redistribution of a very small fraction of phospholipids (< 0.1%) can induce significant shape changes. The unique features of LBPA are also illustrated by observations that, at the acidic late endosome pH, LBPA promotes liposome and virus fusion in vitro. LBPA is thus present in both leaflets of the bilayer and on both ILVs and limiting membranes (Figure 3)—albeit more abundant in intraluminal membranes—and yet it is restricted to the multivesicular regions of late endosomes. Presumably, LBPA is preferentially incorporated into forming ILVs and may in fact play a direct role in ILV biogenesis (see also below), preventing LBPA from being confined to limiting membranes.
redistribution to other membranes and ensuring replenishment of the luminal content.

2.3 | Acyl chain composition

In several cell-types, LBPA is predominantly present as dioleoyl iso-form (50%-80%), but the acyl chain composition of LBPA in rat liver and brain is more complex, including long polyunsaturated acyl chains. In vivo, acyl chains are predominantly present on the 2 and 1 positions of the LBPA glycerol backbone, but these positions are unstable and the acyl chains can migrate to the 3,3' positions (Figure 2). It is not known to what extent acyl chain remodeling occurs in vivo and may accompany changes occurring in the intralumenal membrane organization. However, given the fact that the structures of these isomers are significantly different (Figure 2), it is likely that, in addition to the composition, the position of the acyl chains on the glycerol backbone determine LBPA shape and functions, and thus endosomal membrane dynamics. In fact, the peculiar structure of LBPA combined with its organization in LBPA-rich membrane domains likely explain LBPA antigenicity.

3 | LBPA-CONTAINING MEMBRANES CONTROL ENDOosomal LIPIDS

LBPA-membrane play a crucial role in controlling the fate of other lipids, in particular sphingolipids and cholesterol, which are functionally linked in health and in sphingolipid and cholesterol storage disorders.

3.1 | Glycosphingolipid and ceramide degradation

Elegant biochemical studies have shown that LBPA-rich membranes play a crucial role in the degradation of sphingolipids. This role has been discussed in comprehensive reviews, and will only be briefly summarized below. In this process, sphingolipids are degraded in a stepwise manner by lysosomal enzymes with the help of saposins (Sap-A, -B, -C, -D and GM2-AP) in the presence of anionic phospholipids including LBPA. In vitro experiments showed that the degradation of the ganglioside GM2 can be stimulated 100-fold by 20 mol% LBPA in the presence of GM2-AP—a concentration well in the range of LBPA levels in endosomes.

3.2 | Cholesterol transport

LBPA-rich membranes also play a crucial role in controlling the fate of endosomal cholesterol. Most cells acquire cholesterol from circulating LDL endocytosed by the LDL receptor. Once in late endosomes, cholesteryl esters are de-esterified and free cholesterol is rapidly incorporated into nearby membranes, including LBPA-containing membrane. Cholesterol then reaches the endosome limiting membrane and becomes available for further export to the endoplasmic reticulum for cholesterol-sensing, and to other organelles including the plasma membrane. LBPA-membranes also regulate the flux of cholesterol through endosomes during lipid droplet biogenesis induced by Wnt. Cholesterol transfer from endosomes to the endoplasmic reticulum may be direct or indirect via the plasma membrane. and likely involves nonvesicular transport routes at membrane contact sites.

Within endosomes, cholesterol transfer to the limiting membrane depends on the proteins Niemann-Pick type C1 and C2, and loss-of-function mutations in either of these proteins result in a cholesterol storage disease. NPC1 is a multi-spanning protein of the limiting membrane and NPC2 a globular protein present in the lumen, and both proteins bind cholesterol. Structural and mutagenesis evidence indicate that cholesterol is transferred from NPC2 to NPC1, thereby facilitating export from endosomes, and atomicistic simulations indicate that LBPA is required for NPC2-membrane interactions. Recent studies showed that NPC2 interacts directly with LBPA and that these interactions are necessary for cholesterol trafficking from endo-lysosomes. In addition, endocytosed antibodies against LBPA phenocopy NPC at the cellular level. Conversely, knockdown of the LBPA partner ALIX decreases LBPA levels and endosomal cholesterol, suggesting that LBPA becomes limiting in NPC cells. Consistent with this view, a high-content drug screen revealed that the small compound thio-peramide raises LBPA levels, without affecting other endosomal functions, and concomitantly reduces the cholesterol overload in cells from Niemann-Pick type C patients and in Npc1−/− mice. This compound is an inverse agonist of the histamine receptors H3/H4 and accordingly LBPA levels are inversely correlated with histamine receptor expression levels, but it is not known how this receptor controls LBPA levels. LBPA-membranes may thus serve as platform to accommodate endosomal cholesterol, controlling both the cholesterol storage capacity of late endosomes and the flux of cholesterol through these organelles.

3.3 | LBPA in NPC cells

Elevated levels of LBPA have been found in NPC and other lysosomal storage diseases. This increase may reflect some specific need for LBPA, for example in sphingolipid degradation. Alternatively, this increase may reflect the general expansion of the endolysosomal compartment in storage disorders, upon upregulation of endo-lysosomal gene expression by the transcription factor TFEB. Consistent with the latter view, the increase in LBPA levels in NPC cells are correlated with the general expansion of late endosome volume, protein and lipid. Similarly, the elevated levels of LBPA in macrophages may reflect the higher degradative capacity of these cells.

Eventually, the cellular attempt to compensate for the accumulation of storage materials by an increase in the endosomal system collapses under the excess load in NPC cells and presumably in other
storage disorders, leading to a traffic jam and a breakdown of endosomal membrane dynamics. Given its role in endosomal cholesterol transport, LBPA may then become limiting and its capacity to accommodate or buffer excess cholesterol may be overwhelmed in NPC endosomes. Moreover, a lipolytic analysis revealed that, in addition to LBPA, the amounts of the LBPA-related, minor lipid sLBPA (semi-lysobisphosphatidic acid) increases dramatically in the liver of Npc1−/− mice, up to the physiological levels of LBPA itself in WT mice. This analysis also revealed a profound and highly selective remodeling of the acyl chain composition of both LBPA and sLBPA in NPC mice, but not of any other phospholipid confirming the notion that a metabolic relationship exists between LBPA and sLBPA. One may thus speculate that such changes reflect some additional adjustment in LBPA-membrane chemical and physical properties to better accommodate the changes caused by cholesterol accumulation.

There is no approved treatment against NPC except for Miglustat, which delays but does not arrest the progression of the disease. Cyclodextrins clear cholesterol storage and restore cholesterol feedback regulation in NPC mice, improve symptoms and survival in NPC animal models, and decrease the neurological progression of the disease in phase 1-2 trials in NPC patients, suggesting that cyclodextrins may emerge as a therapeutic strategy. However, the mechanism of action is being debated. Recent studies indicate that hydroxpropyl-cyclodextrin acts by promoting the secretion of the endo-lysosome content, including LBPA, via a mechanism that requires the lysosomal cation channel mucolipin-1 (MCOLN1 or TRPML1) (see Figure 6), which is itself responsible for the lysosome storage disease (LSD) mucolipidosis type 4 when mutated. Interestingly, endo-lysosome secretion elicited by cyclodextrin in NPC cells decreases endosomal cholesterol but not total cell cholesterol, indicating that the secreted cholesterol is presumably incorporated into the plasma membrane or released and recaptured by cells, and eventually redistributed intracellularly. On the whole, these data fit nicely with observations that secretory endosomes or lysosomes mediate the secretion of storage material in lysosome storage disorders via activation of TFEB-family transcription factors and that the secretion of endo-lysosome storage materials depends on MCOLN1 activation.

The current view is that ESCRT-0 initiates the process by binding both PtdIns3P on the membrane and ubiquitin conjugated to cargo molecules, and recruits ESCRT-I, which in turn recruits ESCRT-II as a nucleator for ESCRT-III filaments (Figure 4). In addition to ESCRT-0, -I and -II, the filaments of ESCRT-III can also be nucleated by other factors, including the LBPA partner ALIX, and perhaps HD-PTP, which shares a Bro-1 domain with ALIX. ALIX mediates the ESCRT sorting of the GPCRs PAR1 and P2Y1 while HD-PTP is required for the downregulation of the EGF receptor, PDGF receptor, α5β1 integrin, and virally ubiquitinated MHC class 1. In vivo and in vitro observations show that ESCRT-III filaments drive the membrane deformation process that leads to ILV formation, presumably in conjunction with the triple A ATPase VPS4. LBPA itself may also play a direct role in this process. In addition, ESCRT-III drives other membrane deformation processes that share the same topology, including cytokinetic abscission, viral budding, nuclear envelope reformation, as well as plasma membrane and endo-lysosome membrane repair. Hence, ESCRT-III functions as the general membrane deformation and fission machinery with an orientation opposite to endocytosis, away from the cytoplasm.

In addition to ESCRT-dependent mechanisms, ILVs may also form via ESCRT-independent pathways. In melanocytes, the melanosomal protein PMEL is sorted into ILVs in an ESCRT-independent but CD63-dependent manner (Figure 4). Similarly, different ILV populations may be formed in a Hrs- or CD63-dependent manner in HeLa cells. It should be noted that EGF, which triggers EGF receptor endocytosis and sorting into ILVs, also increases multivesicular endosomes biogenesis and ILV formation in an ESCRT-dependent manner. However, the mechanism driving the increase in ILV formation is not known, perhaps dependent on annexin and SCAMP3. In addition, stress exposure triggers the ligand-independent internalization of EGF receptor via a route that diverts from the canonical pathway and that depends on WASH and Tsg101-ALIX, leading to EGF receptor accumulation in a subset of LBPA-rich multivesicular endosomes.

4 | BIOGENESIS OF INTRALUMENAL MEMBRANES

4.1 | ILV and exosome biogenesis

Downregulated signaling receptors, and other proteins destined for late endosomes and lysosomes, are selectively sorted into ILVs, in a process that begins in early endosomes. Protein sorting into ILVs and ILV formation depend on endosomal sorting complexes required for transport (ESCRT)-0, -I, -II and -III. In yeast an alternative intralumenal fragment pathway may also mediate the ESCRT-independent downregulation of surface transporters delivered to the vacuole limiting membrane. 

4.2 | Microautophagy and exosome biogenesis

In a process clearly reminiscent of ILV biogenesis, cytosolic components can be engulfed within the lumen of nascent ILVs via microautophagy, and then delivered to lysosomes. Microautophagy may be mediated via more than one pathway, dependent or not on autophagy-related (ATG) genes. In budding yeast, the NPC orthologs, Ncr1p and Ncr2p, promote microautophagy presumably by increasing sterol in the vacuole limiting membrane. In fission yeast, Nbr1 was identified as autophagy receptor for the ESCRT-dependent targeting of soluble cargos to the vacuole. Accumulating evidence support the notion that the ESCRT machinery is required for microautophagy.  

In addition, evidence also suggests that proteins encoded by ATG genes have pleiotropic effects on exosome biogenesis and release. In particular, the ATG3-ATG12 conjugate was
reported to interact with ALIX in order to promote autophagy and exosome biogenesis.191

Exosomes correspond to a sub-population of extracellular vesicles that originate from ILVs and are released outside cells upon endosome fusion with the plasma membrane31,192,193 (Figure 1). Consistently, exosome biogenesis depends on ESCRT-III,194 and ALIX71,195,196—although exosomes secreted by oligodendrocytes may form in a ceramide-dependent but ALIX- and ESCRT-independent manner197 (Figure 4). In addition, LBPA is present in exosomes198 and ALIX is considered as one of the best-established exosome markers,31,199,200 which is surprising given the fact that ESCRTs remain cytosolic and are typically excluded from ILVs.201,202

Essentially nothing is known about the mechanisms that control the alternative fates of ILVs—degradation in lysosomes, back-fusion or secretion as exosomes. Neither is anything known about the principles responsible for the lysosomal targeting of ILV cargoes or retrieval to other destinations, including exosomes.

4.3 | Biochemically-distinct populations of ILVs

The sub-cellular distribution of LBPA clearly demonstrates that biochemically-distinct populations of ILVs co-exist within endosomes. Indeed, the lipid cannot be detected in early endosomes,44 where ILV biogenesis begins.148 Neither is the lipid detected in canonical multivesicular endosomes/bodies, which serve as intermediate between early and late endosomes (Figure 1). LBPA is found, and thus likely synthesized, in late endosomes or endo-lysosomes,44 which are filled with internal membranes of various origins, including exosomes in the
making, ILVs destined for lysosomes, as well as remnants of organelles delivered by autophagy (see tomogram of late endosomes in Cos cells—Movie S1). LBPA itself seems to be enriched in one subpopulation of these intraluminal membranes.46 Consistent with this notion, PtdIns3P and LBPA localize to different ILV populations within endosomes.203

The notion than more than one population of ILVs co-exist in endosomes204 is clearly further supported by observations that, in addition to ESCRT-dependent mechanisms, ILVs may also form via ESCRT-independent pathways, as discussed above. One of the future challenges will be to establish what are the overlapping vs unique mechanisms, dependent or not on ESCRT subunits or ESCRT-associated proteins, which may drive the biogenesis of functionally-distinct populations of ILVs, microautophagosomes or exosomes. Interestingly, disruption of the class III PI3-kinase Vps34 in neurons, which is required for both autophagy and ILV formation, triggers the secretion of unique exosomes enriched for undigested lysosomal substrates, specific sphingolipids, and LBPA.205

4.4 | ILVs hijacked by pathogens

Pathogens use all tricks in the book to overcome cellular defenses, and not surprisingly, they also exploit the multivesicular endosome pathway206 (Figure 5). The anthrax toxin penetrates the target cell in a process that depends on LBPA, ALIX and other ESCRTs.71,207 Similarly, during vesicular stomatitis virus (VSV) infection, the release of viral RNA into the cytosol depends on LBPA, ALIX and ESCRTs,57,68,75 as do Lassa virus and lymphocytic choriomeningitis virus69—Lassa virus was also shown to depend on LAMP1.208 Crimean-Congo hemorrhagic fever virus (CCHFV) infection may also depend on ALIX and ESCRTs,209 while Human Papillomavirus (HPV) infection may rely on CD63, syntenin-1 and ALIX210 and Ebola virus on NPC1 and cation two-pore channels (TPC)211-213 (Figures 5 and 6). Influenza A virus (IAV) infection depends on VPS4, as well as ubiquitination,214 the SPOPL/Cullin-3 ubiquitin ligase complex and its target EPS15.215,216

Although the precise role of LBPA, ALIX and ESCRTs in infection or intoxication remains to be elucidated, it has been proposed that anthrax,71,207 VSV67,68 and Japanese encephalitis and yellow fever flaviviruses217 may hijack ILVs so that toxin or nucleic acid be delivered to the cytoplasm by ILV back-fusion with the limiting membrane.29,30,218 Interestingly, in this context, the fusion of dengue virus219 and VSV79,220 during infection depends on anionic phospholipids including LBPA—as does the cytoplasmic entry of the non-enveloped Bluetongue Virus capsid.221

The ESCRT machinery was also recently shown to play additional roles during bacterial infection, in light with a general role for ESCRTs in repairing endo-lysosome membranes153,154 and other membranes.222 Vacuoles containing the intracellular pathogen Coxiella burnetii recruit ESCRTs to maintain an intact vacuole, which presumably provides the bacterium with a replication advantage.154 Similarly, ESCRTs are required to repair small membrane damage in the vacuole containing Mycobacterium marinum in Dictyostelium discoideum174 or Mycobacterium tuberculosis in macrophage,223 presumably to ensure that the pathogen remains contained within intact compartments.

ILVs as exosomes have also been proposed to mediate the spreading of pathogens or pathogenic agents from cell to cell (Figure 5). In fact, it is being discussed whether viruses and exosomes (or other types of extracellular vesicles) share similarities and may be related.224 It has been reported that exosomes may mediate the transmission of hepatitis C virus225 in a process that depends on the ESCRT subunit HRS.226 Similarly, exosomes have also been proposed to transfer hepatitis C viral RNA,227,228 as well as nucleic acids from other viruses including HIV229,230 The non-enveloped hepatitis A virus was also shown to be released after inclusion within a host-derived exosomal-like membrane generated in a process that depends on the ESCRTs, VPS4B and ALIX231,232—an observation that blurs the classic distinction between enveloped and non-enveloped viruses. In addition, urapathogenic Escherichia coli (UPEC), which targets lysosomes but avoids degradation by pH neutralization, can be expelled in exosomes by bladder epithelial cells, upon pH sensing via the calcium channel TRPML3 (TRP channel 3 or mucolipin 3)233 (see Figure 6). Finally, in addition to delivering their toxin cargo to the cytoplasm by back-fusion, ILVs containing anthrax toxin may also be released as exosomes so that the toxin can be transmitted to naïve cells.71 Interestingly, however, anthrax toxin containing ILVs fail to be targeted to lysosomes for degradation.71 It thus appears that the machinery controlling ILV formation and dynamics has been hijacked to mediate viral RNA or toxin release to the cytoplasm during infection/intoxication, or secretion to the extracellular medium as exosomes in order to propagate the infection or to spread the toxin to naïve cells.

5 | LIFE IN THE LUMEN

5.1 | Protons, anions and cations

In the late endosome lumen, where LBPA is found, ILVs and other intraluminal membranes are packed within a highly crowded environment (Movie S1). Beyond the diversity of membranes already discussed above, relatively little is known about the biochemical and biophysical properties of the lumenal milieu,234 although much progress has been made in the characterization of endo-lysosomal ion channels and in the description of the ionic situation within the endo-lysosomal milieu (for recent reviews, see235-239). It is well-established that endo-lysosome acidification depends on the V-ATPase, with early endosomes having a mildly acidic pH $\approx 6.2$ and late endosomes/lysosomes a more acidic pH $\approx 5.0$234,240,241 (Figure 6). Numerous physiological processes, including ligand-receptor uncoupling, lysosomal enzyme activity and membrane traffic are controlled by the acidification properties of endo-lysosomes. The low endo-lysosomal pH is also used by enveloped viruses to trigger fusion of the viral envelope with the endosomal membrane and by some toxins to cross the endo-lysosomal membrane so that the viral nucleic acid or the toxin can reach the host-cell cytoplasm.206,242 In addition to protons, cations and anions also play important roles in the regulation of the endo-lysosomal luminal environment. Chloride controls ion homeostasis...
Viruses, toxin, bacteria and ILV-membrane dynamics

**FIGURE 5** Viruses, toxin and ILV-membrane dynamics. The left side of the figure (penetration) outlines the pathways used by some endocytosed pathogenic agents that enter the host-cell cytoplasm through endosomes, in a process that depends on proteins/lipids involved in ILV membrane dynamics. VSV, Lassa virus, LCMV, and Flaviviruses may penetrate cells in a two-step process. First, the viral enveloped undergoes fusion with the ILV membrane (e.g., in early endosomes) so that the capsid be delivered into the protected environment of the ILV lumen. Then, the capsid is released into the host-cell cytoplasm upon fusion of the ILV membrane with the late endosome limiting membrane (so-called back-fusion). Similarly, the anthrax toxin is first translocated across the ILV membrane and then delivered to the cytoplasm upon ILV back-fusion. Other endocytosed viruses may penetrate cells upon direct fusion of the viral envelope with the late endosome membrane.\(^\text{206}\) The lower part of the figure outlines the role of ESCRT-III and other ESCRT sub-units in repairing damage to vacuoles containing the indicated bacteria. The right side of the figure outlines the inclusion of some viruses and viral particles into exosomes (enclosure) in a process that depends on ESCRT components, and their release as exosomes. The endocytosed anthrax toxin can also be released as exosomes, rather than being delivered to the cytoplasm of the target cell. Membranes shown in the black color imply that it is not known whether the corresponding processes involve LBPA-containing membranes. CCHFV, Crimean-Congo hemorrhagic fever virus; HAV, hepatitis A virus; HCV, hepatitis C virus; HPV, human papillomaviruses; IAV, influenza A virus; LCMV, lymphocytic choriomeningitis; VSV, vesicular stomatitis virus; M marinum, Mycobacterium marinum (in Dictyostelium discoideum cells); M tuberculosis, Mycobacterium tuberculosis; C burnetii, Coxiella burnetii

| PATHOGENIC AGENT | PROTEIN/LIPID REQUIREMENTS | REFERENCES |
|------------------|--------------------------|------------|
| **PENETRATION**  |                          |            |
| VSV              | LBPA, ALIX, ESCRTs       | [61, 62, 69]|
| Lassa, LCMV      | LBPA, ALIX, ESCRTs       | [63]       |
| Flaviviruses     | ALIX, ESCRTs             | [23]       |
| Anthrax toxin    | LBPA, ALIX, ESCRTs       | [65, 201]  |
| Dengue           | LBPA                     | [213]      |
| HPV              | CD63, syntenin-1, ALIX   | [204]      |
| Ebola            | NPC1, TPCs               | [205-207]  |
| Lassa            | LAMP1                    | [202]      |
| CCHFV            | ALIX, ESCRTs             | [203]      |
| IAV              | Ub, SQP1/CUI3, EPS15, ESCRTs | [208-210] |
| **MEMBRANE REPAIR** |                    |            |
| M. Marinum       | TSG101, ESCRT-III, Vps4  | [167]      |
| M. tuberculosis  | ESCRT-III                | [217]      |
| C. burnetii      | TSG101, ESCRT-III        | [147]      |
| **ENCLOSURE AND SECRETION VIA EXOSOMES** | | |
| HCV              | HRS                      | [219, 220] |
| HAV              | ALIX, ESCRT              | [225, 226] |
| Anthrax toxin    | ALIX, TSG10              | [65]       |
and endo-lysosome acidification, and is regulated in endo-lysosomes by 2Cl−/H+ -exchangers of the CLC anion transporter family (CLC-3 through CLC-7), which are responsible for several disorders when mutated.\textsuperscript{238} (Figure 6).

In mammalian cells, endo-lysosomes, in addition to the ER and mitochondria, also serve calcium storage functions—referred to as acidic calcium stores—presumably regulated via ER-endosome membrane contact sites\textsuperscript{12,237,243,244} (Figure 6). Cation channels, including in particular the mucolipin subfamily of TRPML (transient receptor potential) channels or the distantly related TPCs (two-pore channels) maintain endosomal calcium homeostasis,\textsuperscript{237,243-245} and may also function as key regulators of endo-lysosomal trafficking and autophagy-related processes.\textsuperscript{246,247} Calcium is indeed believed to play an important role in the regulation of endo-lysosome and autophagosome membrane dynamics.\textsuperscript{5,246-248} As already mentioned, mutations in TRPML1 (or mucolipin-1, MCOLN1) are responsible for the LSD mucolipidosis type 4\textsuperscript{142} and dysfunction of endo-lysosomal calcium is observed in various LSDs.\textsuperscript{249-251} In addition, TPCs are involved in Ebola virus penetration from endo-lysosomes into host-cells,\textsuperscript{211} while delivery of the viral core to the cytoplasm depends on the NPC1 protein\textsuperscript{212,213} (Figure 5). Finally, efflux of calcium from damaged endosomes serves as a signal to trigger an ESCRT-mediated repair process.\textsuperscript{153}

Much like in the ER,\textsuperscript{252} the free Ca\textsuperscript{2+} in endosomes is estimated to 0.4-0.6 mM.\textsuperscript{249,253} In the ER, most Ca\textsuperscript{2+} is buffered by abundant lumenal Ca\textsuperscript{2+}-binding proteins.\textsuperscript{254,255} However, these proteins or...
their functional homologs are not found in endosomes and lysosomes, and the nature of the Ca$^{2+}$-binding molecules that play similar roles in the acidic calcium stores is unknown. Yet, it can be estimated that $\approx 99.9$% of Ca$^{2+}$ in acidic stores is chelated, supporting the notion that buffer molecules or matrix must exist. It is appealing to propose that the abundant, negatively-charged lipid LBPA serves as calcium buffer in the lumen of late endosome/endo-lysosomes. Indeed, the capacity of calcium to bind negatively-charged lipids is a universal principle, which is best illustrated by the active translocation of the negatively-charged lipid PS from the outer leaflet of the plasma membrane (high calcium environment of the blood) to the inner leaflet (low calcium environment of the cell). Moreover, calcium exhibits a substantial capacity to bind membrane phospholipids and to alter the properties of the bilayer. In fact, accumulation of the divalent cation Zn$^{2+}$ in the LBPA-containing late endosomes of cells expressing the ZnT2 zinc transporter caused cholesterol accumulation much like some/endo-lysosome capacity to modulate calcium-dependent processes, including in NPC cells. It can be anticipated that calcium association to LBPA-rich membranes in the endosome lumen may not only control the fate and dynamics of ILVs, but may also play a key-role in the late endosome/endo-lysosome capacity to modulate calcium-dependent processes, including in lysosomal signaling.

5.2 | The lumen in the lumen: Size matters

In mammalian cells, typical ILVs form one or more fairly homogenous populations of vesicles with a mean diameter around 50 nm, while ILVs in yeast are smaller with a diameter of $\approx 25$ nm. Essentially nothing is known about the chemical conditions that exist within the lumen of ILVs and exosomes, beyond the observations that the pH of newly-formed ILVs is neutral. One should keep in mind that the volume of a 50 nm diameter ILV is exceedingly small, corresponding to $65 \times 10^{-3}$ A$^3$, implying that a fraction of a proton only suffices to reduce the pH by two units, from 7 to 5. Whatever the fate of ILVs, degradation, secretion or retrieval, one may consider these vesicles as unit containers packaging quantum amounts of cargo in the membrane or in the lumen.

This notion becomes important when considering some of the ILV or exosome functions. For example, exosomes presumably transport miRNAs from donor to acceptor cells and thus regulate gene expression in target cells, by repressing translation of target mRNAs and/or by inducing their degradation. One miRNA targets a single RNA molecule, in contrast to enzymes that are regenerated during the catalytic cycle and can process many substrates. Thus, if incorporation into exosome was strictly passive, one would need $2-16 \times 10^5$ exosomes of 50-100 nm diameter to transfer one miRNA species from one typical donor cell with a volume $\approx 1000$ fl to a target cell of the same volume in order to achieve the same miRNA concentration as in the donor—irrespective of what the concentration is—hence, a volume equivalent to the total volume of the donor cell. Thus, a highly efficient mechanism must exist to produce, sort and package miRNAs into exosomes, and to target these exosomes to the recipient cells, for such a transfer mechanism to operate in a physiologically-relevant manner—miRNAs and RNAs associated to extracellular vesicles are reported to be enriched in certain sorting motifs.

Using an assay that measures the biogenesis of ILVs into late endosomes in vitro, the ILV lumenal pH was found to be neutral for a relatively long time, up to 20 minutes after ILV formation. However, given the fact that an ATP-dependent mechanism is unlikely to maintain the pH gradient across the ILV membrane inside endosomes, it is not known whether the pH gradient persists until digestion in the lysosomes, or whether proton permeation across the bilayer eventually acidifies the lumen, prior to degradation. In any case, the asymmetry across the ILV membrane driven by pH and ion gradients, as well as the asymmetric protein and lipid composition of the ILV bilayer likely contribute to regulate the fate of ILVs.

6 | CONCLUSION

Late endosomes/endo-lysosomes are unique organelles of the vertebrate vacuolar apparatus in that they contain membrane vesicles within their luminal environment, which is topologically equivalent to the extracellular space. These vesicles are highly specialized, in particular because some are rich in LBPA—an atypical lipid that is not found elsewhere in the cell. LBPA not only has an unconventional biosynthetic pathway and stereochemistry, but also has a unique shape and acyl chain migration capacity, likely to influence its impact on membrane organization and dynamics.

A fully unanswered and outstanding question is the nature of the mechanism that drive the sorting of ILVs toward one of their possible fates—degradation in lysosomes, secretion as exosomes, or recycling to the limiting membrane via back-fusion. The privileged and secluded environment of ILVs, bathed into the late endosome/endo-lysosome lumen, is fully disconnected from all cytosolic machineries that drive signaling or protein and lipid sorting, and therefore the fate of ILVs cannot rely on these established mechanisms. Future work will be needed to address this issue. However, some speculations are already possible. LBPA-rich membranes are involved in the regulation of several features of the endo-lysosome intralumenal membrane system, including cholesterol transport, sphingolipid degradation, and membrane dynamics, as well as perhaps endosomal Ca$^{2+}$. LBPA also exhibits a rare capacity for adaptive shape changes, via acyl chain remodeling, because of its unique structure. It is therefore attractive to believe that LBPA-rich membranes play a crucial role in modulating trafficking within the endosome and the fate and dynamics of intralumenal membranes. In particular, given the fact that the LBPA partner ALIX is involved in the biogenesis of at least some exosome populations and is itself found in exosome, LBPA-rich endosomal membrane domains may ultimately control the biogenesis of exosomes.

ACKNOWLEDGMENTS

I wish to thank warmly Vincent Mercier (University of Geneva), Oksana Sergeeva (EPFL, Lausanne), Gisou van der Goot (EPFL, Lausanne), Cameron Scott (University of Geneva) and Ueli Schibler...
REFERENCES

1. Huotari J, Helenius A. Endosome maturation. EMBO J. 2011;30(17):3481-3500.
2. Scott CC, Vacca F, Gruenberg J. Endosome maturation, transport and functions. Semin Cell Dev Biol. 2014;31:2-10.
3. Simonetti B, Cullen PJ. Endosomal sorting: architecture of the Retromer coat. Curr Biol. 2018;28(23):R1350-R1352.
4. Hanson PI, Cashikar A. Multivesicular body morphogenesis. Annu Rev Cell Dev Biol. 2012;28:337-362.
5. Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol. 2011;27:107-132.
6. Lamb CA, Yoshimori T, Tooze SA. The autophagosome: origins unknown, biogenesis complex. Nat Rev Mol Cell Biol. 2013;14(12):759-774.
7. Levine B, Kroemer G. Biological functions of autophagy genes: a disease perspective. Cell. 2019;176(1-2):11-42.
8. Wong LH, Eden ER, Futter CE. Roles for ER: endosome membrane contact sites in ligand-stimulated intraluminal vesicle formation. Biochem Soc Trans. 2018;46(5):1055-1062.
9. Berson JF, Harper DC, Tenza D, Raposo G, Marks MS. Pmel17 initiates premelanosome morphogenesis within multivesicular bodies. Mol Biol Cell. 2001;12(11):3451-3464.
10. Delevoye C, Marks MS, Raposo G. Lysosome-related organelles as functional adaptations of the endolysosomal system. Curr Opin Cell Biol. 2019;59:147-158.
11. Raiborg C, Wenzel EM, Pedersen NM, Stenmark H. ER-endosome contact sites in ligand-stimulated intraluminal vesicle formation. Proc Natl Acad Sci U S A. 2008;105(50):19726-19731.
12. Phillips MJ, Voeltz GK. Structure and function of ER membrane contact sites with other organelles. Trends Cell Biol. 2010;20(10):539-545.
13. Marks MS, Heijnen HF, Raposo G. Lysosome-related organelles: unusual compartments become mainstream. Curr Opin Cell Biol. 2013;25(4):495-505.
14. Griffiths GM. Secretion from myeloid cells: secretory lysosomes. Microbiol Spectr. 2016;4(4).
15. Luzzio JP, Hackmann Y, Dieckmann NM, Griffiths GM. The biogenesis of lysosomes and lysosome-related organelles. Cold Spring Harb Perspect Biol. 2014;6(9):a016840.
16. Kowal J, Tkach M, Thery C. Biogenesis and secretion of exosomes. Curr Opin Cell Biol. 2014;29:116-125.
17. Griffiths GM. Secretion from myeloid cells: secretory lysosomes. Microbiol Spectr. 2016;4(4).
18. Luzzio JP, Hackmann Y, Dieckmann NM, Griffiths GM. The biogenesis of lysosomes and lysosome-related organelles. Cold Spring Harb Perspect Biol. 2014;6(9):a016840.
19. Raposo G, Stoorvogel W. Extracellular vesicles: exosomes, microvesicles, and friends. J Cell Biol. 2013;200(4):373-383.
20. Griffiths GM. Secretion from myeloid cells: secretory lysosomes. Microbiol Spectr. 2016;4(4).
21. Latifkar A, Hur YH, Sanchez JC, Cerione RA, Antonyak MA. New insights into extracellular vesicle biogenesis and function. J Cell Sci. 2019;132(13):22406.
22. Lamarr J, Voeltz GK. Structure and function of ER membrane contact sites with other organelles. Trends Cell Biol. 2010;20(10):539-545.
23. Marks MS, Heijnen HF, Raposo G. Lysosome-related organelles as functional adaptations of the endolysosomal system. Curr Opin Cell Biol. 2019;59:147-158.
24. Delevoye C, Marks MS, Raposo G. Lysosome-related organelles as functional adaptations of the endolysosomal system. Curr Opin Cell Biol. 2019;59:147-158.
25. Berson JF, Harper DC, Tenza D, Raposo G, Marks MS. Pmel17 initiates premelanosome morphogenesis within multivesicular bodies. Mol Biol Cell. 2001;12(11):3451-3464.
26. Hurbin I, Geerts WJ, Boudier T, et al. Electron tomography of early melanosomes: implications for melanogenesis and the generation of fibrilar amyloid sheets. Proc Natl Acad Sci U S A. 2008;105(50):19726-19731.
regulates endosome structure and function. Nature. 1998;392 (6672):193-197.

45. Brankatschk B, Pons V, Parton RG, Gruenberg J. Role of SNX16 in the dynamics of tubulo-cisternal membrane domains of late endosomes. PLoS ONE. 2011;6(7):e21771. https://doi.org/10.1371/journal.pone.0021771.

46. Kobayashi T, Beuchat MH, Chevallier J, et al. Separation and character-
tization of late endosomal membrane domains. J Biol Chem. 2002; 277(35):32157-32164.

47. Helenius A, Mellman I, Wall D, Hubbard A. Endosomes. Trends Bio-
chem Sci. 1983;8:245-250.

48. Schink KO, Tan KW, Stemman H. Phosphoinositides in control of membrane dynamics. Annu Rev Cell Dev Biol. 2016;32:143-171.

49. van Meer G, de Kroon AI. Lipid map of the mammalian cell. Nature. 2011;124(1):5-8.

50. Holthuis JC, Menon AK. Lipid landscapes and pipelines in membrane homeostasis. Nature. 2014;510(7503):48-57.

51. Bohdanowicz M, Grinstein S. Role of phospholipids in endocytosis, phagocytosis, and macroinocytosis. Physiol Rev. 2013;93(1):69-106.

52. Brotherus J, Renkonen O, Herrmann J, Fisher W. Novel stereochem-
ic configuration in lysobisphosphatidic acid of cultured BHK cells. Phys Chem Lipids. 1974;13:178-182.

53. Joutti A, Brotherus J, Renkonen O, Laine R, Fischer W. The stereo-
chemical configuration of lysobisphosphatidic acid from rat liver, rabbit lung and pig liver. Biochim Biophys Acta. 1976:450(2):206-209.

54. Tan HH, Makino A, Sudesh K, Greimel P, Kobayashi T. Spectroscopic evidence for the unusual stereochemical configuration of an endosome-specific lipid. Angew Chem. 2012;51(2):533-535.

55. Matsuzawa Y, Hostetler KY. Degradation of bis(monoacylglycero)phosphate by an acid phosphodiesterase in rat liver lysosomes. J Biol Chem. 1979;254(13):5979-6001.

56. Frederick TE, Goff PC, Mair CE, Farver RS, Long JR, Fanucci GE. Effects of the endosomal lipid bis(monoacylglycerol)phosphate on the thermotropic properties of DPPC: a 2H NMR and spin label EPR study. Chem Phys Lipids. 2010;163(7):703-711.

57. Poorthuis BJ, Hostetler KY. Studies on the subcellular localization and properties of bis(monoacylglycerol)phosphate biosynthesis in rat liver. J Biol Chem. 1976;251(15):4596-4602.

58. Somerharju P, Renkonen O. Conversion of phosphatidylglycerol lipids to bis(monoacylglycerol)phosphate in vivo. Biochim Biophys Acta. 1980;618(3):407-419.

59. Amidon B, Schmitt JD, Thuren T, King L, Waite M. Biosynthetic con-
version of phosphatidylglycerol to sn-1:sn-1' bis(monoacylglycerol)phosphate in a macrophase-like cell line. Biochemistry. 1995;34(16):5554-5560.

60. Amidon B, Brown A, Waite M. Transacylation and phospholipases in the synthesis of bis(monoacylglycerol)phosphate. Biochemistry. 1996; 35:13995-14002.

61. Hullin-Matsuda F, Kawasaki K, Delton-Vandenbroucke I, et al. De novo biosynthesis of the late endosome lipid, bis(monoacylglycerol)phosphate. J Lipid Res. 2007;48(9):1997-2008.

62. Mayr JA. Lipid metabolism in mitochondrial membranes. J Inherit Metab Dis. 2015;38(1):137-144.

63. Mijaljica D, Prescott M, Devenish RJ. Different fates of mitochon-
dria: alternative ways for degradation? Autophagy. 2007;3(1):4-9.

64. Kobayashi T, Beuchat MH, Lindsay M, et al. Late endosomal mem-
branes rich in lysobisphosphatidic acid regulate cholesterol transport. Nat Cell Biol. 1999;1(2):113-118.

65. Galve-de Rochemonteix B, Kobayashi T, Rosnolet C, et al. Inter-
action of anti-phospholipid antibodies with late endosomes of human endothelial cells. Arterioscler Thromb Vasc Biol. 2000;20 (2):563-574.

66. Lebrand C, Corti M, Goodson H, et al. Late endosome motility depends on lipids via the small GTPase Rab7. EMBO J. 2002;21(6): 1289-1300.

67. Le Blanc I, Luyet PP, Pons V, et al. Endosome-to-lysosomal transport of viral nucleocapsids. Nat Cell Biol. 2005;7(7):653-664.

68. Luyet PP, Falgüeres T, Pons V, Pattnaik AK, Gruenberg J. The ESCRT-I subunit Tsg101 controls endosome-to-lysosomal release of viral RNA. Traffic. 2008;9:2279-2290.

69. Pasqual G, Rojek JM, Masin M, Chatton JY, Kunz S. Old world arenaviruses enter the host cell via the multivesicular body and depend on the endosomal sorting complex required for transport. PLoS Pathog. 2011;7(9):e1002232.

70. Soric M, Ferro D, Misasi R, et al. Evidence for anti-agulant activity and beta2-GPI accumulation in late endosomes of endothelial cells induced by anti-LBPA antibodies. Thromb Haemost. 2002;88(4):735-741.

71. Abrami L, Brandi L, Moayeri M, et al. Hijacking multivesicular bodies enables long-term and exosome-mediated long-distance action of annexin toxin. Cell Rep. 2013;5:986-996.

72. Dunoyer-Geindre S, Kruthof EK, Galve-de Rochemonteix B, et al. Localization of beta2-glycoprotein I in late endosomes of human endothelial cells. Thromb Haemost. 2001;85(5):903-907.

73. Alessandri C, Bombardieri M, Di Prospero L, et al. Anti-
lysobisphosphatidic acid antibodies in patients with antiphospholipid syndrome and systemic lupus erythematosus. Clin Exp Immunol. 2005;140(1):173-180.

74. Matsuo H, Chevallier J, Mayran N, et al. Role of LBPA and Alix in multivesicular lipidosome formation and endosome organization. Science (New York, NY). 2004;303:531-534.

75. Bissig C, Lenoir M, Velluz MC, et al. Viral infection controlled by a calcium-dependent lipid-binding module in ALIX. Dev Cell. 2013;25 (4):364-373.

76. van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. Nat Rev Mol Cell Biol. 2008;9(2):112-124.

77. Cullis PR, Hope MJ, Bally MB, Madden TD, Mayer LD, Fenske DB. Influence of pH gradients on the transbilayer transport of drugs, lipids, and metal ions into large unilamellar vesicles. Biochim Biophys Acta. 1997;1331(2):187-211.

78. Farge E, Devaux PF. Shape changes of giant liposomes induced by an asymmetric transmembrane distribution of phospholipids. Biophys J. 1992;61(2):247-255.

79. Matos PM, Marin M, Ahn B, Lam W, Santos NC, Melikyan GB. Anionic lipids are required for vesicular stomatitis virus G protein-mediated single particle fusion with supported lipid bilayers. J Biol Chem. 2013;288(18):12416-12425.

80. Akgoc Z, Iosim S, Seyfried TN. Bis(monoacylglycerol)phosphate as a macrophage enriched phospholipid. Lipids. 2015;50(9):907-912.

81. Moreau D, Vacca F, Vossio S, et al. Drug-induced increase in lysobisphosphatidic acid reduces the cholesterol overload in Niemann-pick type C cells and mice. EMBO Rep. 2019;20(7):e47055.

82. Chevallier J, Sakai N, Robert F, Kobayashi T, Gruenberg J, Matile S. Rapid access to synthetic lysobisphosphatidic acids using P(III) chemistry. Org Lett. 2000;2(13):1859-1861.

83. Goursot A, Mineva T, Bissig C, Gruenberg J, Salahub DR. Structure, dynamics, and energetics of lysobisphosphatidic acid (LBPA) iso-
mers. J Phys Chem B. 2010;114(47):15712-15720.

84. Hannah JT, Umebayashi K, Riezman H. Distribution and functions of sterols and sphingolipids. Cold Spring Harb Perspect Biol. 2011;3 (5):a004762.

85. Liscum L. Niemann-pick type C mutations cause lipid traffic jam. Autophagy. 2013;9(1):1859-1861.

86. Lebrand C, Corti M, Goodson H, et al. Late endosome motility depends on lipids via the small GTPase Rab7. EMBO J. 2002;21(6): 1289-1300.
87. Breiden B, Sandhoff K. Lysosomal glycosphingolipid storage diseases. Annu Rev Biochem. 2019;88:461-485.
88. Gallala HD, Sandhoff K. Biological function of the cellular lipid BMP-BMP as a key activator for cholesterol sorting and membrane digestion. Neurochem Res. 2011;36(9):1594-1600.
89. Schulze H, Sandhoff K. Sphingolipids and lysosomal pathologies. Biochim Biophys Acta. 2014;1841(5):799-810.
90. Wilkening G, Linke T, Sandhoff K. Lysosomal degradation on vesicular membrane surfaces. Enhanced glucosylceramide degradation by lysosomal anionic lipids and activators. J Biol Chem. 1998;273(46):30271-30278.
91. Wilkening G, Linke T, Uhlhorn-Dierks G, Sandhoff K. Degradation of membrane-bound ganglioside GM1. Stimulation by bis(monoacylglycerol) phosphate and the activator proteins SAP-B and GM2-AP. J Biol Chem. 2002;277(46):35814-35819.
92. Linke T, Wilkening G, Sadeghfar F, et al. Interfacial regulation of acid ceramidase activity. Stimulation of ceramide degradation by lysosomal lipids and sphingolipid activator proteins. J Biol Chem. 2001;276(8):5760-5768.
93. Worth N, Schuette CG, Wilkening G, Lenn T, Sandhoff K. Degradation of membrane-bound ganglioside GM2 by beta-hexosaminidase a. stimulation by GM2 activator protein and lysosomal lipids. J Biol Chem. 2001;276(12):16625-16630.
94. Goldstein JL, Brown MS. The low-density lipoprotein pathway and its relation to atherosclerosis. Annu Rev Biochem. 1977;46:987-930.
95. Antonny B, Bigay J, Mesmin B. The oxysterol-binding protein cycle: burning off PI(4)P to transport cholesterol. Annu Rev Biochem. 2018;87:809-837.
96. Brown MS, Goldstein JL. Cholesterol feedback: from Schoenheimer's type C2 disease.
97. Vanier MT, van Meer G. Dynamic transbilayer lipid asymmetry.
98. Scott CC, Vossio S, Vacca F, et al. Wnt directs the endosomal flux of LDL-derived cholesterol and lipid droplet homeostasis. EMBO Rep. 2015;16(6):741-752.
99. Scott CC, Vossio S, Rougemont J, Gruenberg J. TFAP2 transcription factors are regulators of lipid droplet biogenesis. elife. 2018;7:e36330.
100. Lange Y, Ye J, Steck TL. How cholesterol homeostasis is regulated by plasma membrane cholesterol in excess of phospholipids. Proc Natl Acad Sci U S A. 2004;101(32):11644-11647.
101. Infante RE, Radhakrishnan A, Kwon HJ, Brown MS, Goldstein JL. NPC2 facilitates bidirectional transfer of cholesterol between NPC1 and lipid bilayers, a step in cholesterol egress from lysosomes. Proc Natl Acad Sci U S A. 2008;105(40):15287-15292.
102. Linke T, Wilkening G, Sadeghfar F, et al. Interfacial regulation of acid ceramidase activity. Stimulation of ceramide degradation by lysosomal lipids and sphingolipid activator proteins. J Biol Chem. 2001;276(8):5760-5768.
103. Carstea ED, Morris JA, Coleman KG, et al. Niemann-pick C1 disease.
104. Schulze H, Sandhoff K. Sphingolipids and lysosomal pathologies.
105. Naureckiene S, Sleat DE, Lackland H, et al. Identification of HE1 as a key activator for cholesterol transfer from the structure of NPC1 middle luminal domain bound to NPC2. Proc Natl Acad Sci U S A. 2016;113(36):10079-10084.
106. Wang ML, Motamed M, Infante RE, et al. Identification of surface residues on Niemann-pick C2 essential for hydrophobic handoff of cholesterol to NPC1 in lysosomes. Cell Metab. 2010;12(2):166-173.
107. Xu S, Qian H, Zhou X, et al. Structural insights into the Niemann-pick C1 (NPC1)-mediated cholesterol transfer and Ebola infection. Cell. 2016;165(6):1467-1478.
108. Infante RE, Wang ML, Radhakrishnan A, Kwon HJ, Brown MS, Goldstein JL. NPC2 facilitates bidirectional transfer of cholesterol between NPC1 and lipid bilayers, a step in cholesterol egress from lysosomes. Proc Natl Acad Sci U S A. 2008;105(40):15287-15292.
109. Trinh MN, Brown MS, Seemann J, Goldstein JL. Lu F. Lysosomal cholesterol export reconstituted from fragments of Niemann-pick C1. elife. 2018;7:e38564.
110. Pfeffer SR. NPC intracellular cholesterol transporter 1 (NPC1)-mediated cholesterol export from lysosomes. J Biol Chem. 2019;294(5):1706-1709.
111. Gong X, Qian H, Zhou X, et al. Structural insights into the Niemann-pick C1 (NPC1)-mediated cholesterol transfer and Ebola infection. Cell. 2016;165(6):1467-1478.
112. Infante RE, Wang ML, Radhakrishnan A, Kwon HJ, Brown MS, Goldstein JL. NPC2 facilitates bidirectional transfer of cholesterol between NPC1 and lipid bilayers, a step in cholesterol egress from lysosomes. Proc Natl Acad Sci U S A. 2008;105(40):15287-15292.
113. Trinh MN, Brown MS, Seemann J, Goldstein JL. Lu F. Lysosomal cholesterol export reconstituted from fragments of Niemann-pick C1. elife. 2018;7:e38564.
the international disease registry for Niemann-pick disease type C: an observational cohort study. Orphanet J Rare Dis. 2015;10:65.

131. Liu B, Ramirez CM, Miller AM, Repa JJ, Turley SD, Dietschy JM. Cyclodextrin overcomes the transport defect in nearly every organ of NPC1 mice leading to excretion of sequestered cholesterol as bile acid. J Lipid Res. 2010;51(5):933-944.

132. Rosenbaum AI, Zhang G, Warren JD, Maxfield FR. Endocytosis of beta-cyclodextrins is responsible for cholesterol reduction in Niemann-pick type C mutant cells. Proc Natl Acad Sci U S A. 2010; 107(12):5477-5482.

133. Abi-Mosleh L, Infante RE, Radhakrishnan A, Goldstein JL, Brown MS. Cyclodextrin overcomes deficient lysosome-to-endoplasmic reticulum transport of cholesterol in Niemann-pick type C cells. Proc Natl Acad Sci U S A. 2009;106(46):19316-19321.

134. Davidson CD, Ali NF, Micsenyi MC, et al. Chronic cyclodextrin treatment of murine Niemann-pick C disease ameliorates neuronal cholesterol and glycosphingolipid storage and disease progression. PLoS ONE. 2009;4(9):e6951.

135. Camargo F, Erickson RP, Garver WS, et al. Intrathecal 2-hydroxypropyl-β-cyclodextrin triggers MCOLN1-dependent endo-lysosome secretion in Niemann-pick type C1 disease. Sci Transl Med. 2015;7(276):276ra226.

136. Vite CH, Bagel JH, Swain GP, et al. Intracisternal cyclodextrin treatment of mucolipidosis type IV. J Lipid Res. 2006;89(4):1109-1123.

137. Vacca F, Vossio S, Mercier V, et al. Cyclodextrin triggers MCOLN1-dependent endo-lysosome secretion in Niemann-pick type C cells. J Lipid Res. 2019;60(4):832-843.

138. Boudewyn LC, Walkley SU. Current concepts in the neuro-pathogenesis of mucopolysaccharidosis type IV. J Neurochem. 2019;148(5):669-689.

139. Medina DL, Fraldi A, Bouche V, et al. Transcriptional activation of lysosomal exocytosis promotes cellular clearance. Dev Cell. 2011;21(3):421-430.

140. Samie MA, Xu H. Lysosomal exocytosis and lipid storage disorders. J Lipid Res. 2014;55(6):995-1009.

141. Martina JA, Diab HI, Lishu L, et al. The nutrient-responsive transcription factor TFE3 promotes autophagy, lysosomal biogenesis, and clearance of cellular debris. Sci Signal. 2014;7(309):ra9.

142. LaPlante JM, Sun M, Falardeau J, et al. Lysosomal exocytosis is impaired in mucolipidosis type IV. Mol Genet Metab. 2006;89(4):339-348.

143. Dong XP, Wang X, Shen D, et al. Activating mutations of the TRPML1 channel revealed by proline-scanning mutagenesis. J Biol Chem. 2009;284(46):32040-32052.

144. Wenzel EM, Schultz SW, Schink KO, et al. Concerted ESCRT and clathrin recruitment waves define the timing and morphology of intraluminal vesicle formation. Nat Commun. 2018;9(1):2932.
169. Olmos Y, Hodgson L, Mantell J, Verkade P, Carlton JG. ESCRT-III controls nuclear envelope reformation. Nature. 2015;522(7555):236-239.

170. Denaes CM, Gilbert RM, Isermann P, et al. Nuclear envelope rupture and repair during cancer cell migration. Science (New York, NY). 2016;352(6283):353-358.

171. Raab M, Gentili M, de Belfi H, et al. ESCRT III repairs nuclear envelope ruptures during cell migration to limit DNA damage and cell death. Science (New York, NY). 2016;352(6283):359-362.

172. Jimenez AJ, Mauri P, Lafaurie-Janvore J, Divoux S, Piel M, Perez F. ESCRT machinery is required for plasma membrane repair. Science (New York, NY). 2014;343(6174):1247136.

173. Scheffer LL, Sreejana SC, Sharma N, et al. Mechanism of Ca(2+) (+)-triggered ESCRT assembly and regulation of cell membrane repair. Nat Commun. 2014;5:5646.

174. Lopez-Jimenez AT, Cardenal-Munoz E, Leuba F, et al. The ESCRT and autophagy machineries cooperate to repair ESX-1-dependent damage at the mycobacterium-containing vacuole but have opposite impact on containing the infection. PLoS Pathog. 2018;14(12):e1007501.

175. Stuffers S, Sem Wegner C, Stenmark H, Breach A. Multivesicular endosome biogenesis in the absence of ESCRTs. Traffic. 2009;10(7):925-937.

176. Theos AC, Truschel ST, Tenza D, et al. A luminal domain-dependent pathway for sorting to intraluminal vesicles of multivesicular endosomes involved in organelle morphogenesis. Dev Cell. 2006;10(3):343-354.

177. van Niel G, Charrin S, Simoes S, et al. The tetraspanin CD63 regulates ESCRT-independent and -dependent endosomal sorting during melanogenesis. Dev Cell. 2011;21(4):708-721.

178. Edgar JR, Eden ER, Futter CE. Hrs- and CD63-dependent competing mechanisms make different sized endosomal intraluminal vesicles. Traffic. 2014;15(2):197-211.

179. White IJ, Bailey LM, Aghakhani MR, Moss SE, Futter CE. EGF stimulates annexin 1-dependent inward vesiculation in a multivesicular endosome subpopulation. EMBO J. 2006;25(1):1-12.

180. Falguieres T, Castle JD, Grueenberg J. Regulation of the MVB pathway by SCAMP3. Traffic. 2012;13:131-142.

181. Tomas A, Vaughan SO, Burgoyne T, et al. WASH and Tsg101/ALIX-dependent diversion of stress-internalized EGFR from the canonical endocytic pathway. Nat Commun. 2015;6:7224.

182. Mizushima N. A brief history of autophagy from cell biology to physiology and disease. Nat Cell Biol. 2018;20(6):521-527.

183. Tsuji T, Fujimoto M, Tatematsu T, et al. Niemann-pick type C proteins promote microautophagy by expanding raft-like membrane domains in the yeast vacuole. eLife. 2017;6.

184. Liu XM, Sun LL, Hu W, Ding YH, Dong MQ, Du LL. ESCRTs cooperate with a selective autophagy receptor to mediate vacuolar-targeting of soluble cargos. Mol Cell. 2015;59(6):1035-1042.

185. Sahu R, Kaushik S, Clement CC, et al. Microautophagy of cytosolic proteins by late endosomes. Dev Cell. 2011;20(1):131-139.

186. Oka M, Maeda Y, Kagohashi Y, et al. Evidence for ESCRT- and clathrin-dependent microautophagy. J Cell Biol. 2017;216(10):3263-3274.

187. Mejlvang J, Olovik H, Svenning S, et al. Starvation induces rapid degradation of selective autophagy receptors by endosomal microautophagy. J Cell Biol. 2018;217(10):3640-3655.

188. Hatakeyama R, De Virgilio C. TORC1 specifically inhibits microautophagy through ESCRT-0.Curr Genet. 2019;65(5):1243-1249.

189. Lefebvre C, Legouix R, Culette E. ESCRT and autophagies: Endosomal functions and beyond. Semin Cell Dev Biol. 2018;74:21-28.

190. Mukherjee A, Patel B, Koga H, Cuervo AM, Jenny A. Selective endosomal microautophagy is starvation-inducible in drosophila. Autophagy. 2016;12(11):1784-1999.

191. Murrow L, Malhotra R, Debnath J. ATG12-ATG3 interacts with Alix to promote basal autophagic flux and late endosome function. Nat Cell Biol. 2015;17(3):300-310.

192. Kowal J, Arras G, Colombo M, et al. Proteomic comparison defines novel markers to characterize heterogeneous populations of extracellular vesicle subtypes. Proc Natl Acad Sci U S A. 2016;113(8):E968-E977.

193. Simons M, Raposo G. Exosomes-vesicular carriers for intercellular communication. Curr Opin Cell Biol. 2009;21(4):575-581.

194. Colombo M, Moita C, van Niel G, et al. Analysis of ESCRT functions in exosome biogenesis, composition and secretion highlights the heterogeneity of extracellular vesicles. J Cell Sci. 2013;126(4):5553-5565.

195. Baietti MF, Zhang Z, Mortier E, et al. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. Nat Cell Biol. 2012;14(7):677-685.

196. Ghosoub R, Lembo F, Rubio A, et al. Syntenin-ALIX exosome biogenesis and budding into multivesicular bodies are controlled by ARF6 and PLD2. Nat Commun. 2014;5:3477.

197. Trajkovic K, Hsu C, Chianta S, et al. Ceramide triggers budding of exosome vesicles into multivesicular endosomes. Science (New York, NY). 2008;319(5867):1244-1247.

198. Denzer K, van Eijk J, Kleijnmeer MJ, Jakobson de Groot C, Geuze HJ. Follicular dendritic cells carry MHC class II-expressing microvesicles at their surface. J Immunol. 2000;165(3):1259-1265.

199. Therey C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. Nat Rev Immunol. 2009;9(8):581-593.

200. Kalra H, Simpson RJ, Ji H, et al. Vesiclepedia: a compendium for extracellular vesicles with continuous community annotation. PLoS Biol. 2012;10(12):e1001450.

201. Katzmann DJ, Odorizzi G, Emr SD. Receptor downregulation and multivesicular-body sorting. Nat Rev Mol Cell Biol. 2002;3(12):893-905.

202. Mageswaran SK, Dixon MG, Curtiss M, Keener JP, Babst M. Binding to any ESCRT can mediate ubiquitin-independent cargo sorting. Traffic. 2014;15(2):212-229.

203. Gillyool DJ, Morrow IC, Lindsay M, et al. Localization of phosphatidylinositol 3-phosphate in yeast and mammalian cells. EMBO J. 2000;19(17):4577-4588.

204. Babst M. MVB vesicle formation: ESCRT-dependent, ESCRT-independent and everything in between. Curr Opin Cell Biol. 2011;23(4):452-457.

205. Miranda AM, Lasiecka ZM, Xu Y, et al. Neuronal lysosomal dysfunction releases exosomes harboring APP C-terminal fragments and unique lipopid signatures. Nat Commun. 2018;9(1):291.

206. Lozach PY, Huotari J, Helenius A. Late-endocytic vesicles. Curr Opin Virol. 2011;1(1):35-43.

207. Abrami L, Lindsay M, Parton RG, Leplla SH, van de Goot FG. Membrane insertion of anthrax protective antigen and cytoplasmic delivery of lethal factor occur at different stages of the endocytic pathway. J Cell Biol. 2004;166(5):645-651.

208. Jay LT, Raaben M, Herbert AS, et al. Virus entry. Lassa virus entry requires a trigger-induced receptor switch. Science (New York, NY). 2014;344(6191):1506-1510.

209. Shitango O, Nikitin AA, Altuntas CZ, Chepurnov AA, Davey RA, Crimean-Congo hemorrhagic fever virus entry into host cells occurs through the multivesicular body and requires ESCRT regulators. PLoS Pathog. 2014;10(9):e1004390.

210. Grassel L, Fast LA, Scheffer KD, et al. The CD63-Syntenin-1 complex controls post-Endocytic trafficking of oncogenic human papillomaviruses. Sci Rep. 2016;6:32337.

211. Sakurai Y, Kolokoltsov AA, Chen CC, et al. Ebola virus. Two-pore channels control Ebola virus host cell entry and are drug targets for disease treatment. Science (New York, NY). 2015;347(6225):995-998.
259. Huster D, Arnold K, Gawrisch K. Strength of Ca(2+) binding to retinal lipid membranes: consequences for lipid organization. Biophys J. 2000;78(6):3011-3018.

260. Melcrova A, Pokorna S, Pullanchery S, et al. The complex nature of calcium cation interactions with phospholipid bilayers. Sci Rep. 2016;6:38035.

261. Melcr J, Martinez-Seara H, Nencini R, Kolafa J, Jungwirth P, Ollila OHS. Accurate binding of sodium and calcium to a POPC bilayer by effective inclusion of electronic polarization. J Phys Chem B. 2018;122(16):4546-4557.

262. Papahadjopoulos D, Nir S, Duzgunes N. Molecular mechanisms of calcium-induced membrane fusion. J Bioenerg Biomembr. 1990;22(2):157-179.

263. Razi M, Futter CE. Distinct roles for Tsg101 and Hrs in multivesicular body formation and inward vesiculation. Mol Biol Cell. 2006;17(8):3469-3483.

264. Pons V, Luyet P-P, Morel E, et al. Hrs and SNX3 functions in sorting and membrane invagination within multivesicular bodies. PLoS Biol. 2008;6(9):e214.

265. Wemmer M, Azmi I, West M, Davies B, Katzmann D, Odorizzi G. Bro1 binding to Snf7 regulates ESCRT-III membrane scission activity in yeast. J Cell Biol. 2011;192(2):295-306.

266. Falguières T, Luyet PP, Bissig C, Scott CC, Velluz M-C, Gruenberg J. In vitro budding of intralumenal vesicles into late endosomes is regulated by Alix and Tsg101. Mol Biol Cell. 2008;19:4942-4955.

267. Garrity AG, Wang W, Collier CM, Levey SA, Gao Q, Xu H. The endoplasmic reticulum, not the pH gradient, drives calcium refilling of lysosomes. elife. 2016;5.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.