The lysosomal membrane—export of metabolites and beyond
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Lysosomes are degradative organelles in eukaryotic cells mediating the hydrolytic catabolism of various macromolecules to small basic building blocks. These low-molecular-weight metabolites are transported across the lysosomal membrane and reused in the cytoplasm and other organelles for biosynthetic pathways. Even though in the past 20 years our understanding of the lysosomal membrane regarding various transporters, other integral and peripheral membrane proteins, the lipid composition, but also its turnover has dramatically improved, there are still many unresolved questions concerning key aspects of the function of the lysosomal membrane. These include a possible function of lysosomes as a cellular storage compartment, yet unidentified transporters mediating the export such as various amino acids, mechanisms mediating the transport of lysosomal membrane proteins from the Golgi apparatus to lysosomes, and the turnover of lysosomal membrane proteins. Here, we review the current knowledge about the lysosomal membrane and identify some of the open questions that need to be solved in the future for a comprehensive and complete understanding of how lysosomes communicate with other organelles, cellular processes, and pathways.

Introduction
Lysosomes are membrane-bound organelles first described by Christian de Duve as early as 1955 and designated as ‘granules rich in hydrolytic enzymes’, summarizing their most important function in the catabolic turnover of various macromolecules [1]. However, in the last 65 years, a tremendous amount of work improved our understanding of the function of lysosomes, their continuous contact with other organelles and cellular pathways, and their contribution to the pathogenesis of a plethora of different common and rare diseases, including lysosomal storage disease [2,3], neurodegenerative diseases like Alzheimer’s disease [4], or cancer [5]. While the first ~50 years of lysosome research mainly focused on the understanding of their lytic capacity mediated by approximately 60 lysosomal, acidic, and mostly soluble enzymes, the past years focused on the understanding of the lysosomal membrane that connects the lysosomal lumen with the cytosol and communicates by different means with cytosolic pathways and processes. These include particularly the mammalian target of rapamycin (mTOR) complex pathway, a central regulator of cell growth and proliferation, and mTOR-mediated signaling [6,7]. These findings have finally placed lysosomes into the center of metabolic regulation of the cellular homeostasis far beyond their initially assumed purely degradative function as the ‘cellular garbage can’. Proteomic studies identified a

Abbreviations
BMP, bis(monoacylglycero)phosphate; BORC, BLOC-one-related complex; ESCRT, endosomal sorting complexes required for transport; GPI, glycosylphosphatidylinositol; mTOR, mammalian target of rapamycin; PIP, phosphatidylinositol phosphate; TGN, trans-Golgi network.
substantial number of > 700 lysosomal membrane proteins, either as predominantly lysosomal proteins (‘resident lysosomal membrane proteins’) or as proteins with a secondary location and enrichment in lysosomes [8–11]. For a high number of those membrane proteins, the molecular function has not yet been clarified.

Here, we review open questions and current findings on the lysosomal membrane and lysosomal membrane proteins. The role of lysosomes in mTOR signaling [6,7,12] but also the transport machinery mediating the microtubule-dependent positioning of lysosomes via dynein/dynactin, the BLOC-one-related complex (BORC), and BORC-independent complexes [12,13] have been extensively reviewed recently, and we, therefore, focus our review on the luminal side of the membrane and transmembrane proteins in the limiting membrane of lysosomes, the degradation of lysosomal membrane proteins, and their transport to lysosomes.

The lipid composition of lysosomal membranes

The limiting lysosomal membrane distinguishes the acidic lumen from the cytosol. This special membrane differs in various aspects from other endomembranes: It has a characteristic composition of lipids [14] and is characterized by a high degree of highly N-glycosylated proteins. Lysosomal membranes are rich in sphingomyelin and contain the uncommon negatively charged lipid, bis(monoacylglycerol)phosphate (BMP) [15], which is absent from other cellular membranes and can be used as a cellular marker [16]. Cholesterol is not enriched in the limiting lysosomal membranes compared to other cellular membranes [15,17]. It should be noted that late endosomal/lysosomal membranes are not uniform: Intraluminal vesicles are rich in BMP, lacking in the limiting membrane. Similarly, the cholesterol content is much higher in intraluminal membranes, membranes that separate intraluminal vesicles from the lysosomal lumen, compared to the limiting membrane [15,18]. The special composition of the intraluminal vesicles was shown to facilitate the hydrolysis of glycosphingolipids by soluble lysosomal enzymes and sphingolipid activator proteins, small proteins that enable binding of the enzymes with their lipid substrate (reviewed in detail in [15]). Just like the distribution of lipids is not uniform between the limiting lysosomal membrane and intraluminal vesicles, there is good evidence that even within the limiting membrane (and presumably also in intraluminal vesicles) of lysosomes and late endosomes, lipids form raft-like microdomains that are typically enriched in lipids like cholesterol and sphingomyelin and contain glycosylphosphatidylinositol (GPI)-anchored proteins [18–21]. This finding is of particular interest regarding the lysosomal membrane, given that raft-like microdomains were shown to act as assembly platforms for fatty acid (palmitoylated-, myristoylated-) modified proteins [22]. Given that several protein complexes on the cytosolic leaflet of lysosomes, including the Regulator complex, and the BORC complex are anchored within the limiting membrane by such fatty acid-modified proteins (Lamtor1 and Myrlysin, respectively), it will be interesting in future work investigating if these complexes are indeed found in such microdomains and if so, if the assembly and maintenance of such microdomains play any regulatory role.

Lysosomes contain special equipment of the minor phospholipids phosphoinositides (PIPs), which define the endomembrane system. Phosphatidylinositol 3-phosphate (PI3P) is the major PIP in the endosomal system, but lysosomes have additionally been shown to contain phosphatidylinositol 3,5-bisphosphate (PI3,5P2) and possibly phosphatidylinositol 5-phosphate (PI5P) (reviewed in [23]). These lysosome-specific PIPs mediate lysosome positioning and dynamics and form signaling platforms for the mTOR complex, but also fusion events and reformation of lysosomes after fusion with autophagosomes [23].

Lysosomes—cellular storage compartments?

The limiting lysosomal membrane discriminates the lumen that contains high concentrations of lytic enzymes from the cytosol. This demarcation requires a sophisticated system of membrane proteins mediating the exchange of metabolites between these two compartments. While some of these transporter proteins facilitating the exchange of metabolites are well described and known for decades, others remain enigmatic. Even the direction of transport, that is, the export of metabolites from the lysosomal lumen to the cytosol versus import from the cytosol to the lysosomal lumen, is not clear for all metabolites. Recent findings challenge the view that transporters are exclusively exporter of catabolic end products from lysosomal the hydrolysis of macromolecules. Eukaryotic lysosomes resemble in different aspects the vacuole in plants and yeast and share some of the key features, including the acidic pH and the equipment with a set of lytic enzymes [24,25]. The yeast and plant vacuole is additionally well known as storage compartments for various metabolites and ions [24,25]. The function of lysosomes as storage compartments in mammalian...
The transport of amino acids and peptides via the lysosomal membrane

While defects in the great majority of soluble lysosomal enzymes result in lysosomal storage disorders due to a defect in the catabolic turnover of their substrates, only 10 lysosomal membrane transporters have been associated with inherited human disorders (reviewed in [29]). These include the long known transporters like Sialin, Cystinosin, exporting sialic acid, and cysteine, respectively, NPC1, which likely exports cholesterol and putative transporters with yet unknown substrates like CLN3 and CLN7. Deficiencies of the latter two proteins cause different subtypes of neuronal ceroid lipofuscinoses. A substantial number of transporters have been functionally characterized, but no human diseases have yet been associated with mutations in their coding genes. The reason for the disparity that mutations in genes coding for most soluble lysosomal proteins cause human disease, but only very few transporters or other lysosomal membrane proteins are causative for human diseases is unclear. It can only be speculated that either these proteins are essential and mutations their coding genes are not compatible with life, or that there is redundancy or functional complementary pathways by which these metabolites can leave lysosomes. Most likely, all three explanations contribute in part to this overall observation. Such a complementary pathway has been described for the export of cystine by Cystinosin, the lysosomal cystine transporter (Fig. 1A). Cystine is an oxidized dimer form of the amino acid cysteine. Deficiency of Cystinosin leads to cystinosis—a rare storage disease characterized by extensive accumulation of intralysosomal cystine crystals. Cystinosis patients can be treated with cysteamine, an amino thiol drug that reacts with lysosomal cystine and forms a chemical intermediate, which in turn can be transported out of lysosomes by the lysosomal transporter for cationic amino acids, PQLC2 [30,31]. Physiological substrates of PQLC2 are positively charged amino acids, including l-ornithine, arginine, histidine, and lysine [31]. Given the increasing recognition of amino acid efflux as a central regulator of the mTOR pathway [6,7], surprisingly, little is known how many amino acids derived from lysosomal proteolysis of substrate proteins are delivered by endocytosis of autophagy are exported from lysosomes. PQLC2 is the transporter for cationic amino acids [31] (Fig. 1A). Small neutral amino acids, including proline, glycine, and alanine are transported by LYAA1 (synonymously called PAT1 or SLC36A1) [32,33]. SNAT7 (synonymously called SLC38A7) has been identified as the principal lysosomal transporter for glutamine and asparagine, uncharged polar amino acids [23]. Alanine and arginine were found to be recognized by SNAT7 as well, but these data could not be reproduced in a later study under conditions mimicking the lysosomal pH [34,35]. SLC15A4 (synonymously called PHT1) was described as a lysosomal histidine transporter [36,37]. SLC38A9, a central component of the amino acid-sensing machinery, can transport glutamine and arginine [38], but is a low-affinity transporter and if it contributes to the bulk export of amino acids questionable. The transporter for several protein degradation-derived amino acids remains enigmatic: These include polar uncharged amino acids like serine and threonine, amino acids with hydrophobic side chains like methionine and valine, amino acids with bulky hydrophobic side chains like tryptophan, phenylalanine, and tyrosine (Fig. 1A). Interestingly, these transporters have been described biochemically a long time ago [3,39,40], but were not cloned end their coding genes have not been identified. A challenging mission in the coming years will be identifying such transporters to better understand the lysosomal recycling of protein degradation-
derived amino acids. Recent methodical developments like the rapid isolation of lysosomes from cultured cells (‘LysoIP’) and recent improvements of targeted and untargeted metabolomics approaches, possibly in the combination of knocking out candidate genes in established cell lines by CRISPR/Cas9, will facilitate this challenging task [26]. Multiple CRISPR/Cas9-mediated knockout of several transporters might provide information about possible functional redundancy. Finally, knockout mice might provide important information about the physiological functions, cell types, and tissues in which lysosomal recycling of amino acids is relevant. Thus far, no knockout mouse strain for any known and well-characterized amino acid transporters has been reported. We have generated SNAT7/SLC38A7 knockout mice. The mice are viable and show no overt phenotype, implicating a redundant system for the export of uncharged polar amino acids like glutamine in vivo (our unpublished data).

It should be noted that not necessarily all peptides might be degraded to single amino acids as the catastrophic end product of lysosomal proteolysis. Lysosomal oligo- and dipeptide transporters have been recognized and described [37,41,42], and some dipeptides are assumed to be resistant against lysosomal proteolysis. It should also be noted that the great majority of cellular hydrolase activity against dipeptides is located in the cytoplasm [41]. Moreover, in vitro experiments validated dipeptide transport via the lysosomal membrane [43]. These data, collectively, hint toward a significant fraction of amino acids that are exported in the form of dipeptides instead of free amino acids. Finally, transport of peptides across the lysosomal membrane is not exclusively one way: In the special case of antigen-presenting immune cells, the oligopeptide transporter ABCB9/TAPL imports cytosolic peptides ranging from 6 up to 59 amino acids into the lysosomal lumen with a substrate preference for peptides with positively charged or large hydrophobic N- and C-terminal residues [44].

The transport of monosaccharides via the lysosomal membrane

Next to amino acid transporter, surprisingly little is understood about the export of another major class of catabolic lysosomal end products: monosaccharides. Monosaccharides derived from the catabolic action of acid glycosidases acting on glycogen, glycolipids, glycoproteins, and mucopolysaccharidases make up a substantial amount of the catabolic end products of lysosomal hydrolysis [45]. The set of soluble hydrolytic

Fig. 1. Schematic overview of lysosomal transporters for amino acids and monosaccharides. (A) Known transporters for the bulk export of luminal amino acids and their corresponding cargo (LYAAT1/SLC36A1, SNAT7/SLC38A7, Cystinosin, PQLC2/SLC66A2, PHT1/SLC15A4) but also predicted but up to now unknown transporters for polar uncharged, hydrophobic or bulky hydrophobic amino acids, and oligo- and dipeptides. (B) Sialin/SLC17A5 and Glut8/SLC2A8 are known monosaccharide transporters of anionic and neutral hexoses, respectively. A transporter for N-acetylated hexoses has been described biochemically, but was not yet cloned. We assume the presence of another, yet unidentified transporter for neutral hexoses.

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enzymes can completely degrade all these physiological higher-molecular weight polymers to monosaccharides. These include mostly neutral hexoses like glucose, mannose, fucose, and galactose but also charged anionic monosaccharides like sialic acid, iduronic acid, glucuronic acid (the latter both derived from the degradation of mucopolysaccharides), and amide derivatives of glucose, galactose (N-acetylgalcosamine (GlcNAc) and N-acetylgalactosamine (GalNAc)) [45]. Disaccharides, like sucrose cannot leave lysosomes by active transport and there is no evidence for transporters of oligosaccharides outside the lysosome [46]. While it is reasonable that a substantial amount of other carbohydrates, for example, intermediates of glycolysis, end up in lysosomes due to autophagy, little is known about the export of such metabolites.

Each of the biochemically distinct monosaccharide groups has its transporter (Fig. 1B). The transporter for anionic monosaccharides is Sialin [47,48], and deficiency of Sialin leads to ‘sialic acid storage disease’, a multisystem disease characterized by extensive accumulation of sialic acid (and presumably other negatively charged monosaccharides like iduronic acid and glucuronic acid) in lysosomes. The export of the major group of monosaccharides, neutral hexoses, remains enigmatic. Facilitative hexose transport is mediated by 14 isoforms of the glucose transporter protein family (GLUT), but only GLUT8 (coded by SLC2A8) was found in lysosomes [49,50]. However, GLUT8 is mainly expressed in testis and, to a lesser extent, in the brain and tissues with an exceptionally high lysosomal turnover of glycogen like the heart, skeletal muscle, or the liver express only minor amounts of GLUT8. Moreover, GLUT8/Slc2a8 knockout mice are viable, develop normally, and show only mild alterations in the brain (increased proliferation of neuronal cells in the dentate gyrus of the hippocampus, hyperactivity), heart (impaired transmission of an electrical wave through the atrium), and sperm cells [50,51]. This is in contrast to the severe phenotypes caused by impaired enzymatic lysosomal glycogen degradation in the case of lysosomal acid glucosidase (GAA) deficiency (Pompe disease), which is characterized by severe lysosomal storage of glycogen, cardiomyopathy, and premature death in knockout mice and humans [2,52]. Assuming that GLUT8 would be the only neutral hexose transporter, a similar or even more severe phenotype would be expected in Slc2a8 knockout mice due to a massive accumulation of glycogen-derived glucose. Just like in the case of the amino acid transporters, future work will hopefully reveal the nature of the N-acetylhexitosamine-transporter and improve our understanding of how neutral hexoses leave the lysosome or if there is any difference in the specificity of hexose transporters (Fig. 1B). An interesting auxiliary finding regarding the transporters of sugars is the fact that there is also evidence for an import of free oligosaccharides from the cytosol into lysosomes [53,54].

**N-glycosylation protects lysosomal membrane proteins from proteolysis**

The harsh conditions of the lysosomal lumen with its low pH, reducing conditions, and particularly high concentration of lytic enzymes (both proteases and lipid-degrading enzymes) forced the development of membranes and membrane proteins that are highly glycosylated as a safeguarding shield protecting proteins and lipids against the hydrolytic enzymes. The N-glycans prevent both endo- and exoproteases from directly accessing the peptide bonds. The sum of this dense layer of glycosylated proteins, largely composed of the abundant structural proteins LAMP1 and LAMP2 [34,55], forms a ‘glycocalyx’-like surface coat lining the luminal side of the lysosomal membrane, though the word ‘glycocalyx’ should be used with care. This coat can be analyzed by electron microscopy and is recognized as an electron translucent halo [56]. The luminal glycosylated halo has a thickness of ~8 nm and corresponds roughly phospholipid bilayer thickness [56]. Interestingly, these microscopy-based descriptive findings are supported by the modeled crystal structure of the glycosylated LAMP proteins [57]: The resulting glycosylated domain structures had diameters of 6 to 9 nm, confirming the observed thickness by electron microscopy [57]. Importantly, the lysosomal ‘glycocalyx’ differs significantly from other ‘classical’ glycocalyces like that of endothelial cells, which is significantly thicker (50 – 100 fold thicker) with up to several micrometers [58]. These apparent differences likely reflect the different functions of the plasma membrane glycocalyces (cell–cell recognition, communication, and intercellular adhesion) and the lysosomal glycocalyx (mainly protection). The thickness of the different glycocalyces can be mainly explained by the types of carbohydrates constituting the glycocalyx, resulting in such structural differences: While the plasma membrane-bound glycocalyces are mainly composed of long O-glycan chains (glycosaminoglycans) attached to proteoglycans, the
lysosomal ‘glycocalyx’ is mainly composed of short N- and rarely O-glycans, for example, attached to the LAMP proteins [59]. LAMP proteins additionally contain N-linked poly-lactosamine carbohydrates, elongated glycan structures with terminal repeats of galactose and GlcNAc [59].

It is also worth noting that not only carbohydrates attached to proteins contribute to the formation of the lysosomal ‘glycocalyx’ but also abundant glycolipids. Approximately equal concentrations of membrane-attached sugars are found in glycoproteins and glycolipids and glucosyl-, lactosyl-, and galactosyl-glucosyl-ceramide are the predominant components of the neutral glycosphingolipid fraction. N-Acetylneuraminyl-lactosyl-ceramide is the main ganglioside [56,60].

**Transporter—accessory protein complexes are common features of lysosomal membrane proteins**

The major function of the lysosomal ‘glycocalyx’ is thought to protect the integral membrane proteins and possibly lipids from proteolysis/hydrolytic attack by the luminal enzymes. The great majority of lysosomal membrane proteins are N-glycosylated themselves, with few exceptions: The Cl(-)/H(+)-exchanger CLC-7 [61], the vitamin B12 transporter ABCD4 [62], the peptide transporter TAPL/ABCB9 [63], and the orphan transporter MFSD1 [64] are among the few unglycosylated lysosomal membrane proteins (Fig. 2). Interestingly, all these proteins have one thing in common: They all have highly N-glycosylated accessory subunits, with which they closely interact, and which presumably takes over the protective function. CLC-7 is tightly linked to the highly N-glycosylated type I transmembrane protein OSTM1 [61]. ABCB9 interacts with LAMP1 and LAMP2 [65], MFSD1 interacts with the highly N-glycosylated type I transmembrane protein GLMP [64,66], and ABCD4 is interacting with LMBD1 [67-69]. In all cases, these accessory proteins seem to share at least their role in protecting the transporter from degradation (Fig. 2). Recently, a structure was solved for CLC-7 in complex with OSTM1 by cryo-electron microscopy independently by two groups, and OSTM1 shields the lumen-exposed loops of CLC-7 like an umbrella with the N-glycans toward the lumen, impressively supporting the protective role by structural biology [70,71]. However, these accessory subunits take over additional functions beyond protection: They often additionally mediate or influence the sorting of the complexes from the ER and the Golgi to lysosomes and in some cases directly affect the transporting capacity, for example, in the case of the Cl(-)/H(+)-exchanger CLC-7 [70,72]. Similarly, we have shown that GLMP affects the trafficking of MFSD1 [66]. Stabilizing ‘chaperoning’ functions of accessory subunits beyond providing an ‘N-glycan shield’ are additionally supported by a couple in which both partners are N-glycosylated, excluding the essential need for protective function: ATRAID and SLC37A3, which both together form a transporter complex for the cytosolic entry of nitrogen-containing bisphosphonates from the lysosomal lumen to the cytosol [55]. Protein levels of both partners were substantially decreased in double knockout cells for both SLC37A3 and ATRAID, suggesting a reciprocal stabilizing/chaperoning function [55]. ATRAID is, like LAMP1, GLMP, and OSTM1, a type I transmembrane protein (Fig. 2). SLC38A9, a central core of the amino acid-sensing machinery [73], interacts with the highly N-glycosylated tetraspanin TM4SF5 [74], but whether it acts

**Fig. 2.** Schematic overview of nonglycosylated integral lysosomal membrane transporters and their corresponding highly N-glycosylated accessory subunits. (N-glycosylation sites are depicted by Y.) The protein complexes MFSD1-GLMP, TAPL-LAMP1/2, SLC37A2-ATRAID, CLC-7-OSTM1, and ABCD4-LMBD1 are illustrated.
as an essential accessory subunit needs further investigation. It should be noted that interaction of SLC38A9 has also been shown with the V-ATPase and NPC1 [73,75]. In summary, the interaction of multitransmembrane spanning lysosomal proteins with accessory proteins, that are often highly glycosylated, seems to be a common feature similar to many plasma membrane-bound transporters [76].

There are additional lysosomal multiple transmembrane spanning proteins that lack N-glycosylation, among them the K(+) channel TMEM175 [77], the glutamine transporter SNTA7/SLC38A7 [34], TMEM192, a four transmembrane spanning protein of unknown biochemical function [78], and p40/SLC35F6, another multitransmembrane protein of unknown biochemical function [79]. It will be interesting figuring out if these nonglycosylated proteins have highly glycosylated accessory proteins stabilizing and protecting them from lysosomal proteolysis.

**Trafficking of lysosomal membrane proteins**

The bulk flow of integral membrane proteins goes from the endoplasmic reticulum to the Golgi apparatus, finally ending up at the plasma membrane [80]. Targeted delivery of transmembrane proteins needs an active sorting mechanism at the TGN for separating lysosomal proteins from plasma membrane proteins for their subsequent delivery to late endosomes and finally lysosomes (designated as the ‘direct pathway’). This sorting mechanism is mediated by cytosolic protein complexes assigned as ‘Adaptor proteins’ (AP) (see below for a more detailed description of these complexes). AP complexes recognize and concentrate cargo proteins into vesicular carriers that mediate transport from a donor membrane to a target organelle membrane [81]. Alternatively, lysosomal membrane proteins are sorted to the plasma membrane and endocytosed upon AP binding, finally ending up in lysosomes (designated as the ‘indirect pathway’) [82]. Both pathways are not mutually exclusively used, but a specific transmembrane protein can often use both pathways. The signals for this separation at the TGN and endocytosis from the plasma membrane are located in the cytosol-exposed tails of lysosomal proteins. The typical sorting signals for lysosomal targeting to which AP bind to, are composed of short linear amino acid sequences from the YXXO (‘tyrosine-based sorting motif’) or [DE][XXXL][LI] (‘dileucine-based sorting motif’) types. Ø in the tyrosine-based sequence represents a bulky hydrophobic residue. X any amino acid. Both sorting motifs are typically found proximal to transmembrane domains, and membrane proximity seems to be more important for tyrosine-based sorting motifs than for dileucine-based motifs [83].

Purely endocytic tyrosine-based sorting motifs are typically situated 10–40 residues from the transmembrane domains; lysosomal-targeting tyrosine-based sorting motifs are commonly at the carboxy-terminus of the proteins and closer to the membrane, usually at 6–9 residues from the transmembrane domain [82]. In proteins targeted to lysosomes, dileucine motifs signals are similar to tyrosine-based signals close (6 – 11 amino acids) to the transmembrane domain and tend to display their signals near their carboxy- or amino terminus [82,84]. However, functional dileucine-based motifs have been described in lysosomal membrane proteins with> 50 amino acids distance to transmembrane domains and factors like the fold of the protein or additional modifications like S-palmitoylation, that might bring such a motif in closer proximity to the membrane, might affect sorting [85–87]. Besides the typical canonical AP-dependent sorting signals of lysosomal membrane proteins, other sorting signals have been described, including ubiquitin-dependent sorting and noncanonical sorting motifs that do not match the YXXO- or [DE][XXXL][LI]-type (reviewed in [88]).

Both types of canonical lysosomal sorting motifs, tyrosine, and dileucine based, are recognized by different adaptor protein complexes: heterotetrameric protein complexes that mediate intracellular membrane trafficking along endocytic and secretory transport pathways. Five different AP complexes have been described (AP-1–AP-5), of which AP1, AP-2, and AP-3 are clathrin-associated [81]. AP-1, AP-2, and AP-3 seem to play important roles in the sorting of lysosomal membrane proteins. All three AP complexes can bind both tyrosine- and dileucine-based sorting motifs, and there is apparently functional redundancy between the complexes [89,90]. While AP-2 plays a pivotal role in endocytosis, mediating the concentration and sorting of cargo from the plasma membrane, the precise function of AP-1 and AP-3 is less clear and still debated. AP-1 is involved in the trafficking of cargo molecules in the biosynthetic pathway from the TGN to endosomes and back. AP-3 was found both in the TGN and post-TGN vesicles and presumably mediates sorting between endosomes and lysosomes or TGN to lysosome transport [90]. Both complexes seem to contribute to the intracellular sorting of at least a subset of lysosomal membrane proteins [82], but the contribution of each AP complex has still not been finally elucidated. Interestingly, even in an AP-1 knockout situation, lysosomal membrane proteins are still...
delivered to lysosomes, and their sorting is independent of the indirect pathway via the plasma membrane [91], indicating that other mechanisms exist for their intracellular delivery. AP-3-deficient cells show mistargeting of some lysosomal membrane proteins to the plasma membrane [92,93], but under steady-state conditions, the majority of these proteins are properly localized to lysosomes, indicating that AP-3 is not essential for their delivery. Notably, LAMP1 is transported properly to lysosomes, even in AP-1/AP-3 double-deficient cells [90]. Given AP-1 is not essential for the TGN-to-endosome transport, the question arises on how the trafficking of lysosomal membrane proteins that are transported via the direct pathway is selectively routed to endosomes. In fact, more recent data suggest sorting mechanisms for lysosomal membrane proteins that are independent of APs, but rather depend on their luminal domains [94]. Deciphering the molecular basis for such mechanisms will improve our knowledge of basic sorting mechanisms tremendously. In summary, though huge efforts have been undertaken in the last 30 years, the trafficking of lysosomal membrane proteins is still not fully understood. CRISPR/Cas9 multiple knockouts of different AP-complex subunits might clarify which AP complexes can functionally replace each other.

**Many lysosomal membrane proteins undergo proteolytic processing**

Limited proteolytic processing is a common feature of many soluble lysosomal enzymes. In some cases, this proteolytic cleave is essential for the hydrolytic activity or modulates enzymatic activity, for example, in the case of some cathepsins [95]. However, in recent years proteolytic processing became also apparent for numerous lysosomal transmembrane proteins. These include the heparan sulfate acetyl-CoA: alpha-glucosaminide N-acetyltransferase (HGSNAT) [96], the cation channel Mucolipin 1 [97], the neuronal ceroid lipofuscinosis-related putative transporter CLN7/MFSD8 [98]. The physiological function of processing/cleavage is yet unknown, and future work will have to clarify if this modification has any consequence on the function of the proteins or is just a first step toward their proteolytic turnover. We observed proteolytic cleavage fragments of the orphan transporter MFSD1 upon overexpression [64]. However, it turned out that proteolysis can be prevented by coexpression of its accessory subunit GLMP or by treatment with inhibitors for lysosomal proteolysis, indicating that proteolytic processing is presumably the first step in the degradation of the transporter protein [99].

Proteolytic processing does not only occur in multiple transmembrane spanning proteins but also in single transmembrane-spanning proteins like lysosomal acid phosphatase (LAP/ACP2) and the lysosomal 5′-exonuclease phospholipase D3 (PLD3) (Fig. 3A). Cleavage leads in this case to the release of the lumen-exposed, enzymatically active domains. LAP is synthesized as a type I transmembrane protein [100], PLD3 as a type II transmembrane protein [101]. Both proteins exhibit enzymatic activity (as acidic phosphatase and 5′-exonuclease, respectively) and are proteolytically cleaved in their luminal domains close to the transmembrane segments after arrival in lysosomes, releasing their larger enzymatically active domains as soluble enzymes. In the case of LAP, the transmembrane form is transported from the trans-Golgi network (TGN) to the plasma membrane, from which it is internalized in endosomes before being finally delivered to dense lysosomes [100] (Fig. 3B). PLD3 is sorted into intraluminal vesicles of multivesicular bodies after ubiquitination before being proteolytically cleaved and released as a soluble lysosomal enzyme [101]. In these examples, proteolytic processing is part of the biosynthetic delivery to lysosomes. In both cases, proteolytic processing seems to prevent retrograde sorting of the precursor forms and might facilitate the interaction of the soluble enzymes with their soluble substrates (Fig. 3B).

**The degradation of lysosomal membrane proteins**

The half-life of most cellular proteins is tightly regulated, and proteins destined for degradation are often marked with ‘degradation-tag’ like ubiquitination [102]. Surprisingly little is known about the regulation of the half-life (and more specifically, the degradation) of lysosomal membrane proteins. Pioneering work from Scot Emr’s laboratory, addressing the turnover of membrane proteins of the yeast vacuole, revealed for selected proteins (Ypq1, a yeast homolog of PQLC2, and the Zinc transporters Cot1 and Zrt1) a mechanism that depends on ubiquitination of the degradation-destined proteins followed by sorting into intraluminal vesicles or an intermediate compartment by the endosomal sorting complexes required for transport (ESCRT) complex and finally degradation by vacuolar proteases [69,103–105] (Fig. 4). Interestingly, this degradation is controlled by a feedback mechanism and the availability of the substrates controls their turnover [69]. Membrane proteins mistargeted to the vacuole membrane (i.e., no resident vacuole proteins) are similarly degraded by a
ubiquitin-dependent mechanism [106]. In the yeast, the membrane-bound ubiquitin E3 ligases Rsp5 and Tul1 catalyze the transfer of ubiquitin from the E2 enzyme to the substrate target [105,106]. Whether mammalian lysosomal membrane proteins follow similar mechanisms for their degradation like in yeast, remain to be established. Increasing evidence points toward the ubiquitination of LMPs not only in the yeast, but also in mammalian cells [107]. However, in mammalian cells ubiquitination of lysosomal membrane proteins is thought rather as a death signal for the entire organelle during damage of lysosomes and subsequent removal of the damaged lysosomes by autophagy, a process called ‘lysophagy’ [107–109]. Very recent data suggest a mechanism for the turnover of individual lysosomal membrane proteins in mammalian cells that is dependent on autophagy and essential autophagy proteins like ATG5, and interestingly also inducible, for example, by glucose starvation, combining these concepts [110].

Other mechanisms regulating the proteolytic turnover of lysosomal membrane proteins in eukaryotes cannot yet be excluded. One possibility would be the cleavage of transmembrane proteins by luminal proteases releasing the lumen-exposed loops and remaining transmembrane segments, which could theoretically serve as substrates for intramembrane proteases (transmembrane proteases cleaving transmembrane segments within the lipid bilayer) (Fig. 4). Lysosomes contain at least one intramembrane protease (SPPL2A), but SPPL2A was shown to exhibit strict substrate specificity for type II transmembrane proteins [111]. Few lysosomal transmembrane proteins were shown to be substrates for SPPL2A, including CD74 and TMEM106B [111,112]. Alternative mechanisms cannot be excluded, for example, the turnover via the proteasome after extraction of the transmembrane protein from the lysosomal membrane upon ubiquitination by the ubiquitin-targeted AAA-ATPase valosin containing protein (VCP)/p97 or proteins with similar functions. VCP is an AAA-ATPase that extracts polyubiquitinated substrates from multimeric macromolecular complexes and biological membranes for proteasomal degradation and was shown to extract

**Fig. 3.** (A) Phospholipase D3 (PLD3) and lysosomal acid phosphatase (LAP/ACP2) are both proteolytically processed single transmembrane proteins. After proteolytic cleavage, the lumen-exposed domains are released and enzymatically active. (B) PLD3 is sorted from TGN into intraluminal vesicle of multivesicular bodies (MVB), proteolytically cleaved and released as a soluble lysosomal enzyme. LAP/ACP2 is transported from the TGN to the plasma membrane and further transported via endosomes to lysosomes. LAP/ACP2 shuttles several times between the plasma membrane and endosomes.
proteins from the ER membrane for degradation. Interestingly, VCP was shown to play a pivotal role in lysophagy as well [108] (Fig. 4). Mechanisms specifically regulating the proteolytic turnover of lysosomal membrane proteins are clearly understudied, and future systematic studies might shed light on this poorly understood process.

**Repair of damaged lysosomal membranes**

The ESCRT complex was not only suggested to play a critical role in the turnover of lysosomal membrane proteins but also in the repair of damaged lysosomal membranes, a process that was just recently described and precedes lysophagy [113,114]. Lysosomes are vulnerable to damage from diverse injuries such as pathogens, molecules that intercalate into or otherwise destabilize the lipid bilayer, or crystals that can rupture the membrane [114]. During membrane damage, ESCRT is recruited to the lysosomal membrane and thought to mediate membrane sealing/scission by forming a membrane-active oligomeric filament. Mechanistically ESCRT was suggested to internalize the membrane patch containing the lesion into intraluminal vesicles by the same mechanism ESCRT is well known for: the ESCRT-mediated endosomal protein sorting and biogenesis of multivesicular endosomes [113]. In this regard, ESCRT restores the compartmental integrity and function of damaged lysosomes [114]. Notably, ESCRT is also involved in the repair of the plasma membrane [115].

**Concluding remarks and open questions**

In summary, recent years have significantly improved the knowledge about the proteome of the lysosomal membrane, exchange processes between the lysosomal lumen and the cytosol, and a key function of lysosomes as central regulators of cell growth and proliferation and signaling hubs, but also brought up new concepts and ideas. As one example, lysosomal hydrolysis generates various metabolites delivered to different cellular compartments where they could act as signaling molecules (so-called ‘lysokines’) for intra-
organellar communication beyond membrane-proximal signaling of mTORC1 [116]. Transport across the lysosomal membrane is a critical control point for such signaling pathways. However, a number of open questions still remain: The precise mechanisms regulating the turnover of lysosomal membranes are still poorly understood, and the transporters of various metabolites like many amino acids are still enigmatic. Likewise, the substrates of long-known lysosomal transporters (like CLN3 or CLN7) are still unknown, and their discovery might pave the way for developing therapeutic approaches. For example, even fundamental questions, about the sorting of lysosomal membrane proteins and the role of their luminal domains in sorting deserve further in-depth investigation. Methodical improvements and novel techniques will hopefully resolve many of the open questions, and a better understanding of the basic biology of lysosomes will be of outstanding importance for modulating their function and, by this means, also selectively target lysosomal function in both rare and common diseases.

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Conflicts of interest

The authors declare no conflict of interest.

Author contributions

MD wrote the manuscript. SR wrote the manuscript and prepared the figures. All authors approved the final version of the manuscript.

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