ANNUAL REVIEWS

Annual Review of Biochemistry

Lipid Dynamics at Membrane Contact Sites

Karin M. Reinisch,[1,2] Pietro De Camilli,[1,2,3] and Thomas J. Melia[1]

[1]Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut, USA; email: karin.reinisch@yale.edu, pietro.decamilli@yale.edu, thomas.melia@yale.edu

[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, Maryland, USA

[3]Department of Neuroscience; Program in Cellular Neuroscience, Neurodegeneration and Repair; and Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, Connecticut, USA

Annu. Rev. Biochem. 2025. 94:479–502

First published as a Review in Advance on March 11, 2025

The *Annual Review of Biochemistry* is online at biochem.annualreviews.org

https://doi.org/10.1146/annurev-biochem-083024-122821

Keywords

membrane expansion, VPS13, ATG2, BLTP, autophagosome, scramblase

Abstract

In eukaryotes, lipid building blocks for cellular membranes are made largely in the endoplasmic reticulum and then redistributed to other organelles. Lipids are transported between organelles by vesicular trafficking or else by proteins located primarily at sites where different organelles are closely apposed. Here we discuss transport at organelle contact sites mediated by shuttle-like proteins that carry single lipids between membranes to fine-tune their composition and by the more recently discovered bridge-like proteins that tether two organelles and provide a path for bulk lipid movement. Protein-mediated lipid transport is assisted by integral membrane proteins that have roles in (*a*) lowering the energy barrier for lipid transfer between the membrane and the lipid transfer protein, a key parameter determining the transfer rate, and (*b*) scrambling lipids to counteract the bilayer asymmetry that would result from such transfer. Advances in this field are shedding new light on a variety of physiological mechanisms.

Contents

INTRODUCTION

Lipid bilayer membranes are a defining feature of all cells, from bacteria to the metazoans. They serve to separate a cell from its environment and, in eukaryotes, delineate functionally distinct compartments within the cell. The lipid compositions of the plasma and various organellar membranes are distinct, in terms of both bulk lipids (including glycerophospholipids, sphingolipids, and sterols) and signaling lipids (including metabolites of bulk lipids), and the composition of the two leaflets of a bilayer may also be asymmetric (1). These differences in membrane lipid composition give rise to different biochemical and biophysical properties critical for physiology.

In eukaryotes, the glycerophospholipid building blocks of membranes are synthesized mostly in the cytosolic leaflet of the endoplasmic reticulum (ER), then equilibrated between the leaflets of this membrane by proteins known as scramblases (2). Lipids can then be redistributed from the ER to other organellar membranes, from which further trafficking is possible. Such redistribution has long been known to take place via vesicle trafficking, a mechanism unique to eukaryotes that also transfers proteins between organelles. Alternatively, lipid redistribution can be mediated by lipid transport proteins (LTPs), and over the last several years, we have come to appreciate that much of this transport occurs at membrane contacts where organellar membranes are closely apposed (10–30 nm apart). Protein-mediated lipid transport evolutionarily predates vesicle-dependent trafficking and is the primary means for moving lipids between the inner and outer membranes of Gram-negative bacteria.

The ER comes into close proximity to all other membrane-bound organelles in cells (3, 4), allowing for protein-mediated lipid transport to and from any other membrane, including those of

mitochondria or peroxisomes, which are disconnected from vesicular trafficking pathways and rely on this form of transport for all their lipids. Contacts also exist between most non-ER organelles, including between peroxisomes and mitochondria; between lipid droplets and mitochondria; and, reminiscent of the apposition of the inner and outer membranes of Gram-negative bacteria, between the inner and outer membranes of mitochondria.

Protein-mediated lipid transfer at contact sites has several advantages over vesicle-dependent lipid transfer. First, as noted above, protein-mediated transfer across contact sites can explain lipid transfer to compartments disconnected from trafficking pathways. Second, transfer across contact sites is faster than vesicle trafficking, allowing for faster cellular responses. And third, while vesicle trafficking between organelles would tend to equilibrate the lipid composition of organelles in the secretory or endocytic pathways, protein-mediated transport can be selective for particular lipids, thereby creating and/or maintaining organelle-specific membrane compositions.

Here we discuss broad concepts regarding the mechanisms underlying protein-mediated lipid transport at membrane contact sites, an area our laboratories are deeply engaged with. We first describe lipid transfer by shuttle-like lipid transporters, which likely fine-tune membrane lipid compositions, and the bridge-like lipid transporters, which were discovered within only the last 5–6 years and are thought to function in bulk lipid transfer. Based on accumulating but still limited data, we then discuss how bridge-like transporters partner with integral membrane proteins that affect lipid dynamics within membranes. Finally, we discuss established and putative roles for nonvesicular bulk lipid transfer in cell physiology, focusing on the best characterized example of a cellular process depending on such transfer: the biogenesis of the autophagosome. Because the field of nonvesicular lipid trafficking is still developing, albeit rapidly, some of the ideas presented are still speculative.

LIPID TRANSFER BY SHUTTLES

Shuttles feature lipid transfer modules that harbor a hydrophobic cavity large enough to accommodate a single lipid or small number of lipids. Often the module also features a lid, which can assume either an open or a closed conformation. At contact sites, this module can capture a lipid molecule from one organellar membrane into the hydrophobic cavity, ferry the lipid across the aqueous cytosolic space between organelles, and deposit the lipid in the membrane of a second organelle (**Figure 1**). While the shuttle is in transit between membranes, the lid assumes a closed conformation, shielding the bound lipid from the cytosol. The shuttle assumes an open conformation while the transport module is associated with the donor or acceptor membranes, allowing the lipid to transfer between the membrane and the hydrophobic cavity.

The transfer of lipid between a membrane and a lipid transfer module is rate determining, relative to the more rapid diffusion of the lipid transfer module and its lipid cargo between membranes (3, 5–7). In the absence of a lipid transfer protein, glycerophospholipid transfer out of the membrane plane and into aqueous cytosol does not occur, as it is too energetically costly. Exactly how lipid transfer proteins lower the energy barrier is neither well-studied nor well-understood, but it is thought to involve a perturbation of interactions between the lipid molecule being extracted and its membrane neighbors (6–8). In their open conformation, lids may insert into the membrane bilayer to disrupt lipid packing, facilitating lipid extraction. The lid also helps to position the transfer protein's hydrophobic cavity optimally to extract lipid from the membrane. Based on biochemical studies and molecular dynamics simulations, examples in which the lid plays a critical role in membrane-to-protein handoff include the Osh6p protein, which transfers either phosphatidylserine (PS) or phosphatidyl inositol-4-phosphate (PI4P); the phosphatidylinositol (PI) transfer proteins; and the ceramide-1-phosphate transfer proteins (7, 9–11). The lid closes

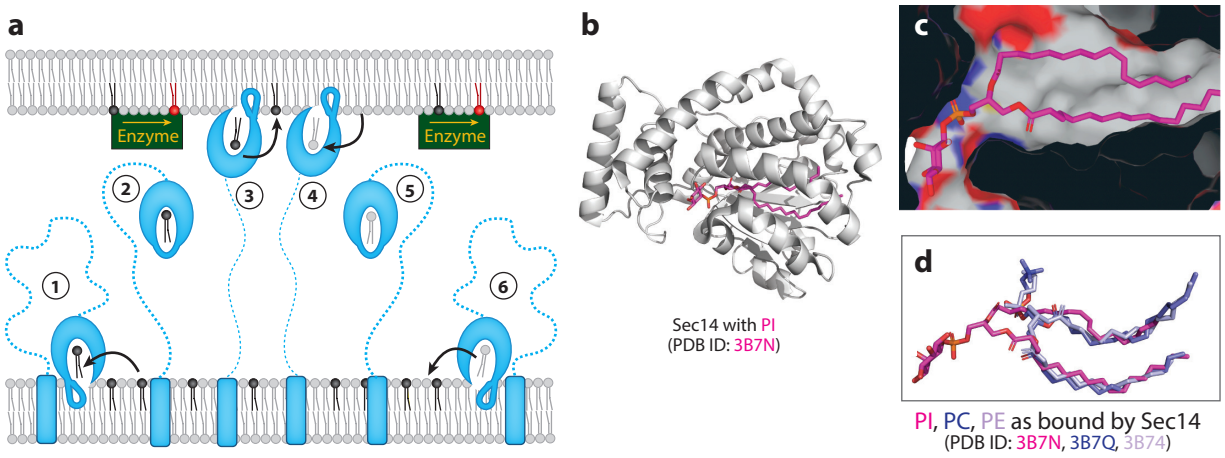

Figure 1

Function and structure of shuttle-like lipid transporters. (*a*) Lipid shuttles typically accommodate one or a few lipids in a hydrophobic cavity and ferry bound lipids across the cytosolic space. The transport steps are numbered to indicate their sequence. The rate-determining step is the removal of the lipid from the donor membrane into the shuttle; there is another energy barrier for lipid release from the shuttle to the acceptor membrane. Shuttles often have lids that may interact with membranes to perturb lipid packing and facilitate lipid extraction or insertion. Similar functions may be carried out by other membrane-inserted domains, as illustrated here (*blue transmembrane helix*), or by partner proteins in the membrane. Many shuttles exchange lipids between membranes rather than carrying lipids unidirectionally, so their hydrophobic cavity is never empty; the empty state would be entropically unfavorable. Enzymes present on either the donor or acceptor membrane provide a concentration gradient, so lipids are either carried down a gradient or counter-exchanged for a lipid that is transferred down such a gradient (see the section titled Lipid Transfer by Shuttles). (*b*) Ribbon diagram of the Sec14 lipid transfer protein, which can bind different lipid species in its hydrophobic cavity and is thought to exchange PI for PC (see the section titled Lipid Transfer by Shuttles). (*c*) PI, shown in stick representation, binds with its acyl chains surrounded by hydrophobic residues in the Sec14 cavity. The Sec14 cavity surface is colored by atom: Carbons are white, oxygens are red, and nitrogens are blue. (*d*) An overlay showing PI, PC, and PE as they are bound within the Sec14 hydrophobic cavity. Abbreviations: PC, phosphatidylcholine; PDB ID, Protein Data Bank identifier; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

subsequent to lipid uptake by the transfer module. This conformational change secures the lipid cargo within the hydrophobic cavity while also lowering the protein's affinity for the membrane, thereby releasing the shuttle from the membrane into the cytosol. In addition to a lidded lipid transfer module, shuttle proteins can feature linkers and adaptor modules that localize and tether the lipid harboring module between membranes at the correct contact site. Some lipid transfer proteins also have a transmembrane segment/domain that permanently anchors them to particular organelles and, importantly, may locally perturb the membrane to facilitate lipid extraction and hence enhance transfer rates.

Lipid transfer by shuttles has been extensively investigated in vitro using purified LTPs and liposomes in place of cellular donor and acceptor membranes. Perhaps unsurprisingly, lipid transfer rates observed during in vitro reconstitutions can be slower than those measured in vivo by an order of magnitude or more (12). This is in part because the composition of liposomes is invariably simpler than that of cellular membranes, for example, consisting of only dipalmitoylphosphatidylcholine to mimic cellular phosphatidylcholine (PC), a major component of all eukaryotic membranes. Addition of other physiological lipids, especially phosphatidylethanolamine (PE), which affects lipid packing, may ease lipid extraction from the membrane. The energy required to extract lipids also depends on the length and saturation of lipid acyl chains, which may differ between cell-free systems and in cells. Anionic lipids such as PS or phosphoinositides are variably enriched in different organelles and might enhance the association of lipid transfer modules

with membranes, increasing the likelihood of lipid extraction; alternatively, they may position the transfer module favorably for lipid extraction into its hydrophobic cavity (8, 13).

Many reconstitutions rely on truncated lipid transfer protein constructs lacking adapter domains, linkers, and/or the transmembrane domains of the intact transfer protein. These missing structural elements may facilitate lipid transfer by the full-length protein, positioning the transfer module proximal to the membrane and optimally orienting the hydrophobic cavity to receive the lipid. Membrane-anchored segments or domains could be instrumental in locally perturbing the membrane to facilitate lipid extraction. Indeed, even if the LTP itself lacks transmembrane segments, in cells, it may partner with membrane-embedded proteins that could locally perturb the membrane in preparation for lipid extraction. For example, many shuttle-transporters localize to ER contact sites via interactions between an FFAT (two phenylalanines in an acidic tract) peptide motif and the major sperm protein (MSP) domain of the ER protein VAPA/B (in metazoans or Scs2/Scs22 in yeast) (14, 15). In addition to the MSP domain, VAPA/B features a C-terminal transmembrane helix.

The importance of lipid dynamics within the membrane for protein-mediated lipid transport has recently begun to be appreciated, especially in the context of the bridge-like LTPs discussed in the section titled Lipid Transport via Protein Bridges. We speculate that integral membrane proteins may also be important for enhancing lipid transfer by shuttle proteins, perhaps via a handoff of lipid from a donor membrane-embedded protein to the shuttle and from the shuttle onto an integral membrane protein in the acceptor membrane.

Rather than transferring lipids unidirectionally, many shuttle proteins exchange lipids between membranes. In this way, the hydrophobic cavity is never empty of lipid, as this would be entropically unfavorable. Some LTPs appear to transfer lipids nonspecifically (or else specific cargo has not yet been identified) and may serve to equilibrate lipid compositions across contact sites. For example, the extended synaptotagmins were proposed to equilibrate lipids between the plasma membrane (PM) and the ER to attenuate signaling lipids at the PM following signal transduction events (16). Other shuttle proteins specifically recognize one or a few distinct lipid species, meaning they have a high binding affinity. LTPs may transfer two distinct, specific cargoes in opposite directions, or they might transfer a single lipid species in one direction in exchange for nonspecific (i.e., lower affinity) cargo on the return journey. These LTPs discriminate phospholipids by specific interactions with their headgroups, often mediated by the lipid transfer module's lid, although some lipids bind with their headgroup at the bottom of the lipid-binding cavity (17) and some shuttles seem to lack lids altogether (18–20).

Shuttle proteins generally transfer lipids down their concentration gradients. For example, in the so-called PI cycle (21), which ensures the presence of PI at the PM even as PI is metabolized during phospholipase C (PLC)–dependent signal transduction, the class IIA PI transfer proteins Nir2 and Nir3 transfer PI from the ER, where it is synthesized and plentiful, to the PM, where PI is rapidly converted to phosphoinositide species. Nir2 and Nir3 also preferentially transfer phosphatidic acid (PA), a lipid generated during PLC-mediated inositol phospholipid hydrolysis at the PM, down a concentration gradient back to the ER, where PA is converted to PI. Similarly, Sec14 binds PI with high affinity and can exchange it for PC, which it binds with lower affinity and which is present at high levels in all membranes. Consistent with the notion that cytosolic forms of LTPs are never empty of lipid cargo, a Sec14 mutant unable to bind PC, and thus unable to exchange lipids, does not transport PI down a gradient: In the absence of PI and unable to take up PC, the mutant protein cannot detach from membranes (10). The PI cycle and PI exchange are described elegantly and more comprehensively elsewhere (22, 23).

Of course, there are exceptions to the generalization that cytosolic shuttle proteins are always lipid occupied. Proteins in the StART family, such as the ceramide transporter CERT or the

sterol transporter Ysp2, can accommodate water in their lipid-binding cavity due to hydrophilic residues at the cavity base. Thus, these proteins may exist in lipid-free forms and transfer lipids unidirectionally (24) (see the structures with Protein Data Bank identifiers 2E3M and 6CAY). Plausibly, some shuttle proteins might also undergo conformational changes that eliminate the hydrophobic cavity, allowing for a lipid-free form.

The lipid counter-exchange mechanism answers the long-standing, fundamental question of how lipids, such as sterols or PS, can be transferred from their place of synthesis in the ER to a compartment where they are already enriched, such as the Golgi or PM. In counter-exchange, shuttle-like proteins of the oxysterol-binding protein (OSBP)–related protein (ORP) family take advantage of energy stored in PI4P gradients to transfer other selected lipids against their concentration gradients (25–27). PI4P is produced from PI at the Golgi, in the endolysosomal system, and also at the PM, so PI4P levels at those locations are elevated with respect to the ER, where levels are low due to the activities of the ER-anchored Sac1 PI4P phosphatase. ORPs bind PI4P via a pleckstrin homology (PH) domain and extract this phosphoinositide from membranes via a so-called ORD, a lipid transport module that prefers PI4P over other lipids. ORPs also preferentially bind a second species of lipid, albeit with lower affinity. Thus, ORPs extract PI4P from the organelle at which it is enriched and transfer it down the PI4P gradient to the ER (to which they are bound via VAP); there, newly delivered PI4P is hydrolyzed to PI by Sac1. In the absence of PI4P at the ER, the ORD then loads up with its second-choice cargo lipid. In the case of OSBP/Osh4p, this other cargo is sterol (25); for yeast Osh6 and mammalian Orp5, Orp8, and Orp10, it is PS (26, 27). The ORP then transfers this second cargo from the ER back across the contact site and counter-exchanges it for PI4P. Transport of the second cargo goes against its concentration gradient, as both sterol and PS are enriched in post-Golgi membranes compared to the ER. Whether all ORP family members transfer lipids via this counter-exchange mechanism, whether LTPs in other families can also counter-exchange, and whether phosphoinositide lipids besides PI4P can be counter-exchanged as has been proposed based on in vitro assays (28) remain under investigation (22, 23). Importantly, as lipid exchangers or counter-exchangers, shuttles serve to fine-tune membrane lipid composition, but they are not well suited for directional bulk lipid transfer, as would be required for rapid membrane expansion or organelle biogenesis.

LIPID TRANSPORT VIA PROTEIN BRIDGES

The first clue regarding the existence of lipid-transferring bridges came from a follow-up to genetic studies in yeast that had identified the ER–mitochondrial encounter structure (ERMES) complex as critical for lipid transfer at contacts between the ER and mitochondria (29), two organelles that are not connected via membrane traffic pathways. While ERMES is not present in metazoans, another large yeast protein that localizes at membrane contact sites, VPS13 (~360 kDa), was shown to rescue cells in cases of ERMES dysfunction, suggesting that it might have at least partially overlapping functions with ERMES (30). That VPS13 is conserved across all eukaryotes, with four isoforms in humans (A–D), suggested that, in higher eukaryotes, VPS13 might fulfill the function of transferring lipids between membranes. Studies of full-length VPS13 by negative stain electron microscopy (31), of a small ~35-kDa N-terminal fragment by X-ray crystallography (3 Å) (32), and of a larger ~160-kDa N-terminal fragment first by negative staining (30) and then by cryo–electron microscopy (cryo-EM) (nominally 3.8 Å) (33) indicated that VPS13 largely consists of a rod-like domain formed by an extended, slightly twisted β-sheet curved to resemble a taco shell (**Figure 2**). Moreover, these proteins were found to contain multiple lipids, suggesting that they are capable of bulk lipid transport (32). The concave surface of the taco is lined by hydrophobic residues, and the domain is long enough to span between apposed organelles

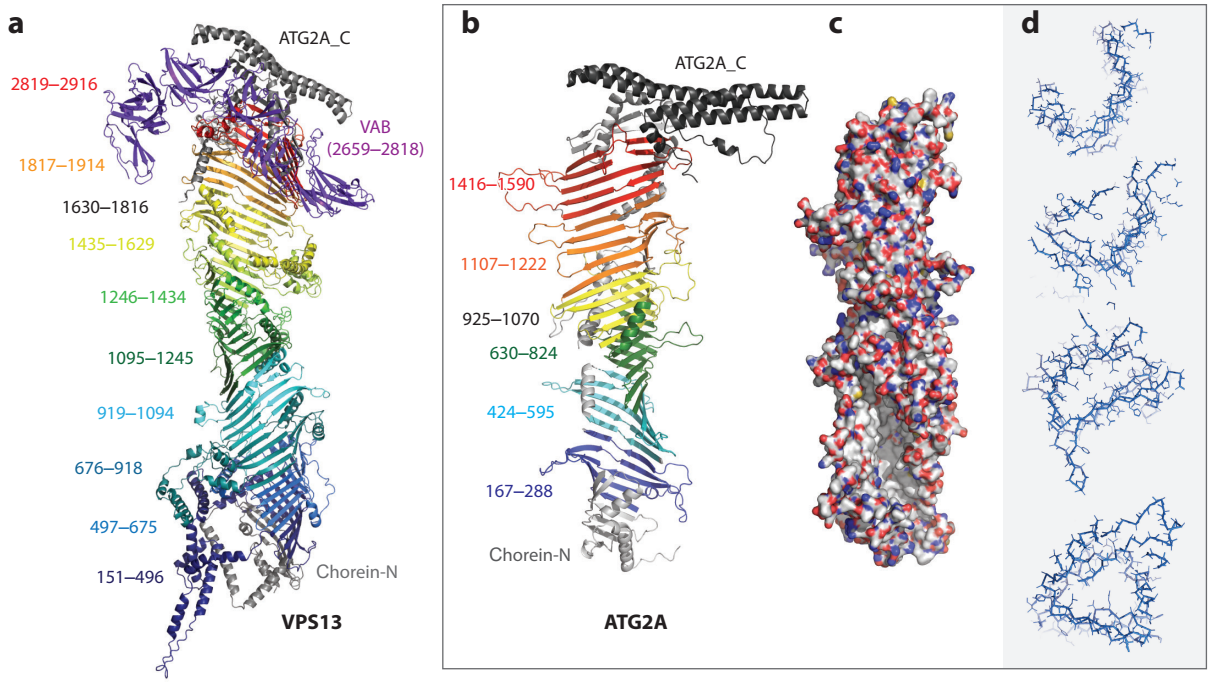

Figure 2

Architecture of bridge-like lipid transfer proteins (BLTPs). (*a*) Ribbon diagram of VPS13, a BLTP from the fungus *Chaetomium thermophilum*, as generated by RoseTTAFold (146). BLTPs feature a taco shell–shaped domain whose cleft is hydrophobic and that can solubilize lipid fatty acyl moieties. In the case of VPS13, the taco shell consists of a series of 10 similar motifs, each with 5 β-strands (49); the 10 motifs are indicated with different colors. VPS13 has additional domains at each end, allowing the ends to interact with donor and acceptor membranes or adaptor proteins. At its C terminus, these include a so-called VAB domain inserted between the ninth and tenth repeat motifs (*purple*); a multi-helix stretch referred to as ATG2A_C (*dark gray*), which may be unstructured in solution; and a pleckstrin homology–like domain (not visible). The N terminus preceding the first β-strand motif, referred to as the chorein-N motif, consists of ∼150 residues and is well-conserved in many BLTPs, including VPS13s and ATG2s. Several of the metazoan VPS13s feature FFAT (two phenylalanines in an acidic tract) peptide motifs in loops at the N-terminal end that allow for interaction with the endoplasmic reticulum protein VAP. (*b*) Ribbon diagram of ATG2A generated by AlphaFold (40) but with the removal of loops that connect the fifth β-strand of one repeat motif and the first β-strand of the next, to allow a better view of the taco shell cavity. ATG2A has six β-strand motifs, each colored differently. The ATG2A_C segment is indicated in dark gray. (*c*) This same structure (without ATG2A_C) is shown in a surface representation, with carbons at the surface shown in white, oxygens in red, and nitrogens in blue. The cavity is hydrophobic (*white*). (*d*) Cross sections of the ATG2A taco shell domain based on cryo–electron microscopy maps (38) (EMDataResource identifier: EMD-37087), showing that the shape and dimensions of the cavity vary along the length of ATG2A as in other BLTPs (see the section titled Lipid Transport via Bridge Proteins). At their narrowest point, they accommodate lipids in single file, suggesting unidirectional transport.

at contact sites: Lipids could move through this bridge with their acyl moieties in the hydrophobic groove of the taco and their hydrophilic headgroups accessible to solvent (33). The width of the hydrophobic groove varies along the length of the taco shell in the 160-kDa fragment and narrows at its most C-terminal end, in approximately the middle of the intact VPS13, where it is just wide enough to accommodate lipids in single file. The identification of the lipid transport properties of VPS13 suggested a similar role for ATG2, a large protein (∼215 kDa) implicated in autophagosome biogenesis, as a region of sequence similarity between the two proteins had been noted (32, 34). Accordingly, an X-ray structure of the very N terminus (∼25 kDa) of ATG2 revealed the same fold as in the VPS13 N-terminal fragment, and several studies showed that ATG2,

like VPS13, has the ability to transfer lipids in vitro (35–37). Moreover, a low-resolution cryo-EM reconstruction indicated that ATG2 is also rod-like, with a groove along its length (36). A subsequent atomic-resolution cryo-EM reconstruction of full-length ATG2 (nominally 3.2 Å) showed a taco-like domain formed from an extended and slightly twisted β-sheet, as in VPS13 (38). The width of the hydrophobic groove varies along ATG2's length, with a constriction near the middle, where lipids can be accommodated only in single file (**Figure 2**). Most recently, the cryo-EM structure of a complex containing another VPS13-like protein, BLTP1 from *Caenorhabditis elegans*, whose ~185-kDa N-terminal half is resolved at atomic resolution (39), showed a similar groove, again with varying diameter. Indeed, at a nominal resolution of 2.7 Å, the BLTP1 study showed the density corresponding to bound lipids, with a single lipid in the narrowest part of the groove and up to five lipids where the groove broadens. Given the hypothesis that these proteins function as bridges, the constrictions in all three proteins suggest that lipid flow is unidirectional (as efficient two-way flow would require accommodation of at least two lipids, traveling in opposite directions, where the groove narrows).

Bioinformatics, including the use of artificial intelligence–based structure-prediction programs (40), identified a number of other proteins with the same taco-like domain as VPS13 and ATG2 (41–46). Referred to as bridge-like lipid-transfer proteins (BLTPs), they include the aforementioned BLTP1 (KIAA1109 in humans, Csf1 in yeast, Tweek in *Drosophila*, LPD3 in *C. elegans*), BLTP2 (KIAA0100 in humans, FMP27/Hob1 and Ypr1170/Hob2 in yeast, Hobbit in *Drosophila*), and BLTP3A/B (UHRF1BP and UHRF1BP1L/SHIP164 in humans), as well as more distantly related proteins in the AsmA family that are thought to function in glycerophospholipid transfer between the inner and outer membranes of Gram-negative bacteria (41, 47, 48). In these structures (called RBG motifs for repeating β-groove motifs), the taco shell's β-sheet contains a repeating motif consisting of five antiparallel strands in eukaryotic proteins (the number is more variable in the AsmA proteins), with tandem motifs arranged so that the last β-strand of one module and the first β-strand of the next module are parallel (44, 49).

Various lines of evidence support the role of these proteins as bridges for bulk intracellular lipid transport (**Figure 3**). First, and most importantly, the founding members of this protein family, VPS13 and ATG2, were identified in unbiased genetic screens for proteins required for the ex novo rapid formation of membranes—the prospore membrane in yeast and the isolation membrane of autophagosomes, respectively (50, 51). Both membranes, and in fact all new organellar membranes, were previously thought to arise from the fusion of hundreds of vesicles whose source was largely controversial. The discovery of BLTPs was paradigm shifting in raising the possibility that an organelle could instead grow largely via BLTP-mediated lipid transport, and BLTPs were proposed to play a role in bulk lipid transfer for membrane expansion or maintenance (32, 35, 36). Second, BLTP family members are long enough to span the typical distances observed between membranes at contact sites (10–30 nm). Third, for many of them, localization at specific membrane contact sites has been documented and motifs that mediate direct or indirect interactions with the participating membranes have been identified (reviewed in 52). Fourth, studies of VPS13 and ATG2 have shown that these proteins are not functional when swathes of hydrophobic residues in the taco groove are replaced by hydrophilic residues to interrupt lipid flow (33, 36, 53), consistent with these proteins functioning as channels rather than shuttles. Fifth, most convincingly, in situ cryo–electron tomography of overexpressed VPS13C showed bridging structures corresponding to VPS13 between organelle contact sites (54), and tomographic studies of autophagosome biogenesis showed rod-like structures between the ER and the forming autophagosome that were postulated to correspond to ATG2 (55).

Analogously, a comprehensive, in situ cryo–electron tomography study suggested that the ERMES complex may transport lipids by a bridge-like mechanism between the ER and

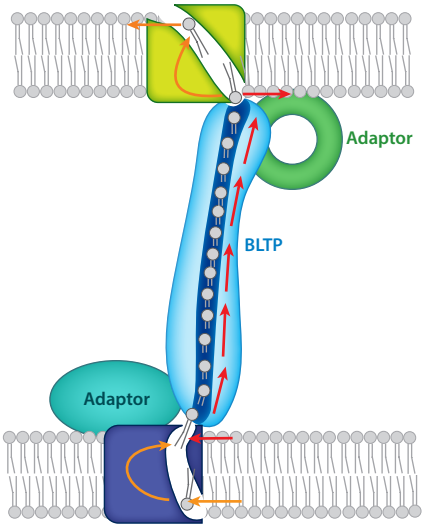

Figure 3

Bridge-like lipid-transfer proteins (BLTPs) are components of large complexes that include integral membrane proteins. BLTPs (*light blue*) span membrane contact sites, allowing lipids to move through their hydrophobic cavity to cross between membranes. BLTPs are thought to interact with integral membrane proteins (*purple* and *yellow-green*) as well as additional adaptors (*green* and *turquoise*) at both ends. Integral membrane components likely lower the energy barrier for lipid transfer between the BLTP and membrane. Several of the integral membrane protein partners have a scrambling activity that is key for directional bulk lipid transfer. This is because BLTPs transfer lipids only between the cytosolic leaflets of apposed membranes, necessitating lipid equilibration between bilayer leaflets to restore bilayer symmetry in donor and/or acceptor membranes.

mitochondria (56), although ERMES proteins are not bona fide members of the BLTP family in terms of their fold. Rather, SMP (synaptotagmin-like mitochondrial-lipid-binding protein) domains of ERMES subunits are assembled in a linear fashion to form a kinked, rod-like structure (56). Based on structures of the SMP domain subcomplexes (57) and AlphaFold predictions for the rod-like assembly, ERMES proteins also feature a continuous hydrophobic groove that would allow lipids to travel between membranes (56), and it seems likely that the basic molecular basis underlying lipid transport by BLTPs and ERMES is similar. In support of this proposal, the Gem1 GTPase functions together with ERMES in yeast, and its Miro GTPase homolog in metazoans, which lack ERMES, works with VPS13D (58, 59).

INTRAMEMBRANE LIPID DYNAMICS IN BRIDGE-LIKE LIPID-TRANSFER PROTEIN–MEDIATED TRANSPORT BETWEEN MEMBRANES

The BLTP taco shell is a soluble domain that bridges the cytosolic leaflets of apposed organellar membranes and thus should transfer lipids only between cytosolic leaflets. This poses a conundrum if BLTPs function in membrane bilayer expansion, unless there exist mechanisms to equilibrate lipids between bilayer leaflets, as lipids are extracted or added to just one leaflet of the donor or acceptor membrane, respectively. Indeed, the two best studied BLTPs, VPS13 and ATG2, are now known to work in conjunction and to interact physically with integral membrane proteins that have lipid scrambling activity. The C-terminal end of ATG2 interacts directly with ATG9 (60, 61) in Golgi-derived vesicles that seed the autophagosome (62, 63), and ATG2's N-terminal end

can interact directly with the ER proteins TMEM41B and VMP1 (60). ATG9, TMEM41B, and VMP1 have all been shown to have scrambling activity in vitro (60, 64–66). Integral membrane proteins with scramblase activity have also been identified as interacting with the C-terminal ends of yeast Vps13 (Mcp1) and mammalian VPS13A (XK, ATG9) (67–71).

Scramblases are thought to transfer lipids between membrane leaflets via a credit-card mechanism (72). They feature hydrophilic grooves through which polar lipid headgroups are believed to transfer across the hydrophobic membrane to reach the opposite membrane leaflet, akin to a credit card sliding through a reader. Low-resolution studies of the ATG2–ATG9 complex (38, 61) indicate that the hydrophobic groove of the ATG2 taco domain is aligned with the hydrophilic groove of the ATG9 scramblase, so that lipids can be handed off from ATG2 to ATG9 to either insert into the cytosolic-facing leaflet of the acceptor membrane or move across it to the lumenal leaflet.

It has long been appreciated that scramblases are required at the ER, as lipids are synthesized asymmetrically on its cytosolic leaflet and must be equilibrated between both leaflets to permit ER expansion (73). The concept that scramblases are also required for the growth of other membranes that expand via BLTP-mediated lipid transfer to their cytosolic leaflets is only now emerging (60, 64, 65, 74) and, with it, the discovery of new classes of proteins that harbor lipid scrambling activity. These include a subset of proteins that have other previously well-established functions. For example, the voltage-dependent anion channel can move lipids along its dimer interface to drive lipid scrambling in the mitochondria (75). Additionally, a number of protein insertase complexes that are known to function in organelle biogenesis by delivering integral membrane proteins to nascent organelles or translocating peptides into their lumen have recently been reported to have scrambling activity as assessed in vitro or in silico (74). For some of these insertases, their deletion or dysfunction in cells leads to defects in lipid dynamics. For example, before its identification as an insertase (76), the yeast ER membrane protein complex was reported to function together with ERMES in PS transport between the ER and mitochondria (77); an insertase resident in the thylakoid sack of the plant chloroplast is also required for the growth of this organelle's membrane (Abl3) (78). It is still unclear whether all BLTPs function in membrane expansion (see the section titled Physiological Roles for Bridge-Like Lipid-Transfer Proteins) and, when they do, whether they all interact with scramblases physically at one or both ends.

LIPID BRIDGES AS COMPONENTS OF LIPID TRANSFER MACHINES

It is becoming clear that LTPs that function by a bridge-like mechanism do not work in isolation: They work as part of a transport machine that also includes scramblases or other membrane-embedded proteins, as well as adaptor proteins. In Gram-negative bacteria, lipopolysaccharide transfer from the inner membrane to the outer membrane surface has been well-studied and may serve as a model system to understand bridge-like mediated lipid transport (79, 80), although this transport system does not involve bona fide BLTP family members as discussed in the section titled Lipid Transport via Protein Bridges. Lipopolysaccharide transfer is carried out by the lipopolysaccharide transporter (Lpt), an elaborate protein machine consisting of six distinct components. Lpt consists of a β-sheet lipid transport module (LptA) surmised to multimerize into bridge-like structures, reminiscent of the BLTPs or ERMES; this bridge interacts with integral membrane protein complexes in both the inner and outer membrane. The integral membrane subcomplex in the inner membrane (LptF, LptG, LptC) is an ABC transporter, which uses ATP hydrolysis to fuel lipopolysaccharide desorption from the bilayer, transfer into the LptA bridge, and movement of the lipid along the bridge. Lipids are thought to first pass along the LptA bridge much as they do along BLTPs, with the hydrophobic lipid moieties in a hydrophobic groove and the hydrophilic

headgroup accessible to cytosol. Finally, the lipid is handed off to the integral membrane complex in the outer membrane (LptD, LptE); this complex is thought to facilitate lipid insertion into the periplasmic leaflet of the outer membrane and lipid transfer across the membrane bilayer to the surface.

We expect some general aspects of bridge-like lipid transport to be similar. As noted in the section titled Intramembrane Lipid Dynamics in Bridge-Like Lipid-Transfer Protein–Mediated Transport Between Membranes, at least some BLTPs (ATG2, VPS13) interact with a group of integral membrane proteins that have scrambling activity. Moreover, two BLTPs, BLTP1 and BLTP2, have an N-terminal transmembrane region, and as recently reported (39), in BLTP1, this helix forms a five-helix transmembrane domain with two other ER proteins at the entry to the lipid transfer channel. This transmembrane complex forms a conduit for lipids to pass out of the membrane and into BLTP1's lipid transfer groove, and it might have scrambling activity. We note, however, that while all LTPs that function as bridges in membrane expansion require the function of scramblases, they may not directly interact with scramblases, as lipid export/import and lipid scrambling may be spatially separated within a membrane. On the other hand, we expect that all BLTPs interact with integral membrane proteins in both the donor and the acceptor bilayers and that these proteins function in intramembrane lipid dynamics, facilitating the transfer of lipids between the membrane and the BLTP to expedite the transfer rate. Such integral membrane components could include lipid biosynthetic machinery, which has been reported to accumulate at membrane contact sites (81). Notably, no ATPases analogous to LptF, LptG, or LptC in the LPS transporter have so far been identified in the BLTP interactome, consistent with use of a distinct energy source for directional glycerophospholipid transfer in eukaryotes.

An unanswered question concerns whether the BLTPs are specific for the transport of a particular lipid. Arguably, if these proteins function in bulk transport for membrane expansion, they should transport glycerophospholipids nonspecifically. Supporting this hypothesis, overexpressed VPS13, ATG2, and BLTP3 copurify with all glycerophospholipids according to their prevalence in cells (32, 36, 82). However, it is still possible that at endogenous levels in vivo, when the BLTPs associate with integral membrane protein partners in the donor membrane, the latter select the lipids that enter the BLTP taco domain for transport. Identification of the integral membrane interactomes of the various BLTPs and their functions should help in evaluating this possibility.

The energy source for directional lipid transport remains the major and most intriguing unresolved question regarding BLTP-based lipid transport. Noting that many BLTPs function at contact sites with the ER, where new glycerophospholipids are synthesized solely in its cytosolic leaflet, we favor a model in which asymmetric lipid biosynthesis could drive lipid flow. Synthesis of new lipids would significantly increase the lateral pressure in the ER's cytosolic leaflet (at least in the absence of local scrambling activity), leading to cessation of synthesis until this pressure is relieved. Pressure could be relieved by lipid redistribution between the ER cytosolic and lumenal leaflets, leading to ER membrane growth. Alternatively, transport of lipids to the cytosolic leaflet of an acceptor organelle could relieve lateral pressure, with scrambling occurring there. Another hypothesis is that changes in membrane tension and subsequent organellar lipid transport are driven by lipid osmosis, in which lipids migrate from one organelle to another to equilibrate the concentration of lipids in relation to that of integral or membrane associated proteins (83). A full understanding of the biochemical and biophysical bases of BLTP-mediated lipid trafficking requires detailed analyses regarding the composition and organization of membrane domains at contact sites and more information on the proteins that interact with BLTPs and/or assemble at specific contact sites.

In cells, BLTP-mediated transfer is estimated to occur at rates of tens to hundreds of lipids per second per BLTP complex (84, 85). Rates for BLTP-mediated trafficking have not yet been

studied in a physiologically relevant manner in vitro. This is because in vitro studies so far have been carried out in the absence of integral membrane proteins and other interaction partners that stably anchor the BLTP between membranes, so that the BLTPs in these assays most likely function as shuttles rather than physiological bridges. In addition, the liposome compositions used in vitro to date have not been physiological. Moreover, the driving forces for directional transfer are unknown and so are lacking in these studies, even as they should be intimately linked with lipid transfer rates. The hypothesis that lateral membrane pressure or lipid osmosis can drive directionality is important, albeit challenging, to test in more physiologically relevant reconstituted systems.

PHYSIOLOGICAL ROLES FOR BRIDGE-LIKE LIPID-TRANSFER PROTEINS

While there is strong evidence that a major function of BLTPs is to deliver lipids in bulk, details related to the physiological functions of such transport are available only in a few cases. Elucidating the roles of these proteins is complicated by several factors. First, they may act at more than one type of membrane contact site; second, their site(s) of action may depend upon the functional state of the cell, and their tethering and lipid transport functions may be transient; and third, some BLTPs appear to have overlapping functions. Information gathered from genetic screens in model organisms, human diseases, and cell biological studies have suggested a variety of functions that generally, but not always, involve traffic from the ER to other membranes.

Autophagosome Biogenesis

Bulk phospholipid transport allows a membrane to expand, independent of protein addition. The physiological roles of yeast VPS13 and ATG2 are to mediate bulk phospholipid transport, contributing lipid to the sporulation membrane and the autophagosome isolation membrane, respectively (33, 36, 50, 51). As autophagy is such an intensely studied process, autophagosome formation is currently the best understood example, not only of BLTP-mediated organelle biogenesis but also of BLTP-mediated activity in general. Because many concepts discussed in the section titled Intramembrane Lipid Dynamics in Bridge-Like Lipid-Transfer Protein–Mediated Transport Between Membranes are based on mechanistic insights gained from the study of autophagosome growth, a detailed exploration of this process is merited before discussing other emerging examples. Several recent reviews provide additional detail (86–88).

Generation of autophagosomes starts with the expansion of seed membranes to generate cup-like structures called phagophores (**Figure 4**). As the phagophore expands, its lumen remains extremely small (55) and the surface density of transmembrane proteins decreases, such that mature autophagosomes have very few detectable integral membrane proteins (89, 90). A dearth of integral membrane proteins and minimal volume expansion each suggested a biogenesis pathway distinct from the paradigm of vesicle-mediated membrane delivery through membrane–membrane fusion. Furthermore, geometric considerations about how the surface area and volume change during phagophore growth imply that at least 60–80% of the autophagosome membrane must derive from nonvesicular lipid transport (55). The discovery that the autophagy protein Atg2/ATG2 (yeast/mammalian) is a BLTP (32, 35–37) suggested a new model for autophagosome biogenesis, whereby protein-mediated bulk lipid transfer might drive membrane expansion (**Figure 4**). In this model, a small seed membrane is recruited to an ER contact site bridged by ATG2 proteins. Lipid flow from the ER into the seed drives membrane expansion, and a seed-localized scramblase—the integral membrane protein Atg9/ATG9—ensures that these incoming lipids populate both leaflets. Both human and yeast ATG9 proteins can scramble lipids in vitro

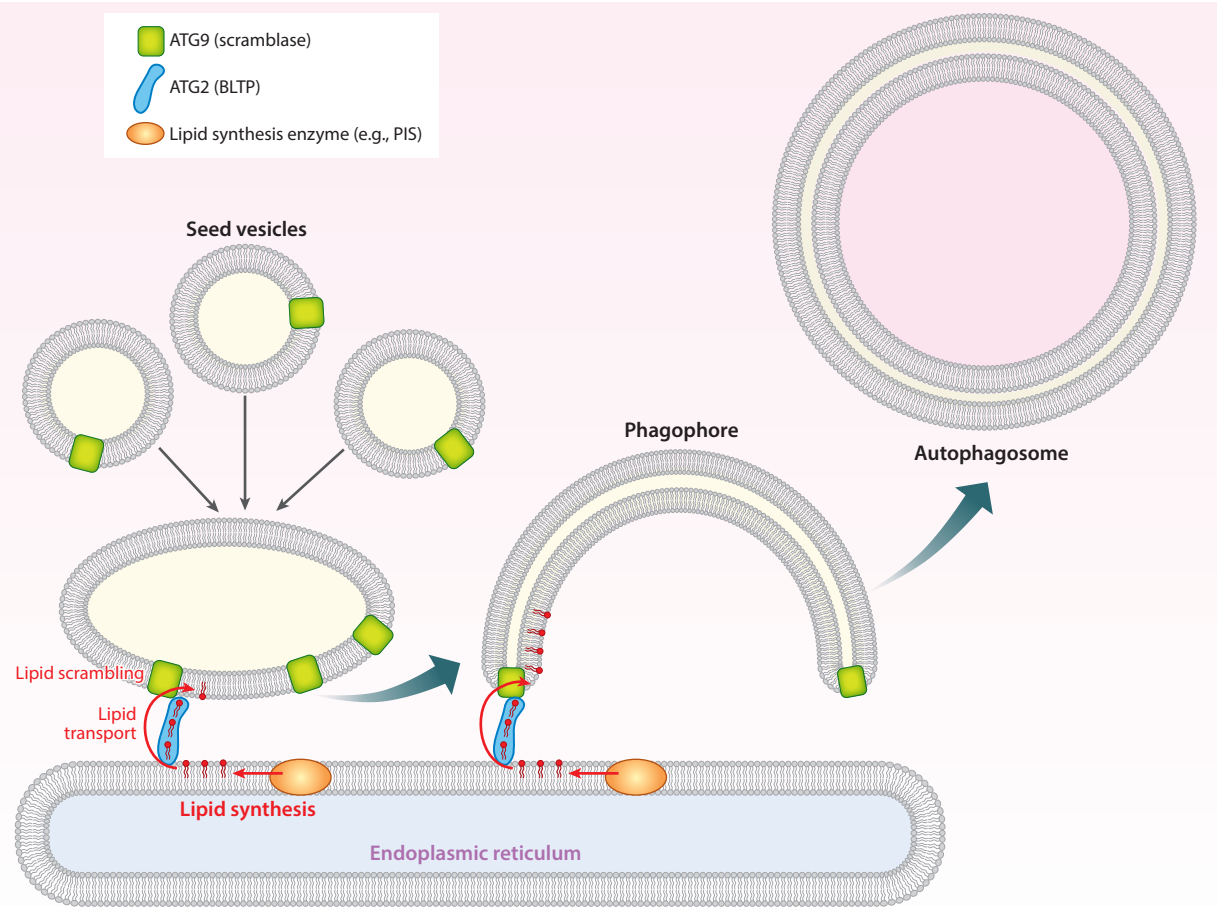

Figure 4

General model for membrane expansion during autophagosome biogenesis. Autophagosome biogenesis initiates with the coalescence of small vesicles, at least some of which harbor ATG9 lipid scramblases. The ATG2 BLTP is recruited to and stabilizes contact sites between these seeds and the ER. The seeds then expand through an ATG2-mediated lipid transport process in which lipid deposited into the developing phagophore populates both leaflets via ATG9-mediated lipid scrambling. Flux of lipid into the developing autophagosome is thought to be coordinated with lipid synthesis on the ER. A plausible, yet untested model is that as newly synthesized lipids on the cytoplasmic leaflet of the ER accumulate, the difference in membrane tension between the ER and the ATG9 seeds triggers transport of phospholipid to equilibrate this tension. Abbreviations: BLTP, bridge-like lipid-transfer protein; ER, endoplasmic reticulum; PIS, phosphatidylinositol synthase.

(60, 64, 65), and each physically engages the C terminus of ATG2 proteins (60, 91), forming a potentially unbroken hydrophobic conduit from the BLTP directly into the scramblase interior (38, 61). Consistent with this model, mutations in Atg9 that disrupt the hydrophilic channel that supports lipid scrambling block autophagosome biogenesis and prevent incorporation of newly synthesized lipids into the lumenal leaflet of nascent phagophores (92).

It is unclear whether ER-localized scrambling activity is also required for lipid transfer. As noted in the section titled Intramembrane Lipid Dynamics in Bridge-Like Lipid-Transfer Protein–Mediated Transport Between Membranes, the ER integral membrane proteins TMEM41B and VMP1, which bind the ATG2 N terminus, have scrambling activity (60, 66, 93). Their genetic depletion largely phenocopies autophagy defects in ATG2 knockouts,

suggesting that they may work together with ATG2 during autophagosome biogenesis (94–97). Direct evidence for these scramblases engaging ATG2 during autophagy is still missing, but intriguingly, TMEM41B localizes with ATG2 at mitochondrial–ER contact sites during PS transfer into the mitochondria (98) and localizes with ATG9 during lipid droplet catabolism (99). The latter depends upon ATG2, strongly implying the presence of a scramblase–transporter–scramblase complex. Whether the scrambling activity itself is needed to support any of these functions, or whether instead TMEM41B and VMP1 locally disturb membrane integrity to simply facilitate lipid handoff to and from ATG2, remains to be established.

In the current model, highly motile, ATG9-positive vesicles derived from Golgi and endosomal sources transit to sites where autophagosomes form. Their docking and the consequent formation of a stable contact site can be inferred from a loss of mobility, and this process depends in part on the presence of ATG2 (100). As is true for other BLTPs, ATG2 proteins are recruited on demand, relocating from the cytosol or from other organelles, including lipid droplets, to support autophagosome formation (101, 102). Although autophagosome biogenesis may initiate at multiple organelles, the best described mechanism involves Atg2/ATG2 sitting between the ER and the developing phagophore (36, 91, 103, 104) in a topologically restricted orientation where its C terminus binds Atg9/ATG9 proteins on the phagophore (60, 91) and its N terminus engages ER factors (60, 105). In vitro, ATG2 proteins directly bind membranes through motifs at either end of the protein (105, 106), but in cells, ATG2 recruitment to the ATG9-containing phagophore depends upon the recruitment of two families of lipid-associated peripheral proteins: WIPI4, which binds phosphatidylinositol-3-phosphate, and GABARAP, which becomes covalently attached to PE (reviewed in 86). WIPI4 and GABARAP bind loops on ATG2, and at least one of the proteins is needed for ATG2 localization (105–108). Mutations in WIPI4's ATG2-binding site are associated with neurodegeneration (109). In addition, ATG2 engages ATG9 on the phagophore and eventually assembles into a large phagophore initiation complex with FIP200 and several more autophagy proteins (110), as well as plausibly TMEM41B and VMP1 on the ER side.

In yeast, where the microscopy has been easiest to interpret, Atg2 proteins engage highly curved membranes on both sides of the contact site, bridging the phagophore rim to tubular ER (55). Autophagosome formation is further promoted by the membrane curvature-inducing motifs of the reticulon-like protein Rop1, which colocalizes with Atg2 (111). The poor lipid packing in highly curved membranes may alter the energetic penalties associated with both lipid extraction and lipid deposition. At these sites, 30–60 Atg2 proteins collaborate, each moving ~200 lipids/s to support autophagosome formation over a few minutes (85). Atg2 copy number directly impacts the size and eventual constriction of the phagophore rim, implying that lipid transport is connected to the complex geometry of the cup-like membrane (112). The initial phagophore membrane is thought to be formed from 1–3 Atg9 vesicles merged via a poorly described fusion event (113). Phagophore-integrated Atg9 also accumulates at the rim (103), and the total Atg9 copy number is similar to that of Atg2, suggesting a stochiometric assembly of Atg2–Atg9 complexes. Vps13 proteins can further augment the membrane expansion rate by also assembling between the ER and the phagophore (85), though they are not sufficient to drive autophagosome biogenesis.

Although it is not yet clear how directional lipid flux toward the phagophore is promoted, several lines of evidence implicate a role for local ER lipid synthesis. Lipid synthesis machinery is concentrated at phagophore–ER contact sites in both mammalian cells and yeast. In mammals, ER-resident PI synthase colocalizes with autophagosome biogenesis markers, and local degradation of PI by an exogenous, ER-targeted PI lipase blocks autophagosome formation (114). Cholinephosphotransferase 1 (CPT1), phospholipase D1 (PLD1), and phosphatidylserine synthase 1 (PSS1) can also be found at these sites when autophagosome biogenesis is blocked

(114). Recruitment of PLD1 to phagophores and production of PA are each correlated with increased autophagosome biogenesis (115). In yeast, the phagophore recruits the soluble acyl CoA synthetase Faa1, to drive local fatty acid production (116) through a motif that recognizes anionic lipids (117). Moreover, metabolic labeling experiments revealed that when yeast undergo nutrient deprivation, the vast majority of newly synthesized PC is routed directly into the growing autophagosome (92). Collectively, these findings suggest a model where newly made fatty acids produced on the yeast phagophore might diffuse or otherwise be transported to the ER, where they would immediately be incorporated into newly synthesized glycerophospholipids and then transported back to drive phagophore growth (87).

There may also be a parallel, lipid osmosis–dependent driving force (83) that causes lipids to flow from regions of low protein density to regions of higher protein density in the forming autophagosome. Atg9 seeds begin at an extremely high protein-to-lipid ratio, with Atg9 proteins packed into small, ~60-nm vesicles. Atg2-mediated lipid transport from the ER into these vesicles might be essential to relax local protein packing enough to support other forms of local biochemistry on the membrane surface (62). As the phagophore expands, the density of ATG9 proteins rapidly decreases (63), but total surface protein might remain high enough to maintain a lipid osmotic gradient as new proteins are constantly added, either through peripheral membrane interactions or through covalent linkages to phagophore-associated lipids (83).

More Examples of Organelle Formation Mediated by Bridge-Like Lipid-Transfer Proteins

Lipid delivery by BLTPs also likely underlies the role of VPS13 family proteins in the growth of mitochondria and peroxisomes that do not communicate with the ER by membrane traffic and which also acquire their proteins by vesicle-independent mechanisms (32, 58, 118, 119). Recently reported lipid-scrambling activity of proteins of the outer mitochondrial membrane (68, 74, 75) could provide an efficient way to coordinate protein and lipid addition to this membrane. VPS13B is required for the formation of the acrosome (120), a double membrane structure that is topologically similar to the autophagosome. The acrosome forms proximal to the Golgi complex and eventually surrounds the head of spermatids. Another attractive role for BLTPs could be in the massive expansion of the PM that accompanies the development of specialized cell types.

Controlling Lipid-to-Protein Ratio in Membranes of the Secretory and Endocytic Pathways

Lipid transport by BLTPs may provide a mechanism to modify the protein-to-lipid ratio along the secretory and endocytic pathways, as the membranes of some organelles, like the large secretory granules of exocrine cells, have a much lower abundance of intramembranous particles than the ER or the PM (121). In yeast, VPS13 was reported to provide bilayer lipids to late endosomes to facilitate the formation of the intralumenal vesicles of multivesicular bodies (122). Such a mechanism would allow for the bilayer expansion needed to generate intralumenal vesicles independent of the protein cargo that is loaded into these vesicles. It remains to be seen whether a similar function applies to mammalian VPS13C, which is localized to contacts between the ER and late endosomes and lysosomes (32). BLTP3B and BLTP2 are two other proteins reported to function in the endocytic pathway (82, 123). VPS13B is localized on the Golgi, and, in its absence, Golgi cisternae reformation after their brefeldin A–induced dispersion is delayed, raising the possibility that BLTP-mediated flow of lipids between Golgi cisternae may play a role in membrane traffic progression within the Golgi complex in cooperation with vesicular transport (124).

Phagocytosis

Internalization of pathogens and cell debris starts with the formation of an endocytic cup that surrounds the particle to be internalized. It has long been known that cup formation correlates with the emergence of tight appositions between the ER and the portions of the PM that develop into the cup. Most interestingly, BLTP1 was a major hit in two genetic screens for factors critically implicated in early steps of phagocytosis—during the internalization of *Legionella* by mammalian cells and the clearance of neuronal debris by astrocytes in *C. elegans* (125, 126). C1orf43/Spigot, a binding partner of BLTP1 at the ER membrane, is also required for these processes (39). An interesting possibility is that lipid delivery by BLTP1 at ER–PM contact sites at the phagocytic cup helps to either expand the cup or modify its lipid composition.

Bulk Delivery of Specific, Newly Synthesized Lipids by Bridge-Like Lipid-Transfer Proteins

Neither lipidomics nor in vitro lipid transport assays have revealed significant lipid specificity for the various BLTPs studied to date (32, 36, 82). Nevertheless, many BLTPs have been associated with processes involving the bulk delivery of particular lipids. For example, Fmp27, one of the two yeast BLTP2 orthologs, was reported to increase the concentration of PE in the PM via direct transport from the ER (127), and the BLTP1 yeast ortholog, Csf1, was proposed to support the delivery of mitochondrially derived PE to the ER for glycosylphosphatidylinositol synthesis (43). Implicit in these studies is a connection, either physical or via proximity, between BLTPs and the lipid synthesis machinery.

Membrane Resilience and Repair

Recent studies have demonstrated that perturbations to lysosomal membranes are rapidly followed by the recruitment of factors that prevent their rupture or reseal them, as leakage of lysosome material would be highly damaging for cells. Among such responses is the expansion of ER–lysosome membrane contact sites, mediated by shuttle-type LTPs in the OSBP/ORP family and by BLTPs (ATG2 and VPS13C) (53, 128). OSBP/ORPs bind PI4P on lysosomes via a PH domain, and their recruitment is triggered by the acute formation of PI4P on these organelles that accompanies lysosomal damage (53). How ATG2 and VPS13C are acutely recruited to lysosomes by membrane damage remains to be determined, although in the case of ATG2, CASM-dependent accumulation of lipid-bound LC3A in the lysosome membrane plays a role (129, 130). ATG2 and VPS13C BLTPs could cooperate in the bulk delivery of lipids required to prevent membrane damage or mediate repair; at the same time, OSBP/ORPs can ensure enrichment of cholesterol and PS in the lysosome membrane via their ability to mediate PI4P–cholesterol and PI4P–PS counter-transport. A role for VPS13C in the maintenance of lysosome health is of special interest, as lysosome dysfunction has been implicated in several neurodegenerative diseases including Parkinson's disease, and VPS13C loss of function is associated with this condition (131). BLTPs may also play a role in PM membrane repair, as a pool of ATG9 present at the PM was shown to protect against its damage (132). Given the well-established partnership between ATG9 and ATG2 and the more recently reported interaction between VPS13A and ATG9 (71), such a protective role may be mediated by bulk lipid transport from the ER to the PM.

Homeoviscous Adaptation

Homeoviscous adaptation is the process by which cells that can live at different temperatures rapidly adapt their lipidome in response to temperature changes. As growth temperatures decrease,

cells respond by increasing the abundance of desaturated fatty acid lipid chains to normalize membrane fluidity. Yeast Cfs1 and its *C. elegans* ortholog, BLTP1, localize at ER–PM contacts and are required for this rapid adaptation, which also involves synthesis of new phospholipids with unsaturated fatty acids in the ER (46, 126, 133, 134). BLTP1/CFS1 may provide a pathway for the rapid distribution of these newly synthesized lipids to the PM, while preexisting lipids may be progressively diluted out by endocytic removal. Fmp27/BLTP2 (see the section titled Bulk Delivery of Specific, Newly Synthesized Lipids by Bridge-Like Lipid-Transfer Proteins) was also implicated in maintaining physiological membrane fluidity at low temperatures by delivering PE to the PM from the ER (127).

Surface Exposure of Phosphatidylserine

The bilayer of the PM is highly asymmetric, with acidic phospholipids such as PS concentrated primarily within its cytosolic leaflet. Surface exposure of PS functions as an eat-me signal from dying cells that triggers their recognition by phagocytic cells, leading to their removal. Interestingly, in T cells, VPS13A and the lipid scramblase XK are binding partners at the PM (69, 135) and are required to trigger PS exposure during necrosis induced by activation of the purinergic receptor P2X7 (136). Similar diseases result from loss-of-function mutations in VPS13A and XK (67)—chorea-acanthocytosis and McLeod syndrome, respectively—suggesting a functional partnership between delivery of PS to the PM by VPS13A and its scrambling by XK (67, 68, 70). How this process is regulated is an important question for further study.

Lipid Droplet Biology

Lipid droplets form contact sites with many other organelles, including the ER and the mitochondria, where fatty acid oxidation occurs following lipolysis-derived breakdown of TAG and other neutral lipid droplets. These contacts are replete with lipid transport machinery, including both shuttles (20, 137–140) and BLTPs (VPS13A/C, ATG2) (32, 101, 141). It has been unclear why LTP-mediated lipid trafficking to and from lipid droplets might be necessary, as lipid droplets originate from and spend much of their lifetime connected to the ER via a membranous stalk: The phospholipid monolayer encapsulating the neutral lipid core of the lipid droplet is contiguous with the cytoplasmic leaflet of the ER bilayer until a very late stage in lipid droplet maturation (142, 143). Nevertheless, recent studies indicate that shuttle-mediated lipid trafficking, at least, takes place at these sites (20, 137, 138, 144). Genetic depletion of BLTPs can lead to either a reduction (145) or an accumulation (101) of lipid droplet mass and number, suggesting that BLTPs move lipids or lipolytic products in bulk between stores in lipid droplets and other organelles. The changes in lipid droplet abundance and size upon BLTP depletion could also reflect broader changes in cellular lipid homeostasis, rather than direct protein-mediated bulk lipid trafficking to/from lipid droplets. For a recent review on this topic, see Mathiowetz & Olzmann (142).

OUTLOOK

As the structure and function of individual LTPs come into focus, we can begin considering these players in a larger physiological context. Uncovering which types of proteins collaborate with LTPs may address questions about how lipid flux is controlled and how lipid movement is carried out efficiently by large lipid transport machines. A comprehensive determination of when and where LTPs function will inevitably lead to further questions about how local lipid compositions within cells are established and modified in response to physiological changes or specific stresses. Rapidly advancing work in this field will help determine the coordination of the bulk lipid flow

mediated by BLTPs and the more specific lipid transport achieved by shuttle-like LTPs, as well as the interplay between protein-mediated lipid transfer and classic vesicle-mediated lipid delivery.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

K.M.R. thanks Stefano Vanni and Tim Levine for many discussions. Research by the authors described in this review was funded by grants from the National Institutes of Health (R35-GM131715 to K.M.R., R35-GM153482 to T.J.M., and R01-NS36251 and P30-DA018343 to P.D.C.), the Parkinson's Foundation (CPF-RCE-1946 to P.D.C.), and Aligning Science Across Parkinson's through the Michael J. Fox Foundation for Parkinson's Research (ASAP-000580 to P.D.C. and K.M.R.).

LITERATURE CITED

1. Holthuis JC, Menon AK. 2014. Lipid landscapes and pipelines in membrane homeostasis. *Nature* 510:48–57
2. Chauhan N, Farine L, Pandey K, Menon AK, Butikofer P. 2016. Lipid topogenesis – 35 years on. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1861:757–66
3. Balla T, Kim YJ, Alvarez-Prats A, Pemberton J. 2019. Lipid dynamics at contact sites between the endoplasmic reticulum and other organelles. *Annu. Rev. Cell Dev. Biol.* 35:85–109
4. Voeltz GK, Sawyer EM, Hajnoczky G, Prinz WA. 2024. Making the connection: how membrane contact sites have changed our view of organelle biology. *Cell* 187:257–70
5. Dittman JS, Menon AK. 2017. Speed limits for nonvesicular intracellular sterol transport. *Trends Biochem. Sci.* 42:90–97
6. Rogers JR, Geissler PL. 2020. Breakage of hydrophobic contacts limits the rate of passive lipid exchange between membranes. *J. Phys. Chem. B* 124:5884–98
7. Rogers JR, Geissler PL. 2023. Ceramide-1-phosphate transfer protein enhances lipid transport by disrupting hydrophobic lipid-membrane contacts. *PLOS Comput. Biol.* 19:e1010992
8. Rogers JR, Espinoza Garcia G, Geissler PL. 2021. Membrane hydrophobicity determines the activation free energy of passive lipid transport. *Biophys. J.* 120:3718–31
9. Shadan S, Holic R, Carvou N, Ee P, Li M, et al. 2008. Dynamics of lipid transfer by phosphatidylinositol transfer proteins in cells. *Traffic* 9:1743–56
10. Sugiura T, Takahashi C, Chuma Y, Fukuda M, Yamada M, et al. 2019. Biophysical parameters of the Sec14 phospholipid exchange cycle. *Biophys. J.* 116:92–103
11. Lipp NF, Gautier R, Magdeleine M, Renard M, Albanese V, et al. 2019. An electrostatic switching mechanism to control the lipid transfer activity of Osh6p. *Nat. Commun.* 10:3926
12. Wong LH, Copic A, Levine TP. 2017. Advances on the transfer of lipids by lipid transfer proteins. *Trends Biochem. Sci.* 42:516–30
13. Sugiura T, Nakao H, Ikeda K, Khan D, Nile AH, et al. 2021. Biophysical parameters of the Sec14 phospholipid exchange cycle – effect of lipid packing in membranes. *Biochim. Biophys. Acta Biomembr.* 1863:183450
14. Loewen CJ, Roy A, Levine TP. 2003. A conserved ER targeting motif in three families of lipid binding proteins and in Opi1p binds VAP. *EMBO J.* 22:2025–35
15. Murphy SE, Levine TP. 2016. VAP, a versatile access point for the endoplasmic reticulum: review and analysis of FFAT-like motifs in the VAPome. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1861:952–61
16. Saheki Y, Bian X, Schauder CM, Sawaki Y, Surma MA, et al. 2016. Control of plasma membrane lipid homeostasis by the extended synaptotagmins. *Nat. Cell Biol.* 18:504–15

17. Kudo N, Kumagai K, Tomishige N, Yamaji T, Wakatsuki S, et al. 2008. Structural basis for specific lipid recognition by CERT responsible for nonvesicular trafficking of ceramide. *PNAS* 105:488–93
18. Schauder CM, Wu X, Saheki Y, Narayanaswamy P, Torta F, et al. 2014. Structure of a lipid-bound extended synaptotagmin indicates a role in lipid transfer. *Nature* 510:552–55
19. Kim H, Lee S, Jun Y, Lee C. 2022. Structural basis for mitoguardin-2 mediated lipid transport at ER-mitochondrial membrane contact sites. *Nat. Commun.* 13:3702
20. Hong Z, Adlakha J, Wan N, Guinn E, Giska F, et al. 2022. Mitoguardin-2–mediated lipid transfer preserves mitochondrial morphology and lipid droplet formation. *J. Cell Biol.* 221:e202207022
21. Michell RH. 1975. Inositol phospholipids and cell surface receptor function. *Biochim. Biophys. Acta Rev. Biomembr.* 415:81–147
22. Pemberton JG, Kim YJ, Balla T. 2020. Integrated regulation of the phosphatidylinositol cycle and phosphoinositide-driven lipid transport at ER-PM contact sites. *Traffic* 21:200–19
23. Lipp NF, Ikhlef S, Milanini J, Drin G. 2020. Lipid exchangers: cellular functions and mechanistic links with phosphoinositide metabolism. *Front. Cell Dev. Biol.* 8:663
24. Khelashvili G, Chauhan N, Pandey K, Eliezer D, Menon AK. 2019. Exchange of water for sterol underlies sterol egress from a StARkin domain. *eLife* 8:e53444
25. Mesmin B, Bigay J, Moser von Filseck J, Lacas-Gervais S, Drin G, Antonny B. 2013. A four-step cycle driven by PI(4)P hydrolysis directs sterol/PI(4)P exchange by the ER-Golgi tether OSBP. *Cell* 155:830–43
26. Chung J, Torta F, Masai K, Lucast L, Czapla H, et al. 2015. PI4P/phosphatidylserine countertransport at ORP5- and ORP8-mediated ER–plasma membrane contacts. *Science* 349:428–32
27. Moser von Filseck J, Copic A, Delfosse V, Vanni S, Jackson CL, et al. 2015. Phosphatidylserine transport by ORP/Osh proteins is driven by phosphatidylinositol 4-phosphate. *Science* 349:432–36
28. Ghai R, Du X, Wang H, Dong J, Ferguson C, et al. 2017. ORP5 and ORP8 bind phosphatidylinositol-4,5-biphosphate (PtdIns(4,5)P_2) and regulate its level at the plasma membrane. *Nat. Commun.* 8:757
29. Kornmann B, Currie E, Collins SR, Schuldiner M, Nunnari J, et al. 2009. An ER-mitochondria tethering complex revealed by a synthetic biology screen. *Science* 325:477–81
30. Lang AB, John Peter AT, Walter P, Kornmann B. 2015. ER–mitochondrial junctions can be bypassed by dominant mutations in the endosomal protein Vps13. *J. Cell Biol.* 210:883–90
31. De M, Oleskie AN, Ayyash M, Dutta S, Mancour L, et al. 2017. The Vps13p–Cdc31p complex is directly required for TGN late endosome transport and TGN homotypic fusion. *J. Cell Biol.* 216:425–39
32. Kumar N, Leonzino M, Hancock-Cerutti W, Horenkamp FA, Li P, et al. 2018. VPS13A and VPS13C are lipid transport proteins differentially localized at ER contact sites. *J. Cell Biol.* 217:3625–39
33. Li P, Lees JA, Lusk CP, Reinisch KM. 2020. Cryo-EM reconstruction of a VPS13 fragment reveals a long groove to channel lipids between membranes. *J. Cell Biol.* 219:e202001161
34. Munoz-Braceras S, Calvo R, Escalante R. 2015. TipC and the chorea-acanthocytosis protein VPS13A regulate autophagy in *Dictyostelium* and human HeLa cells. *Autophagy* 11:918–27
35. Osawa T, Kotani T, Kawaoka T, Hirata E, Suzuki K, et al. 2019. Atg2 mediates direct lipid transfer between membranes for autophagosome formation. *Nat. Struct. Mol. Biol.* 26:281–88
36. Valverde DP, Yu S, Boggavarapu V, Kumar N, Lees JA, et al. 2019. ATG2 transports lipids to promote autophagosome biogenesis. *J. Cell Biol.* 218:1787–98
37. Maeda S, Otomo C, Otomo T. 2019. The autophagic membrane tether ATG2A transfers lipids between membranes. *eLife* 8:e45777
38. Wang Y, Dahmane S, Ti R, Mai X, Zhu L, et al. 2025. Structural basis for lipid transfer by the ATG2A–ATG9A complex. *Nat. Struct. Mol. Biol.* 32:35–47
39. Kang Y, Lehmann KS, Vanegas J, Long H, Jefferson A, et al. 2024. Structural basis of bulk lipid transfer by bridge-like lipid transfer protein LPD-3. bioRxiv 2024.06.21.600134. **https://doi.org/10.1101/2024.06.21.600134**
40. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, et al. 2021. Highly accurate protein structure prediction with AlphaFold. *Nature* 596:583–89
41. Levine TP. 2019. Remote homology searches identify bacterial homologues of eukaryotic lipid transfer proteins, including Chorein-N domains in TamB and AsmA and Mdm31p. *BMC Mol. Cell Biol.* 20:43

42. Neuman SD, Jorgensen JR, Cavanagh AT, Smyth JT, Selegue JE, et al. 2022. The Hob proteins are novel and conserved lipid-binding proteins at ER–PM contact sites. *J. Cell Sci.* 135:jcs259086
43. Toulmay A, Whittle FB, Yang J, Bai X, Diarra J, et al. 2022. Vps13-like proteins provide phosphatidylethanolamine for GPI anchor synthesis in the ER. *J. Cell Biol.* 221:e202111095
44. Neuman SD, Levine TP, Bashirullah A. 2022. A novel superfamily of bridge-like lipid transfer proteins. *Trends Cell Biol.* 32:962–74
45. Castro IG, Shortill SP, Dziurdzik SK, Cadou A, Ganesan S, et al. 2022. Systematic analysis of membrane contact sites in *Saccharomyces cerevisiae* uncovers modulators of cellular lipid distribution. *eLife* 11:e74602
46. John Peter AT, Cheung NJ, Kornmann B. 2022. Csf1: a putative lipid transport protein required for homeoviscous adaptation of the lipidome. *Contact* 5. **https://doi.org/10.1177/25152564221101974**
47. Grimm J, Shi H, Wang W, Mitchell AM, Wingreen NS, et al. 2020. The inner membrane protein YhdP modulates the rate of anterograde phospholipid flow in *Escherichia coli*. *PNAS* 117:26907–14
48. Kumar S, Ruiz N. 2023. Bacterial AsmA-like proteins: bridging the gap in intermembrane phospholipid transport. *Contact* 6. **https://doi.org/10.1177/25152564231185931**
49. Levine TP. 2022. Sequence analysis and structural predictions of lipid transfer bridges in the repeating beta groove (RBG) superfamily reveal past and present domain variations affecting form, function and interactions of VPS13, ATG2, SHIP164, Hobbit and Tweek. *Contact* 5. **https://doi.org/10.1177/25152564221134328**
50. Tsukada M, Ohsumi Y. 1993. Isolation and characterization of autophagy-defective mutants of *Saccharomyces cerevisiae*. *FEBS Lett.* 333:169–74
51. Park JS, Neiman AM. 2012. VPS13 regulates membrane morphogenesis during sporulation in *Saccharomyces cerevisiae*. *J. Cell Sci.* 125:3004–11
52. Hanna M, Guillen-Samander A, De Camilli P. 2023. RBG motif bridge-like lipid transport proteins: structure, functions, and open questions. *Annu. Rev. Cell Dev. Biol.* 39:409–34
53. Tan JX, Finkel T. 2022. A phosphoinositide signalling pathway mediates rapid lysosomal repair. *Nature* 609:815–21
54. Cai S, Wu Y, Guillen-Samander A, Hancock-Cerutti W, Liu J, De Camilli P. 2022. In situ architecture of the lipid transport protein VPS13C at ER–lysosome membrane contacts. *PNAS* 119:e2203769119
55. Bieber A, Capitanio C, Erdmann PS, Fiedler F, Beck F, et al. 2022. In situ structural analysis reveals membrane shape transitions during autophagosome formation. *PNAS* 119:e2209823119
56. Wozny MR, Di Luca A, Morado DR, Picco A, Khaddaj R, et al. 2023. In situ architecture of the ER–mitochondria encounter structure. *Nature* 618:188–92
57. Jeong H, Park J, Jun Y, Lee C. 2017. Crystal structures of Mmm1 and Mdm12–Mmm1 reveal mechanistic insight into phospholipid trafficking at ER-mitochondria contact sites. *PNAS* 114:E9502–11
58. Guillen-Samander A, Leonzino M, Hanna MG, Tang N, Shen H, De Camilli P. 2021. VPS13D bridges the ER to mitochondria and peroxisomes via Miro. *J. Cell Biol.* 220:e202010004
59. Covill-Cooke C, Kwizera B, Lopez-Domenech G, Thompson CO, Cheung NJ, et al. 2024. Shared structural features of Miro binding control mitochondrial homeostasis. *EMBO J.* 43:595–614
60. Ghanbarpour A, Valverde DP, Melia TJ, Reinisch KM. 2021. A model for a partnership of lipid transfer proteins and scramblases in membrane expansion and organelle biogenesis. *PNAS* 118:e2101562118
61. van Vliet AR, Chiduza GN, Maslen SL, Pye VE, Joshi D, et al. 2022. ATG9A and ATG2A form a heteromeric complex essential for autophagosome formation. *Mol. Cell* 82:4324–39.e8
62. Sawa-Makarska J, Baumann V, Coudevylle N, von Bulow S, Nogellova V, et al. 2020. Reconstitution of autophagosome nucleation defines Atg9 vesicles as seeds for membrane formation. *Science* 369:eaaz7714
63. Olivas TJ, Wu Y, Yu S, Luan L, Choi P, et al. 2023. ATG9 vesicles comprise the seed membrane of mammalian autophagosomes. *J. Cell Biol.* 222:e202208088
64. Matoba K, Kotani T, Tsutsumi A, Tsuji T, Mori T, et al. 2020. Atg9 is a lipid scramblase that mediates autophagosomal membrane expansion. *Nat. Struct. Mol. Biol.* 27:1185–93
65. Maeda S, Yamamoto H, Kinch LN, Garza CM, Takahashi S, et al. 2020. Structure, lipid scrambling activity and role in autophagosome formation of ATG9A. *Nat. Struct. Mol. Biol.* 27:1194–201
66. Li YE, Wang Y, Du X, Zhang T, Mak HY, et al. 2021. TMEM41B and VMP1 are scramblases and regulate the distribution of cholesterol and phosphatidylserine. *J. Cell Biol.* 220:e202103105

67. Park JS, Neiman AM. 2020. XK is a partner for VPS13A: a molecular link between chorea-acanthocytosis and McLeod syndrome. *Mol. Biol. Cell* 31:2425–36
68. Adlakha J, Hong Z, Li P, Reinisch KM. 2022. Structural and biochemical insights into lipid transport by VPS13 proteins. *J. Cell Biol.* 221:e202202030
69. Park JS, Hu Y, Hollingsworth NM, Miltenberger-Miltenyi G, Neiman AM. 2022. Interaction between VPS13A and the XK scramblase is important for VPS13A function in humans. *J. Cell Sci.* 135:jcs260227
70. Guillen-Samander A, Wu Y, Pineda SS, Garcia FJ, Eisen JN, et al. 2022. A partnership between the lipid scramblase XK and the lipid transfer protein VPS13A at the plasma membrane. *PNAS* 119:e2205425119
71. van Vliet AR, Jefferies HBJ, Faull PA, Chadwick J, Ibrahim F, et al. 2024. Exploring the ATG9A interactome uncovers interaction with VPS13A. *J. Cell Sci.* 137:jcs261081
72. Pomorski TG, Menon AK. 2016. Lipid somersaults: uncovering the mechanisms of protein-mediated lipid flipping. *Prog. Lipid Res.* 64:69–84
73. Vance DE, Choy PC, Farren SB, Lim PH, Schneider WJ. 1977. Asymmetry of phospholipid biosynthesis. *Nature* 270:268–69
74. Li D, Rocha-Roa C, Schilling MA, Reinisch KM, Vanni S. 2024. Lipid scrambling is a general feature of protein insertases. *PNAS* 121:e2319476121
75. Jahn H, Bartos L, Dearden GI, Dittman JS, Holthuis JCM, et al. 2023. Phospholipids are imported into mitochondria by VDAC, a dimeric beta barrel scramblase. *Nat. Commun.* 14:8115
76. Guna A, Volkmar N, Christianson JC, Hegde RS. 2018. The ER membrane protein complex is a transmembrane domain insertase. *Science* 359:470–73
77. Lahiri S, Chao JT, Tavassoli S, Wong AK, Choudhary V, et al. 2014. A conserved endoplasmic reticulum membrane protein complex (EMC) facilitates phospholipid transfer from the ER to mitochondria. *PLOS Biol.* 12:e1001969
78. Sundberg E, Slagter JG, Fridborg I, Cleary SP, Robinson C, Coupland G. 1997. ALBINO3, an Arabidopsis nuclear gene essential for chloroplast differentiation, encodes a chloroplast protein that shows homology to proteins present in bacterial membranes and yeast mitochondria. *Plant Cell* 9:717–30
79. Sperandeo P, Martorana AM, Polissi A. 2017. Lipopolysaccharide biogenesis and transport at the outer membrane of Gram-negative bacteria. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* 1862:1451–60
80. Owens TW, Taylor RJ, Pahil KS, Bertani BR, Ruiz N, et al. 2019. Structural basis of unidirectional export of lipopolysaccharide to the cell surface. *Nature* 567:550–53
81. Prinz WA, Toulmay A, Balla T. 2020. The functional universe of membrane contact sites. *Nat. Rev. Mol. Cell Biol.* 21:7–24
82. Hanna MG, Suen PH, Wu Y, Reinisch KM, De Camilli P. 2022. SHIP164 is a chorein motif lipid transfer protein that controls endosome–Golgi membrane traffic. *J. Cell Biol.* 221:e202111018
83. Zhang Y, Lin C. 2024. Lipid osmosis, membrane tension, and other mechanochemical driving forces of lipid flow. *Curr. Opin. Cell Biol.* 88:102377
84. Petrungaro C, Kornmann B. 2019. Lipid exchange at ER-mitochondria contact sites: a puzzle falling into place with quite a few pieces missing. *Curr. Opin. Cell Biol.* 57:71–76
85. Dabrowski R, Tulli S, Graef M. 2023. Parallel phospholipid transfer by Vps13 and Atg2 determines autophagosome biogenesis dynamics. *J. Cell Biol.* 222:e202211039
86. Melia TJ, Lystad AH, Simonsen A. 2020. Autophagosome biogenesis: from membrane growth to closure. *J. Cell Biol.* 219:e202002085
87. Chang C, Jensen LE, Hurley JH. 2021. Autophagosome biogenesis comes out of the black box. *Nat. Cell Biol.* 23:450–56
88. Zhen Y, Stenmark H. 2023. Autophagosome biogenesis. *Cells* 12:668
89. Baba M, Takeshige K, Baba N, Ohsumi Y. 1994. Ultrastructural analysis of the autophagic process in yeast: detection of autophagosomes and their characterization. *J. Cell Biol.* 124:903–13
90. Fengsrud M, Erichsen ES, Berg TO, Raiborg C, Seglen PO. 2000. Ultrastructural characterization of the delimiting membranes of isolated autophagosomes and amphisomes by freeze-fracture electron microscopy. *Eur. J. Cell Biol.* 79:871–82
91. Gomez-Sanchez R, Rose J, Guimaraes R, Mari M, Papinski D, et al. 2018. Atg9 establishes Atg2-dependent contact sites between the endoplasmic reticulum and phagophores. *J. Cell Biol.* 217:2743–63

92. Orii M, Tsuji T, Ogasawara Y, Fujimoto T. 2021. Transmembrane phospholipid translocation mediated by Atg9 is involved in autophagosome formation. *J. Cell Biol.* 220:e202009194
93. Huang D, Xu B, Liu L, Wu L, Zhu Y, et al. 2021. TMEM41B acts as an ER scramblase required for lipoprotein biogenesis and lipid homeostasis. *Cell Metab.* 33:1655–70.e8
94. Itakura E, Mizushima N. 2010. Characterization of autophagosome formation site by a hierarchical analysis of mammalian Atg proteins. *Autophagy* 6:764–76
95. Morita K, Hama Y, Izume T, Tamura N, Ueno T, et al. 2018. Genome-wide CRISPR screen identifies TMEM41B as a gene required for autophagosome formation. *J. Cell Biol.* 217:3817–28
96. Moretti F, Bergman P, Dodgson S, Marcellin D, Claerr I, et al. 2018. TMEM41B is a novel regulator of autophagy and lipid mobilization. *EMBO Rep.* 19:e45889
97. Shoemaker CJ, Huang TQ, Weir NR, Polyakov NJ, Schultz SW, Denic V. 2019. CRISPR screening using an expanded toolkit of autophagy reporters identifies TMEM41B as a novel autophagy factor. *PLOS Biol.* 17:e2007044
98. Zhu Y, Fujimaki M, Snape L, Lopez A, Fleming A, Rubinsztein DC. 2024. Loss of WIPI4 in neurodegeneration causes autophagy-independent ferroptosis. *Nat. Cell Biol.* 26:542–51
99. Mailler E, Guardia CM, Bai X, Jarnik M, Williamson CD, et al. 2021. The autophagy protein ATG9A enables lipid mobilization from lipid droplets. *Nat. Commun.* 12:6750
100. Broadbent DG, Barnaba C, Perez GI, Schmidt JC. 2023. Quantitative analysis of autophagy reveals the role of ATG9 and ATG2 in autophagosome formation. *J. Cell Biol.* 222:e202210078
101. Velikkakath AK, Nishimura T, Oita E, Ishihara N, Mizushima N. 2012. Mammalian Atg2 proteins are essential for autophagosome formation and important for regulation of size and distribution of lipid droplets. *Mol. Biol. Cell* 23:896–909
102. Pfisterer SG, Bakula D, Frickey T, Cezanne A, Brigger D, et al. 2014. Lipid droplet and early autophagosomal membrane targeting of Atg2A and Atg14L in human tumor cells. *J. Lipid Res.* 55:1267–78
103. Suzuki K, Akioka M, Kondo-Kakuta C, Yamamoto H, Ohsumi Y. 2013. Fine mapping of autophagy-related proteins during autophagosome formation in *Saccharomyces cerevisiae*. *J. Cell Sci.* 126:2534–44
104. Graef M, Friedman JR, Graham C, Babu M, Nunnari J. 2013. ER exit sites are physical and functional core autophagosome biogenesis components. *Mol. Biol. Cell* 24:2918–31
105. Kotani T, Kirisako H, Koizumi M, Ohsumi Y, Nakatogawa H. 2018. The Atg2-Atg18 complex tethers pre-autophagosomal membranes to the endoplasmic reticulum for autophagosome formation. *PNAS* 115:10363–68
106. Zheng JX, Li Y, Ding YH, Liu JJ, Zhang MJ, et al. 2017. Architecture of the ATG2B-WDR45 complex and an aromatic Y/HF motif crucial for complex formation. *Autophagy* 13:1870–83
107. Bozic M, van den Bekerom L, Milne BA, Goodman N, Roberston L, et al. 2020. A conserved ATG2-GABARAP family interaction is critical for phagophore formation. *EMBO Rep.* 21:e48412
108. Proikas-Cezanne T, Haas ML, Pastor-Maldonado CJ, Schussele DS. 2024. Human WIPI β-propeller function in autophagy and neurodegeneration. *FEBS Lett.* 598:127–39
109. Bueno-Arribas M, Blanca I, Cruz-Cuevas C, Escalante R, Navas MA, Vincent O. 2021. A conserved ATG2 binding site in WIPI4 and yeast Hsv2 is disrupted by mutations causing β-propeller protein-associated neurodegeneration. *Hum. Mol. Genet.* 31:111–21
110. Nguyen A, Lugarini F, David C, Hosnani P, Alagoz C, et al. 2023. Metamorphic proteins at the basis of human autophagy initiation and lipid transfer. *Mol. Cell* 83:2077–90.e12
111. Wang N, Shibata Y, Paulo JA, Gygi SP, Rapoport TA. 2023. A conserved membrane curvature-generating protein is crucial for autophagosome formation in fission yeast. *Nat. Commun.* 14:4765
112. Shatz O, Fraiberg M, Isola D, Das S, Gogoi O, et al. 2024. Rim aperture of yeast autophagic membranes balances cargo inclusion with vesicle maturation. *Dev. Cell* 59:911–23.e4
113. Yamamoto H, Kakuta S, Watanabe TM, Kitamura A, Sekito T, et al. 2012. Atg9 vesicles are an important membrane source during early steps of autophagosome formation. *J. Cell Biol.* 198:219–33
114. Nishimura T, Tamura N, Kono N, Shimanaka Y, Arai H, et al. 2017. Autophagosome formation is initiated at phosphatidylinositol synthase-enriched ER subdomains. *EMBO J.* 36:1719–35
115. Holland P, Knaevelsrud H, Soreng K, Mathai BJ, Lystad AH, et al. 2016. HS1BP3 negatively regulates autophagy by modulation of phosphatidic acid levels. *Nat. Commun.* 7:13889

116. Schutter M, Giavalisco P, Brodesser S, Graef M. 2020. Local fatty acid channeling into phospholipid synthesis drives phagophore expansion during autophagy. *Cell* 180:135–49.e14
117. Baumann V, Achleitner S, Tulli S, Schuschnig M, Klune L, Martens S. 2024. Faa1 membrane binding drives positive feedback in autophagosome biogenesis via fatty acid activation. *J. Cell Biol.* 223:e202309057
118. Anding AL, Wang C, Chang TK, Sliter DA, Powers CM, et al. 2018. Vps13D encodes a ubiquitin-binding protein that is required for the regulation of mitochondrial size and clearance. *Curr. Biol.* 28:287–95.e6
119. Baldwin HA, Wang C, Kanfer G, Shah HV, Velayos-Baeza A, et al. 2021. VPS13D promotes peroxisome biogenesis. *J. Cell Biol.* 220:e202001188
120. Da Costa R, Bordessoules M, Guilleman M, Carmignac V, Lhussiez V, et al. 2020. Vps13b is required for acrosome biogenesis through functions in Golgi dynamic and membrane trafficking. *Cell. Mol. Life Sci.* 77:511–29
121. De Camilli P, Peluchetti D, Meldolesi J. 1976. Dynamic changes of the luminal plasmalemma in stimulated parotid acinar cells. A freeze-fracture study. *J. Cell Biol.* 70:59–74
122. Suzuki SW, West M, Zhang Y, Fan JS, Roberts RT, et al. 2024. A role for Vps13-mediated lipid transfer at the ER–endosome contact site in ESCRT-mediated sorting. *J. Cell Biol.* 223:e202307094
123. Parolek J, Burd CG. 2024. Bridge-like lipid transfer protein family member 2 suppresses ciliogenesis. *Mol. Biol. Cell* 35:br11
124. Ugur B, Schueder F, Shin J, Hanna MG, Wu Y, et al. 2023. VPS13B is localized at the interface between Golgi cisternae and is a functional partner of FAM177A1. *J. Cell Biol.* 223:e202311189
125. Jeng EE, Bhadkamkar V, Ibe NU, Gause H, Jiang L, et al. 2019. Systematic identification of host cell regulators of *Legionella pneumophila* pathogenesis using a genome-wide CRISPR screen. *Cell Host Microbe* 26:551–63.e6
126. Kang Y, Jefferson A, Sheehan A, La Torre RD, Jay T, et al. 2023. Tweek-dependent formation of ER-PM contact sites enables astrocyte phagocytic function and remodeling of neurons. bioRxiv 2023.11.06.565932. **https://doi.org/10.1101/2023.11.06.565932**
127. Banerjee S, Daetwyler S, Bai X, Michaud M, Jouhet J, et al. 2024. The Vps13-like protein BLTP2 is pro-survival and regulates phosphatidylethanolamine levels in the plasma membrane to maintain its fluidity and function. bioRxiv 2024.02.04.578804. **https://doi.org/10.1101/2024.02.04.578804**
128. Wang X, Xu P, Bentley-DeSousa A, Hancock-Cerutti W, Cai S, et al. 2024. Lysosome damage triggers acute formation of ER to lysosomes membrane tethers mediated by the bridge-like lipid transport protein VPS13C. bioRxiv 2024.06.08.598070. **https://doi.org/10.1101/2024.06.08.598070**
129. Cross J, Durgan J, McEwan DG, Tayler M, Ryan KM, Florey O. 2023. Lysosome damage triggers direct ATG8 conjugation and ATG2 engagement via non-canonical autophagy. *J. Cell Biol.* 222:e202303078
130. De Tito S, Almacellas E, Yu DD, Zhang W, Millard E, et al. 2024. ATG9A and ARFIP2 cooperate to regulate PI4P levels for lysosomal repair. bioRxiv 2024.07.23.604321. **https://doi.org/10.1101/2024.07.23.604321**
131. Lesage S, Drouet V, Majounie E, Deramecourt V, Jacoupy M, et al. 2016. Loss of VPS13C function in autosomal-recessive parkinsonism causes mitochondrial dysfunction and increases PINK1/Parkin-dependent mitophagy. *Am. J. Hum. Genet.* 98:500–13
132. Claude-Taupin A, Jia J, Bhujabal Z, Garfa-Traore M, Kumar S, et al. 2021. ATG9A protects the plasma membrane from programmed and incidental permeabilization. *Nat. Cell Biol.* 23:846–58
133. Tokai M, Kawasaki H, Kikuchi Y, Ouchi K. 2000. Cloning and characterization of the CSF1 gene of *Saccharomyces cerevisiae*, which is required for nutrient uptake at low temperature. *J. Bacteriol.* 182:2865–68
134. Wang C, Wang B, Pandey T, Long Y, Zhang J, et al. 2022. A conserved megaprotein-based molecular bridge critical for lipid trafficking and cold resilience. *Nat. Commun.* 13:6805
135. Amos C, Xu P, De Camilli P. 2023. Erythroid differentiation dependent interaction of VPS13A with XK at the plasma membrane of K562 cells. *Contact* 6. **https://doi.org/10.1177/25152564231215**
136. Ryoden Y, Segawa K, Nagata S. 2022. Requirement of Xk and Vps13a for the P2X7-mediated phospholipid scrambling and cell lysis in mouse T cells. *PNAS* 119:e2119286119

137. Freyre CAC, Rauher PC, Ejsing CS, Klemm RW. 2019. MIGA2 links mitochondria, the ER, and lipid droplets and promotes de novo lipogenesis in adipocytes. *Mol. Cell* 76:811–25.e14
138. Du X, Zhou L, Aw YC, Mak HY, Xu Y, et al. 2020. ORP5 localizes to ER–lipid droplet contacts and regulates the level of PI(4)P on lipid droplets. *J. Cell Biol.* 219:e201905162
139. Pu M, Zheng W, Zhang H, Wan W, Peng C, et al. 2023. ORP8 acts as a lipophagy receptor to mediate lipid droplet turnover. *Protein Cell* 14:653–67
140. Wan N, Hong Z, Parson MAH, Korfhage JL, Burke JE, et al. 2024. Spartin-mediated lipid transfer facilitates lipid droplet turnover. *PNAS* 121:e2314093121
141. Ramseyer VD, Kimler VA, Granneman JG. 2018. Vacuolar protein sorting 13C is a novel lipid droplet protein that inhibits lipolysis in brown adipocytes. *Mol. Metab.* 7:57–70
142. Mathiowetz AJ, Olzmann JA. 2024. Lipid droplets and cellular lipid flux. *Nat. Cell Biol.* 26:331–45
143. Walther TC, Chung J, Farese RV Jr. 2017. Lipid droplet biogenesis. *Annu. Rev. Cell Dev. Biol.* 33:491–510
144. Wu J, Gao M, Wu X, Liu Y, Du X, et al. 2024. PI(4)P recruits CIDE proteins to promote the formation of unilocular lipid droplets during adipogenesis and hepatic steatosis. bioRxiv 2024.08.22.609036. **https://doi.org/10.1101/2024.08.22.609036**
145. Chen S, Roberts MA, Chen CY, Markmiller S, Wei HG, et al. 2022. VPS13A and VPS13C influence lipid droplet abundance. *Contact* 5. **https://doi.org/10.1177/25152564221125613**
146. Baek M, DiMaio F, Anishchenko I, Dauparas J, Ovchinnikov S, et al. 2021. Accurate prediction of protein structures and interactions using a three-track neural network. *Science* 373:871–76