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# Role of VPS13, a protein with similarity to ATG2, in physiology and disease

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## Abstract

The evolutionarily conserved VPS13 family proteins have been implicated in several cellular processes. Mutations in each of the four human VPS13s cause neurodevelopmental or neurodegenerative disorders. Until recently, the molecular function of VPS13 remained elusive. Genetic, functional and structural studies have now revealed that VPS13 acts at contact sites between intracellular organelles to transport lipids by a novel mechanism: direct transfer between bilayers via a hydrophobic channel that spans its entire rod-like N-terminal half. Predicted similarities to the autophagy protein ATG2 suggested a similar role for ATG2 that has now been confirmed by structural and functional studies. Here, after a brief review of this evidence, we discuss what is known of human VPS13 proteins in physiology and disease.

In 2001 Rampoldi *et al.* [1] mapped mutations responsible for chorea acanthocytosis to a gene encoding a very large and uncharacterized protein (hence called chorein) which showed similarities to the yeast SOI1/VPS13 protein [2,3]. Chorein is one of 4 mammalian paralogues that are encoded by 4 distinct genes broadly expressed in different tissues [4]. Mutations in the other three paralogues also result in neurodegenerative or neurodevelopmental diseases: Cohen Syndrome (VPS13B) [5], Parkinson's disease (VPS13C) [6–8] and spinocerebellar ataxia (VPS13D) [9,10]. Studies in a variety of model organisms and cell types over the last several years had identified cellular processes which are affected by the loss of VPS13 family proteins, including autophagy, cytoskeletal organization, $Ca^{2+}$ signaling and mitochondria homeostasis, but a mechanistic understanding of VPS13 function had remained elusive [11]. Very recent studies have shed light on such function by demonstrating that VPS13 is the founding member of a new family of lipid transport proteins that act at contact sites between different organelles. This brief review will discuss these new findings and, where possible, will attempt to relate these new findings to the pathological manifestations resulting from VPS13 mutations.

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## Early clues from yeast genetics for a role of VPS13 in lipid transport at membrane contact sites not leading to fusion

The single Vps13 yeast protein (alias: SOI1 [12], Vpt2 [3] was originally identified in a screen for proteins involved in **V**acuolar **P**rotein **S**orting [2,3]. Subsequently, yeast Vps13 was implicated in recycling traffic between endosome and the Golgi complex [2,12], in the maintenance of mitochondrial membrane integrity [13], and in the growth of the prospore membrane [14,15]. This is a special membrane generated during the second meiotic division that ultimately becomes the plasma membrane of the 4 daughter spore cells. A first insight into a link between Vps13 and lipid dynamics emerged from the discovery that the function of yeast Vps13 is partially redundant with that of the ERMES complex (**ER** to **M**itochondria **E**ncounter **S**tructure (ERMES) [16]. ERMES is a multi-subunit complex localized at contacts between the ER and mitochondria thought to mediate lipid transfer between these two organelles, which are not connected by vesicular transport [17]. Yeast cells with ERMES deletions exhibit growth defects but are viable, suggesting the existence of additional pathways for lipid exchange between ER and mitochondria. A search for such bypass pathways identified spontaneous dominant VPS13 mutations as suppressors of the ERMES-knockout (KO) phenotype. Conversely, the combined deficiency of ERMES and VPS13 resulted in lethality [13,16]. It was also shown that Vps13 localizes to contacts between the yeast vacuole (which corresponds to lysosomes in mammalian cells) and either the nuclear envelope (which is part of the ER), or mitochondria [13,16]. Collectively, these findings raised the possibility that yeast Vps13 may cooperate in lipid transfer between the ER and mitochondria via an indirect route involving the vacuole. A lipid transport function of VPS13 was indeed established by Kumar, Leonzino *et al.* [18] and subsequent studies as described below.

## VPS13 family proteins mediate lipid transport at intracellular membrane contact sites.

VPS13 is a large (> 3,000 a.a.) evolutionary conserved protein. Structural predictions had revealed repetitive modules comprising primarily ß-strands throughout its N-terminal half, followed by WD40-like elements (alias VAB domain [19]), a DH-like (DH-L) fold and a PH domain at the C-terminus [18,20] (Fig.1). Additional motifs present in VPS13 proteins are shown in Fig.1. A cryo-EM reconstruction of an N-terminal fragment (a.a. 1–1390) of VPS13 from the fungus *Chaetomium thermophilum* (VPS131–1390) showed that it resembles a highly elongated open-ended basket, including a handle. The bottom of “basket” is represented by an extended ß-sheet, while α-helices between ß-strands form the “handle” [21,22] (Fig.1). A previously reported crystal structure of a smaller fragment of the same protein (a.a. 1–335) [18] could be docked into one end of the basket. All of the residues lining the concave surface of the basket in this smaller fragment are hydrophobic [21], and sequence analysis suggests that the remainder of the concave basket interior is also hydrophobic. Further, both fragments of VPS13 can bind lipids (the fragment comprising residues 1–1390 can bind at least 10 lipids per protein), presumably in the hydrophobic groove that corresponds to the basket interior, and VPS131–1390 can transport lipids between artificial membranes *in vitro*, supporting a role for the protein in lipid transport

[18]. The cryo-EM study suggests that VPS13s may channel lipids directly and unidirectionally between membrane bilayers via the hydrophobic groove [21] (Fig. 2), thus contributing to membrane expansion independently of vesicular transport. Such role, for example, could explain the role of yeast VPS13 in the growth of the sporulation membrane [14]. Most interestingly, in this respect, VPS13 has structural and functional similarities to the autophagy factor ATG2 (Fig. 1) [18,23–26], a protein required for the growth of the autophagic membrane, another example of de-novo growth of a membrane. More specifically, 1) VPS13 and ATG2 share small stretches of primary a.a. similarity (chorein and ATG-C homology domain) (Fig. 1) [4,18], 2) the entire ATG2 protein has a rod-like shape with an elongated cavity resembling the N-terminal half of VPS13 [24] and 3) the crystal structures of the N-terminal portions of the ATG2 and VPS13 rods are nearly superimposable [25]. Moreover, 4) like VPS13, ATG2 is localized at membrane contact sites (contacts between the ER and the preautophagic membrane [27,28] and 5) can transport lipids *in vitro* [24–26]. ATG2 does not contain a WD40-L region but binds WD40 proteins of the ATG18/WIPI family [26,27]. Interestingly, VPS13 itself was implicated in autophagy in several model organisms [29,30] (see also below).

While the overall architecture of VPS13 is conserved from yeast to humans, significant differences in the subcellular localization of different VPS13s in different organisms, and therefore likely in their specific roles in lipid transport, have been observed. VPS13A is localized at contacts between ER and mitochondria [18,31,32] while VPS13C is localized at contacts between the ER and late endosomes/lysosomes [18]. In addition, both proteins are localized at contacts between the ER and lipid droplets [18,33,34] (Fig.3). Binding to the ER of both proteins is mediated by an FFAT motif-dependent interaction with the ER integral membrane protein VAP [18,32,35]. Binding to lipid droplets requires an amphipathic helix present in their C-terminal region, the so-called ATG-C homology region (Fig. 1), as a similar lipid droplet binding amphipathic helix occurs in ATG2 [18,36]. The binding of VPS13C to endosomes/lysosomes is mediated by its WD40-L region and involves at least in part Rab7 on their surface [18]. Conversely, binding of VPS13A to mitochondria is mediated by its $DH_L$-PH domain, but its binding factor on mitochondria remains unknown [18]. While yeast Vps13 binds the outer mitochondrial membrane protein Mcp1, an Mcp1 paralogue has not been identified in mammals [37]. Furthermore, binding to Mcp1 is mediated by the WD40-L/VAB region of yeast Vps13, which recognizes a PXP motif in Mcp1, suggesting a different mitochondrial binding mechanism. The PxP motif is also found in two other yeast Vps13 binding proteins: sorting nexin Ypt35, which is responsible for Vps13 recruitment to the vacuole, and Spo71p, which recruits Vps13 to the prospore membrane [19]. PxP containing VPS13 interactors have not been identified yet in mammals, however several disease-causing mutations in VPS13B and VPS13D reside in the WD40-L/VAB region [38].

For VPS13B (also known as COH1) and VPS13D, evidence for a localization at membrane contact sites is still missing. VPS13B was reported to be localized in the Golgi complex region and to interact with Rab6 [39,40]. In another study, VPS13B was proposed to function as a tethering factor involved in vesicle trafficking between early and recycling endosomes [41]. Concerning VPS13D, its knockdown was shown to cause striking defects in mitochondrial morphology [9,10,42]. However, there is no evidence so far that VPS13D

localizes to mitochondria and its *Drosophila* orthologue was shown by immunofluorescence to colocalize with the integral lysosomal protein LAMP1 [42].

## Genetic diseases resulting from VPS13 mutations and disease models

### VPS13A

Chorea-acanthocytosis (ChAc) is a rare, Huntington-like autosomal recessive neurodegenerative disorder caused by mutations in VPS13A [1,43]. It is characterized by involuntary hyperkinetic movements (chorea; hence the name “chorein” for the approximately 150 a.a. N-terminal fragment which represents the most conserved region among VPS13s and between VPS13 and ATG2). Movement disorders correlate with the degeneration of striatal neurons, and with the presence of abnormally shaped blood cells (acanthocytosis). The age of onset is typically around the third decade of life, and core symptoms are often accompanied by seizures, dystonia and behavioral changes [44]. Given the localization of VPS13A at ER-mitochondrial contacts, subtle chronic alterations of the lipid composition of mitochondria or defects in the growth of their membranes may play a role in disease pathogenesis. Similar clinical conditions, referred to as neuroacanthocytosis are McLeod syndrome (MLS), Huntington disease-like 2 (HDL 2) and pantothenate kinase-associated neurodegeneration (PKAN) (see Peikert *et al.* [44]). Given the lipid transport function of VPS13, it is of special interest that several of these other conditions involve alterations of lipid metabolism.

*Vps13a*$^{-/-}$ mice were reported to have acanthocytosis, mild neurological and behavioral abnormalities that vary strongly depending on the strain background [45,46], and male infertility. The latter was attributed to a defect in sperm motility resulting from abnormal mitochondria in the sperm midpiece [47]. Flies with loss-of-function mutations in the *Vps13* gene (Drosophila *Vps13* is most similar to human VPS13A and C, while the two other fly paralogues, *Vps13b* and *Vps13d*, represent the orthologues of mammalian VPS13B and VPS13D) have reduced lifespan, age dependent decline in climbing ability, and large vacuoles in the brain [48]. In addition, *Vps13* mutant adult fly eyes display lipid droplet accumulation [32], which is a common hallmark observed with neurodegeneration induced by ROS due to mitochondrial defects [49]. Expression of human *VPS13A* in *Vps13* mutant flies rescues a subset of these phenotypes [32]. Interestingly, in a genome-wide comparative study to identify causal genes involved in migration behavior, VPS13A was isolated as the only gene whose polymorphism correlates with migratory behavior differences [50].

### VPS13B

Autosomal recessive mutations in VPS13B cause Cohen Syndrome (CS) [5], a neurodevelopmental disorder characterized by postnatal microcephaly, intellectual disability, craniofacial anomalies, hypotonia, progressive retinal dysfunction and truncal fat accumulation [51]. Clinical manifestations, which can be heterogeneous, generally start to manifest by two years of age. In agreement with the reported accumulation of VPS13B in the Golgi complex, an abnormal Golgi structure was observed in fibroblasts from Cohen syndrome patients and in various mammalian cell lines upon RNAi-dependent knockdown [39,52]. Moreover, consistent with Golgi complex’s role in glycosylation, serum proteins

derived from CS patients have an unusual glycosylation pattern [52]. Abnormal glycosylation of cell surface molecules may play a role in developmental defects, given the important role of glycoproteins in cell-cell interactions. However, in view of the lipid transport function of Vps13, glycosylation defects are likely to be only an indirect consequence of a functional perturbation of Golgi function that remains to be elucidated.

*Vps13b*$^{-/-}$ KO mice display behavioral/neurological impairments, including defects in spatial learning and reduced activity in the open field test [53]. Furthermore, as in the case of the *Vps13a* KO mouse model, *Vps13b* KO mice display male infertility, but via a different mechanism. Lack of Vps13B results in impaired formation of the acrosomal membrane of sperm cells, a process which occurs in proximity of the Golgi complex area and which is required for subsequent egg fertilization [54]. An intriguing hypothesis is that impaired acrosomal membrane growth may reflect a role of Vps13B in *de novo* membrane biogenesis, akin to the proposed role of yeast Vps13 in growth of the sporulation membrane (see above) and of ATG2 in the growth of the phagophore.

## VPS13C

Biallelic mutations resulting in VPS13C loss-of-function cause rare cases of early-onset, autosomal recessive Parkinson's Disease (PD) [6–8] (hence the VPS13C alias PARK23) and genetic variations in the *VPS13C* locus have been associated with PD by GWAS studies [55–57]. PD due to VPS13 loss-of-function initially presents with akinetic asymmetrical rigid syndrome and variable tremor and dystonia that is responsive to levodopa, but subsequently cognitive decline and motor neuron signs may also develop. Post-mortem examination of a patient brain revealed widespread diffuse alpha-synuclein- and ubiquitin-positive Lewy body pathology. VPS13C KO mice do not have obvious neurological phenotypes (our unpublished observations), in line with the lack of such phenotypes in mouse models of other forms of familial PD.

The identification of yeast Vps13 as a protein with a role in mitochondria biology [13,16] initially suggested a primary action of VPS13C at mitochondria. Accordingly, it was reported that siRNA-mediated knockdown of VPS13C in Cos7 cells leads to multiple mitochondrial defects [6]. Most interestingly, the same study also showed that VPS13C knockdown increased mitochondrial recruitment of pink1 and parkin, upregulation of parkin transcripts, and an exacerbation of pink1/parkin dependent mitophagy upon CCCP treatment [6]. Though this study described the presence of VPS13C on the mitochondrial outer membrane by subcellular fractionation, this localization was not observed by fluorescence microscopy in cells expressing, or overexpressing, tagged VPS13C constructs, which instead localized at contacts between the ER and either late-endososomes/lysosomes or lipid droplets [18]. A close proximity of VPS13C to lysosomes was further supported by proximity biotinylation studies [58]. Thus, the mechanism(s) underlying the reported mitochondrial defects in VPS13C KO cells remain unknown. It is important to note, however, that many recent studies have suggested the occurrence of lysosomes-to-mitochondria crosstalk, and of mitochondrial impairments in response to defects in lysosome function [59–61]. In view of these considerations, it remains possible that perturbation of lipid homeostasis in late endosomes/lysosomes may results in PD by having

a negative impact on mitochondrial metabolism or quality control mechanisms. Consistent with the localization of a pool of VPS13C at ER lipid droplets contacts, it was shown that VPS13C plays a role in adipogenesis and lipolysis in adipocytes [33,34].

### VPS13D

Bi-allelic mutations in *VPS13D* have been identified as a cause of early-onset, clinically heterogenous movement disorders in more than 20 patients from three separate studies [9,10,62]. The most common core clinical diagnosis was cerebellar ataxia with or without spasticity, as well as dystonia, hypotonia, spastic paraparesis, and chorea. Two patients had confirmed microcephaly and three suffered from seizures. Three patients presented as a pure hereditary spastic paraplegia, and were also the oldest at time of onset (40, 42, and 63), all features suggestive of a milder phenotype [9,62].

Of all the VPS13 proteins, VPS13D seems to be the most important for cell viability. The KO of VPS13D is embryonically lethal in both mice and flies, and VPS13D was found to be essential in several human cell lines [9,63]. Moreover, none of the human cases appear to be homozygous for total loss-of-function alleles [9,10,62], consistent with the possibility that biallelic total loss of function mutations may be lethal. Accordingly, the VPS13D gene is predicted to be intolerant to mutations, with a pLI score of 1.00 [9].

Genetic studies in flies have implicated VPS13D in the clearance of mitochondria in the gut and in neurons [9,42]. More specifically, knockdown of *Vps13D* in the fly intestine causes a defect in mitochondrial clearance and mitophagy, as well as in the accumulation of large spherical mitochondria [42]. The UBA domain of VPS13D is thought to be important for this function (Fig. 1). Relevant to neurological diseases, knockdown of *Vps13D* in fly motor neurons causes a reduction in mitochondria in the axon as well as at the neuromuscular junction [9], suggesting a link of VPS13D to mitochondria motility. The defect in mitophagy was proposed to be mediated by an impairment of phagophore enlargement around mitochondria [64] reminiscent of the phenotype displayed by ATG2 defects. Abnormal mitochondria and a defect in mitophagy were also observed in a VPS13D KO human cell line [42] and in fibroblasts from patients with VPS13D mutations [9]. Mitochondrial pathology in progressive cerebellar ataxias have been previously described [65] consistent with a primary role of mitochondrial dysfunction in VPS13D-dependent neurological manifestations.

## Concluding remarks

The molecular function of VPS13 had remained elusive for more than two decades after its first identification in yeast. The discovery of severe neurological conditions resulting from mutations of human VPS13s invigorated interest in the role of this protein family. As discussed in this brief review, recent studies have drastically advanced knowledge about the molecular properties and function of this protein family, whose members are now thought to function as conduits for the transport of lipids between bilayers independently of vesicular transport. The predicted similarity of VPS13 to ATG2 had first suggested a similar lipid transport function for ATG2 [18,25,36,66], which has now been confirmed by structural and functional studies [24]. While VPS13 and ATG2 are the first eukaryotic proteins thought to

function by providing hydrophobic channels that allow lipid flow between bilayers, such mechanism has been described for the translocation of lipids from the inner to the outer membrane of Gram-negative bacteria [67–70]. Interestingly, evolutionary relations between bacterial proteins involved in this transport and VPS13 family protein have been suggested [71]. As discussed in the review, this mechanism could account for the reported roles of VPS13 and ATG2 in membrane expansion.

Important future priorities include the determination of the structure of full length VPS13 and the precise elucidation of the direction, energetics, selectivity and regulation of lipid transport mediated by VPS13. It will also be important to determine how mutations in different VPS13 genes result in different pathological conditions. While all four VPS13 genes are broadly expressed in the body, partially different patterns of cellular expression and/or different intracellular sites at which they transport lipids may explain the different manifestations resulting from their mutations. Importantly, however, the identification of VPS13 as a lipid transport protein has opened the possibility of elucidating mechanisms of disease with potential implications for preventive and/or therapeutic strategies.

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between a subset of eukaryotic lipid transport proteins that act at membrane contact sites and proteins that transport lipids between the inner and outer membrane of Gram-negative bacteria.

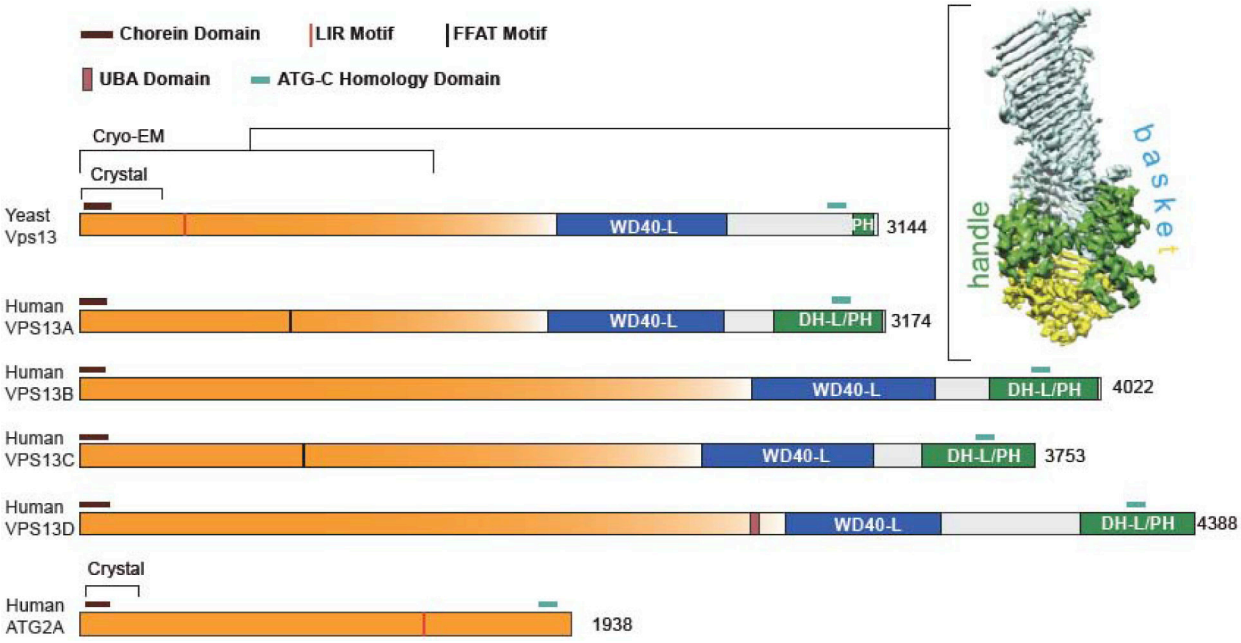


**Figure 1. Schematic representation of VPS13 protein domains and similarity to ATG2.**

The N-terminal half of VPS13 proteins, which folds into an elongated rod with a long hydrophobic groove along its length [18,21], is indicated in orange. Primary sequence similarities occur in the chorein and ATG-C domains. Portions of VPS13 corresponding to regions solved by crystallography [18] or Cryo-EM [21] in VPS13 from *Chaetomiun thermophilum* are indicated by brackets. The color gradient is meant to reflect the unclear C-terminal boundary of the rod solved by Cryo-EM, which may extend further. The entire ATG2 protein has structural similarities to the rod portion of VPS13, as shown by Cryo-EM [24]. The crystal structure of the N-terminal region of ATG2 (bracket) is nearly identical to the corresponding crystallized region of VPS13 [18,25]. A clear FFAT motif is present in VPS13A and VPS13C. VPS13D has an additional ubiquitin-associated domain (UBA in pink). Both yeast Vps13 and human ATG2A have LC3 interacting regions (LIR motif). The density map of the N-terminal region of VPS13 from *Chaetomiun thermophilum* solved by Cryo-EM is shown at top right, where the portion also solved by crystallography is shown in yellow. The “basket” is colored light blue and yellow, helices comprising the “handle” are green (from ref 21, reprinted with permission ©2020 Li et al. Originally published in JCB https://doi.org/10.1083/jcb.202001161).

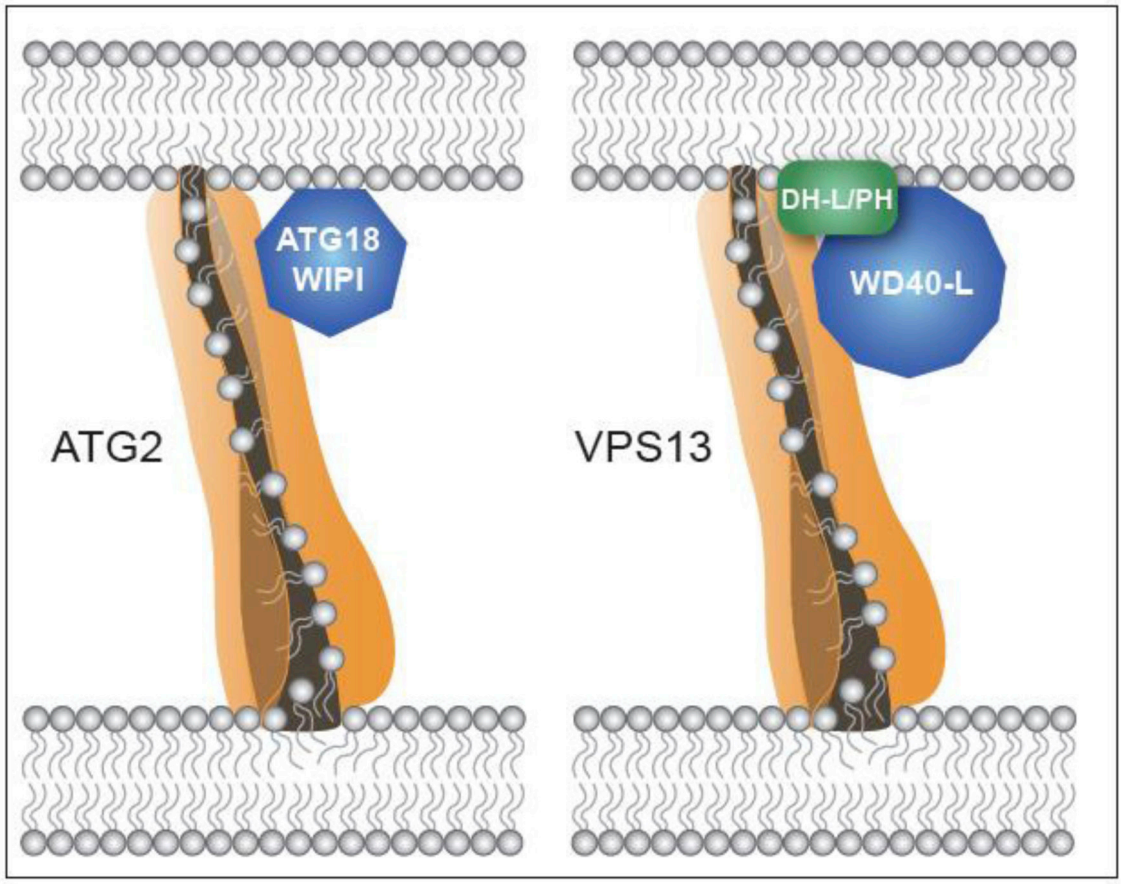


**Figure 2. Putative organization and lipid transport mechanism of VPS13 and ATG2 at membrane contact sites.**

Schematic cartoon illustrating how the N-terminal half of VPS13 and ATG2 could directly bridge two bilayers thus allowing membrane lipid flow between them. At least for VPS13A and VPS13C, the bottom membrane is represented by the ER membrane, where these two proteins are tethered via an interaction with the ER protein VAP. Anchoring to the target membrane is mediated by the WD40-L and DH-L/PH domain regions (Model based on Kumar *et al.* [18] and Li *et al.* [21]). ATG2 is proposed to have a similar organization at contacts between the ER and the pre-autophagosomal membrane. Anchoring to the target membrane is mediated by WD40 proteins ATG18/WIPI [26].

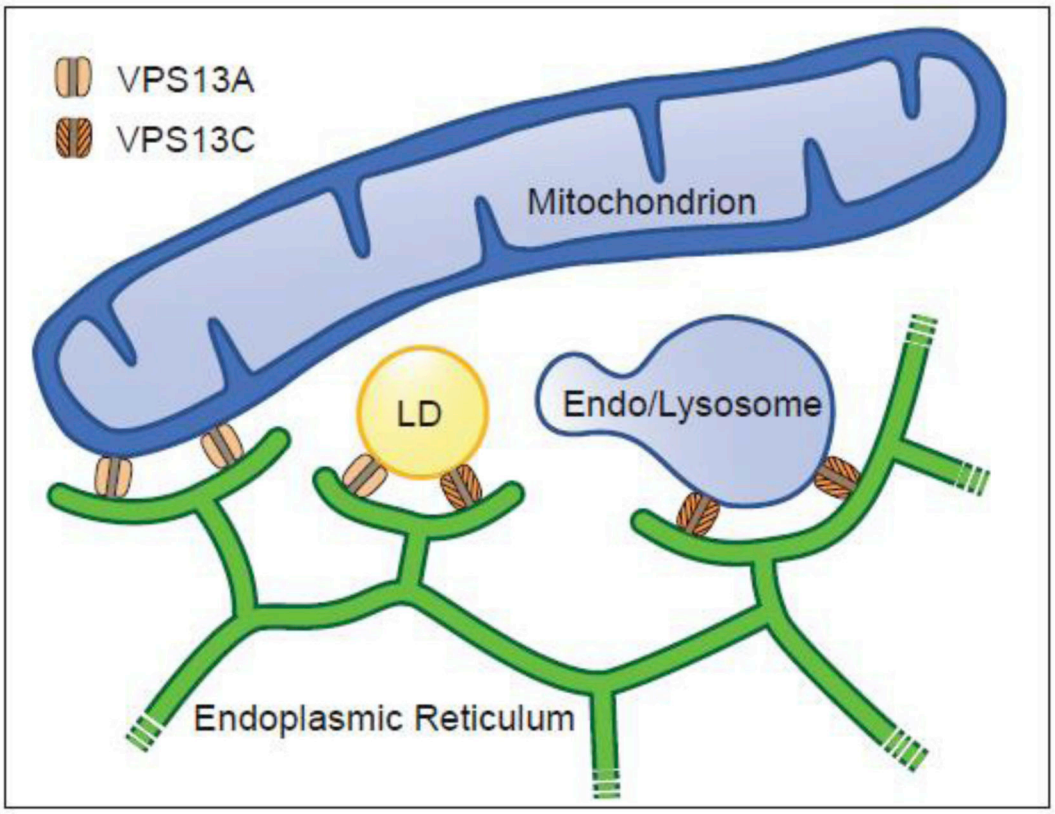


**Figure 3. VPS13 localization at membrane contact sites.**

Schematic drawing illustrating the localization of VPS13A and VPS13C at contacts between the ER and either mitochondria or endosomes/lysosomes respectively. Both proteins are also localized at contacts of the ER with lipid droplets (LD).