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SPOTLIGHT

VPS13: A lipid transfer protein making contacts at multiple cellular locations

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The evolutionarily conserved VPS13 proteins localize to multiple membrane contact sites though their function and regulation has been elusive. Bean et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201804111) found that competitive adaptors control the different localizations of yeast Vps13p, while Kumar et al. (2018. *J. Cell Biol.* https://doi.org/10.1083/jcb.201807019) provide biochemical and structural evidence for VPS13 proteins in the nonvesicular transport of phospholipids.

Cellular organelles in eukaryotes are characterized by distinct lipid compositions. The growth and expansion of organelles require targeted delivery of lipids directly or indirectly from their site of synthesis, the ER. Like membrane proteins, lipids can be transported between organelles by membrane-enclosed transport vesicles. There is also growing evidence that nonvesicular lipid transport represents a major transport route for most if not all lipids (Wong et al., 2017). Such nonvesicular mechanisms are critical for mitochondria, which are not known to be connected with the ER through transport vesicles. Lipid transfer proteins (LTPs) shuttle lipids from the donor membrane to the acceptor membrane through the cytosol during nonvesicular transport. Although different LTP families differ structurally, they all have a hydrophobic lipid-binding pocket that can shield the lipid cargo from the aqueous phase (Wong et al., 2017). Moreover, many LTPs have membrane-targeting domains that can facilitate the formation of or direct the LTPs to membrane contact sites (MCSs; Prinz, 2014), distinct regions between organelles that align within close proximity of one another with a gap of 10–30 nm. In general, MCSs are important domains where lipids and ions are—given the short distance—very efficiently exchanged between intracellular organelles.

VPS13 proteins are large, evolutionarily conserved proteins with a single orthologue (Vps13p) in the yeast *Saccharomyces cerevisiae* and four orthologues in mammals (VPS13A–D; Lang et al., 2015). In yeast, Vps13p localizes to two MCSs: the vacuole–mitochondria patch (vCLAMP) and the nuclear vacuole junction (NVJ) as well as to endosomes and the prospore membrane. The relative abundance of Vps13p at different sites varies with nutrient supply and growth stages, though how Vps13p localization is regulated is unclear. Vps13p appears to function in parallel with the ER–mitochondria encounter structure (ERMES), which tethers the ER to mitochondria and mediates lipid transfer. Gain-of-function mutations of *VPS13* can rescue the growth defect of ERMES mutants, and loss of both *VPS13* and ERMES results in synthetic lethality (Lang et al., 2015). Genetic and cell biological data suggest that through vCLAMP, Vps13p may represent an alternative pathway for delivering lipids to mitochondria. However, the precise function of Vps13p in mitochondrial lipid homeostasis remains to be elucidated. Notably, each of the four mammalian *VPS13* genes is associated with a genetic disorder: chorea acanthocytosis (*VPS13A*); Cohen syndrome, a condition characterized by global developmental delay and intellectual disability (*VPS13B*); an early onset form of Parkinson's disease (*VPS13C*); and a form of ataxia with spasticity (*VPS13D*). Understanding the molecular function of VPS13A–D and their precise cellular localization will shed light on the pathogenesis of these diseases.

In this issue, two studies provide mechanistic insights into both the localization and molecular function of VPS13 proteins as well as the role of LTP-mediated lipid transfer in organelle growth (Bean et al.; Kumar et al.). To understand mechanisms mediating Vps13p localization, Bean et al. (2018) examined large-scale datasets for possible Vps13p-interacting proteins. An endosomal protein Ypt35p was identified and confirmed to interact with Vps13p by colocalization and coimmunoprecipitation analyses. Notably, Ypt35p can recruit Vps13p to endosomes and the NVJ. Through truncation and mutagenesis analyses, Bean et al. (2018) identified an N-terminal PxP motif in Ypt35p as both necessary and sufficient for Vps13 recruitment, binding to the DUF1162 domain of Vps13p. Bean et al. (2018) extended these findings by identifying PxP motifs in Spo71p (a prospore membrane protein) and Mcp1p (a mitochondrial protein), demonstrating that their PxP motifs were required for interaction with Vps13p and for target-

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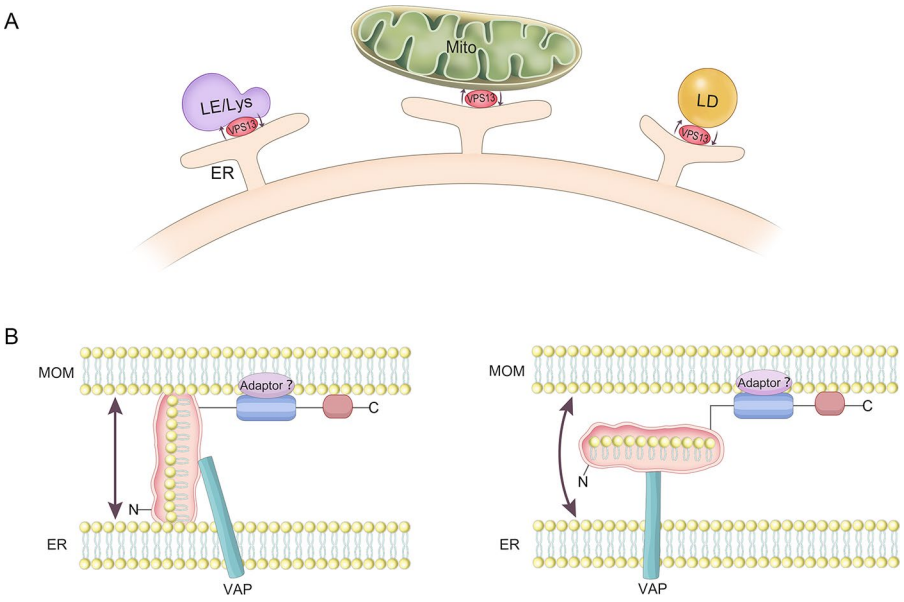

Figure 1. **Mammalian VPS13 proteins localize to different contact sites to mediate lipid transfer. (A)** VPS13 proteins localize to different MCSs. LE/Lys: late endosome/lysosome; Mito, mitochondrion. **(B)** Hypothetical models of VPS13A function as an LTP. Through interacting with VAMP-associated protein (VAP) on the ER as well as lipids and putative adaptor proteins on the mitochondria outer membrane (MOM), VPS13A localizes to the ER–mitochondria contact site. Left: The N-terminal domain of VPS13A may form a hydrophobic tunnel connecting two membranes, allowing glycerophospholipids to "slide" through. Right: VPS13A may function as a large carrier, shuttling glycerophospholipids between membranes.

ing Vps13p to membranes. Thus, Vps13p localizes to different subcellular locations via interacting with distinct adaptor proteins including Mcp1p, Spo71p, Ypt35p, and possibly more. Finally, Bean et al. (2018) manipulated the expression of Vps13p adaptor proteins and found that Vps13p concentrated with the higher expressing adaptor. Thus, the relative abundance of Vps13p adaptors may dictate the cellular distribution of Vps13p. Overall, this elegant study identified a novel Vps13p-interacting motif and unveiled an important mechanism by which Vps13p distribution may be controlled in response to environmental changes. It is worth noting that key residues in the DUF1162 regions of human VPS13B and VPS13D are associated with human diseases: the N2993S mutation of VPS13B causes Cohen syndrome, and the N3521S mutation of VPS13D is associated with a form of ataxia with spasticity. Thus, the interaction of VPS13 proteins with their adaptors could be important in disease settings. Future work is needed to identify putative VPS13 adaptor proteins in mammals.

In mammalian cells, Kumar et al. (2018) found that VPS13A primarily localizes to ER–mitochondria contacts, whereas VPS13C localizes to contacts between ER and the endolysosomal system. Both proteins were also found on lipid droplets (LDs; Fig. 1 A). Kumar et al. (2018) identified key regions of the proteins responsible for ER targeting, mitochondria targeting (VPS13A), endosome/lysosome targeting (VPS13C), and LD targeting. Notably, the ∼1,350-aa N-terminal VPS13α region was well conserved within the VPS13 family, and there was also homology between the most N-terminal ∼330-aa region of VPS13 (the Chorein_N domain) and other proteins including the autophagy protein ATG2. The yeast Vps13α region was purified and shown to bind glycerophospholipids but not sterols or ceramide. Interestingly, each purified Vps13α contains ∼10 glycerolipids, suggesting that Vps13α may bind and move multiple lipid molecules simultaneously. Indeed, FRET- and liposome-based in vitro transport assays confirmed Vps13α transfers fluorescently labeled phosphatidylserine (PS) and phosphatidylethanolamine between membranes. As a major breakthrough in the study of VPS13 proteins, Kumar et al. (2018) were able to resolve the structure of a Vps13 ($Vps13_{crystal}$; aa 1–335) fragment from the fungus *Chaetomium thermophilum*, which has a unique scoop-shaped structure, with the concave side lined with hydrophobic residues. Of particular interest is the large diameter of the hydrophobic cavity, which measures ∼20 Å across, consistent with the notion that Vps13p can accommodate several lipid molecules simultaneously. Furthermore, the full-length Vps13p contains several $Vps13_{crystal}$ -like modules, which may stack up to form an extended lipid-binding cavity (Fig. 1 B). Together, these biochemical and structural results support a direct role of VPS13 proteins in the transport of glycerophospholipids, which should help understand the different pathologies associated with mutations of VPS13 genes. Finally, given the homology between the N-terminal regions of VPS13 and ATG2, ATG2 may also function as a lipid transporter. Future efforts should be aimed at examining the role of ATG2 and other proteins with the Chorein_N homology domain in lipid transport and metabolism.

These results have broad implications in organelle expansion. Similar to mitochondria, vesicular transport from the ER to LDs has not been reported. Therefore, nonvesicular lipid transport pathways should play a major role in supplying lipids to LDs. During LD expansion, the need for surface phospholipids may be met by targeting key enzymes of phospholipid synthesis to LDs. VPS13A/C are the first LTPs found at ER–LD contacts, and they may therefore represent an alternative pathway to supply phospholipids to growing LDs. In autophagy, membrane contacts between the ER and preautophagosomal structure (PAS)/isolation membrane (IM) have also been observed. Thus, ATG2 may function at these sites to deliver phospholipids to aid the elongation of the PAS/IM and/or membrane closure. Interestingly, ATG2 also localizes to LDs (Velikkakath et al., 2012); thus, it is tantalizing to speculate that ATG2 may mediate the transfer of lipids between LDs and PAS/IM under certain conditions.

The structural and localization data supporting a role for VPS13 proteins as lipid transporters bring up a few key questions. The hydrophobic cavity of $Vps13_{crystal}$ is perhaps the largest among known lipid transport domains and could indeed

accommodate several lipid molecules at once. Therefore, it is highly likely that VPS13 may mediate the bulk transfer of glycerophospholipids. However, a major challenge in the study of nonvesicular lipid transport is to accurately measure the rate of lipid transfer. This challenge is further complicated by different modes of lipid transfer mediated by different types of LTPs. For instance, the oxysterol binding protein (OSBP) and OSBP-related proteins (ORPs) employ a counter-transport mechanism to mediate the presumable selective transfer of lipids one molecule at a time. With technological advances in the future, it would be interesting to compare the rates of PS transfer by VPS13 and ORP5/8 since both were reported to transfer PS (Chung et al., 2015; Ghai et al., 2017; Kumar et al., 2018). Most important will be to elucidate the potential role of the lipid transfer function of VPS13 proteins in vivo, especially in mammals. VPS13A-deficient cells are known to exhibit abnormal mitochondrial morphology and membrane potential (Glaß et al., 2018). Given the critical role of nonvesicular lipid transfer in mitochondrial lipid homeostasis, it will be interesting to assess how VPS13A may regulate mitochondrial lipids. Notably, VPS13A is the fourth mammalian protein with a lipid-binding domain reported to be localized at ER–mitochondria contact sites, after ORP5/8 and PDZD8 (Galmes et al., 2016; Hirabayashi et al., 2017). While a role for PDZD8 in lipid transfer remains unexplored, ORP5/8 was reported to transfer PS and regulate mitochondrial function. Thus, the impact on mitochondrial lipids and functional integrity may be assessed in cells deficient in VPS13A alone or in combination with ORP5/8 or PDZD8. Finally, future structural and biochemical studies should help determine whether VPS13 proteins form a hydrophobic tunnel bridging two membranes, allowing lipids to slide through, or function as lipid carriers that shuttle lipids between membranes (Fig. 1 B).

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