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SPOTLIGHT

A firehose for phospholipids

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All lipid transport proteins in eukaryotes are thought to shuttle lipids between cellular membranes. In this issue, Li et al. (2020. *J. Cell Biol.* https://doi.org/10.1083/jcb.202001161) show that Vps13 has a channel-like domain that may allow lipids to flow between closely apposed membranes at contact sites.

The many membranes of eukaryotic cells grow, shrink, and exchange their contents through vesicular and nonvesicular lipid transport systems. Vesicular transport is well-suited for the long-range motor-dependent movement of large amounts of lipid mixtures and accompanying membrane proteins. Non-vesicular systems rely on lipid-binding proteins and protein complexes localized to the closely apposed membranes of organellar contact sites. The latter class are ideally suited for the short-range transfer of specific classes of lipids between adjacent membranes. The capacity of this system is, however, limited. Li et al. (2020) aptly compare the typical short-range lipid transporter to a lidded teacup, ferrying one lipid molecule at a time between membranes. Sometimes it is necessary to rapidly transfer large amounts of lipid from one adjacent membrane to another, and lidded teacups are not enough. For example, the mammalian autophagosome grows adjacent to an ER domain known as the omegasome. The de novo formation of a 500-nm autophagosome over the course of 10 min requires the input of 4,000 phospholipid molecules per second from the omegasome. Sporulation in some yeasts is another example of a major membrane rearrangement that requires rapid lipid transfer.

To extend Li et al.'s metaphor, trying to do this with traditional lipid transporters would be like trying to put out a house fire with a bucket brigade of firemen using teacups. Evidence emerging from several laboratories (Maeda et al., 2019; Osawa et al., 2019; Valverde et al., 2019) suggests that the cell has solved this problem with a system more akin to a wide-bore firehose than a teacup. Li et al. have provided the clearest image yet of what this system looks like.

Traditional lipid transport proteins that shuttle one lipid monomer at a time (Fig. 1 A) may not be efficient or abundant enough to facilitate the rapid bulk lipid transport needed for events like sporulation and autophagy. Since the most efficient phospholipid-shuttling proteins, such as Osh6p in yeast, transport up to ∼0.2 phospholipids per second (Moser von Filseck et al., 2015), it would require at least 20,000 such transporters working at top speed to provide the 4,000 phospholipids per second required to grow an autophagosome. Lipid transport proteins that form tubes or hydrophobic conduits that bridge two membranes and allow lipids to flow between them would seem to be a more efficient way to move bulk amounts of lipids (Fig. 1 B). Bacteria have these types of transporters. The protein LptA, for example, has a hydrophobic cleft and multimerizes to form a hydrophobic channel between the inner and outer membranes of some Gram-positive bacteria. It binds the acyl chains of lipopolysaccharides, enabling them to flow between membranes (Suits et al., 2008). Similar lipid-transporting bridges had not been found in eukaryotes. Some eukaryotic lipid transport proteins, such as the extended synaptotagmins, have been proposed to function as bridges, but most evidence suggests they do not (Wong et al., 2019).

In this issue, Li et al. (2020) present compelling evidence that the eukaryotic protein Vps13 has a lipid-transporting channel that allows lipids to flow between membranes. VPS13s are very large proteins, typically ∼4,500 amino acids, found in all eurkaryotes. There are four in humans and mutations in some cause chorea acanthocytosis and some types of Parkinson's disease. There is some evidence that Vps13s transport lipids and are enriched at contact sites. Li et al. used single particle cryo-EM to solve the structure N-terminal 1390 amino acids of the Vps13 from the yeast *Chaetomium thermophilum*. Amino acids 131–1390 are rich in β-strands that form a long hydrophobic channel that could bind tens of phospholipids, with the acyl chains in the channel and hydrophilic headgroups extending into the cytoplasm. This study and a previous one by this group suggest Vps13 can indeed bind many lipids simultaneously.

These features support the idea that Vps13s transports lipids between membranes at contact sites, but do they shuttle lipids or do lipids flow from one membrane to another through the channel in Vps13s? To distinguish between these possibilities, Li et al. engineered a block in the channel by replacing a number of adjacent hydrophobic residues in the channel with charged

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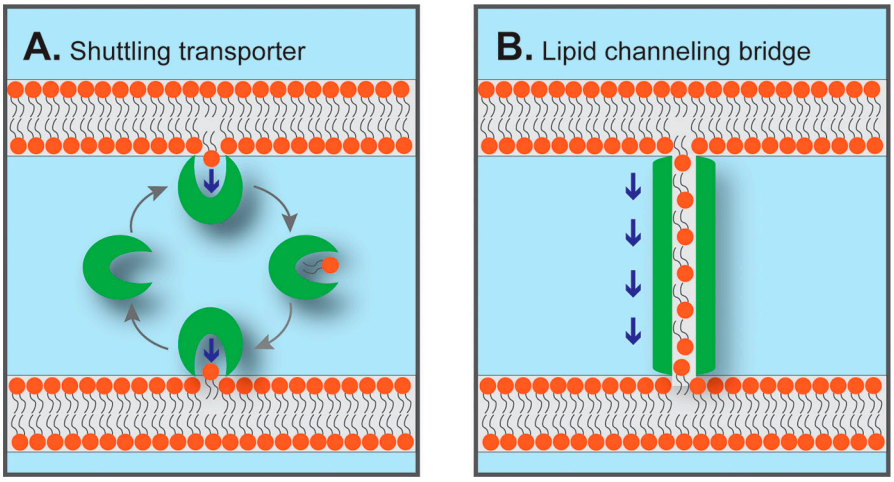

Figure 1. **Two types of intramembrane lipid transporters. (A)** Shuttling transporters move one lipid at a time between membranes. **(B)** Bridging transporters allow lipids to flow between membranes though a hydrophobic tunnel or conduit.

residues. This changed only affected one part of the channel and did not prevent lipids from being bound by Vps13, they found. If Vps13 functions as a channel, the mutations should render it nonfunctional because they would prevent lipids from flowing through the channel. On the other hand, if Vps13 shuttles lipids between membranes, the lipids would remain stationary in the proteins, and occluding part of the channel would not prevent Vps13 from transporting lipids. To assess whether the mutant Vps13 is functional, Li et al. determined whether the mutant proteins support prospore formation in yeast. During sporulation of yeast, there is a massive membrane reorganization, and it has previously been found that sporulation is blocked in cells lacking Vps13 (Park and Neiman, 2012). The mutant Vps13 proteins did not support sporulation, even when overexpressed, but retained the ability to fold and localize properly. This is strong evidence that lipids must be able to flow through the Vps13 channel for the protein to function. However, it should be noted that this indirect evidence, and a direct demonstration that lipids flow through Vps13 remains an important task for the future.

Interestingly, previous work from this group and others has shown that a protein required for autophagy, Atg2, has a similar long hydrophobic channel (Maeda et al., 2019; Osawa et al., 2019; Valverde et al., 2019). The questions as to the lipid source for autophagosome expansion has been one of the longest running debates in the autophagy field, and the answer finally seems to be coming into view. One of the most interesting questions is what provides the thermodynamic driving force for transfer. In the case of the autophagosome, fatty acids are synthesized locally at sites of expansion (Schütter et al., 2020). An appealing model is that localized synthesis of phospholipids in the omegasome/ER close to ATG2 proteins generates a local excess of phospholipids, which is relieved by transfer through the ATG2 tunnel.

This study raises a number of fascinating questions about the mechanism of lipid transport by Vps13. One is whether Vps13 must bind two membranes simultaneously to function; alternatively, it could bind the two membranes at a contact site sequentially. Rapid improvements in cryoelectron tomography of cellular samples may provide the answer before long. Another question arises from a puzzling feature of the channel: it is about twice as wide at one end than at the other. Perhaps this helps facilitate lipid loading into one end of the channel. How lipids enter and exit the tunnel and what provides the driving force for transport are other important issues that remain to be resolved. Another important question is how the rate and selectivity of lipid movement though the channel are controlled. Li et al. have given us the most detailed picture yet of the firehose of bulk intracellular lipid trafficking.

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