HHS Public Access

Author manuscript

Contact (Thousand Oaks). Author manuscript; available in PMC 2022 September 19.

Published in final edited form as:

Contact (Thousand Oaks). 2022 ; 5: . doi:10.1177/25152564221103080.

Moving Lipids, by the Numbers

Pascal F. Egea

Department of Biological Chemistry, School of Medicine, University of California Los Angeles, Los Angeles, USA

Lipid trafficking in eukaryotic cells can follow vesicular and non-vesicular pathways (Antonny et al., 2018). However, some organelles do rely exclusively on non-vesicular trafficking to obtain or distribute some of the lipids essential to their functions and biogenesis. In the past decades membrane contact sites between organelles have emerged as hubs for the non-vesicular trafficking of lipids, highlighting the importance of lipid transfer proteins (LTPs), a large functional class of structurally diverse proteins involved in this process (Egea, 2021).

In a previous issue of *Contact*, Zhang et al. (Zhang et al., 2022) review two different types of LTPs, shuttles and bridges, that transfer lipids between heterotypic membranes using distinct mechanisms (Figure 1) and develop some physical and mathematical kinetic models to better describe lipid transfer in qualitative *and* quantitative terms. While shuttle LTPs such as Osh4 extract specific lipids from membranes and ferry them back and forth across the aqueous space separating the close yet physically distinct membranes, bridge LTPs such as Atg2 form continuous proteinaceous nanotubes through which lipids are unidirectionally channeled in single file from one organelle to the other, thus bypassing the protein diffusion step.

Rates of lipid transfer have been primarily measured using recombinant proteins and fluorescent lipids substrates reconstituted in liposomes acting as membrane surrogates (Wong et al., 2017). More recently, single molecule methods such as optical tweezers, DNA origami, total internal reflection fluorescence and atomic force microscopies, have enabled a better understanding of some of the physicochemical (*i.e.*, membrane composition) and geometrical parameters (*i.e.*, distance between membranes, tethering interactions and protein domain dynamics) controlling lipid transfer (Bian et al., 2019). Nevertheless, we lack a clear qualitative and quantitative understanding of *all* the physico-chemical forces driving lipid fluxes at the molecular and cellular levels. Numbers simply do not add up as rates measured *in vitro* do not match the lipid fluxes estimated *in vivo*. These discrepancies observed for both shuttle and bridge LTPs have been discussed in more detail in the case of yeast mitochondrial contact sites (Petrungaro & Kornmann, 2019) and are rooted in our

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

incomplete understanding of the physicochemical parameters controlling protein-assisted lipid exchange at the molecular level but also in our incomplete survey of all existing (and redundant) trafficking pathways at the cellular level (John Peter et al., 2022).

Zhang et al. first provide an explicit kinetic model to quantify the specific counter-exchange of lipids by a shuttle LTP such as Osh4 (Figure 1A). The Authors assume that molecules of shuttle are predominantly bound to either one of their two cognate lipid substrates while the amount of *apo* forms (*i.e.*, membrane-bound and cytosolic forms) remain negligible. Furthermore, the two exchange reactions at the membranes follow simple bimolecular kinetic laws and are the rate limiting steps while protein diffusion between membranes is not a limiting factor (Figure 1B). These assumptions constitute a simplification of the prior analytical models developed by other groups; with these approximations, the analytical solutions of the kinetic equations fit experimental data obtained using liposomes and purified proteins and show that counter-exchange of two lipids occurs even against a concentration gradient and without apparent energy expenditure.

The Authors next elaborate on the case of the Atg2 bridge LTP involved in the formation and growth of autophagosomes, large double membrane structures that engulf cellular content destined for degradation and recycling. Autophagosome biogenesis is a complex and highly coordinated process involving a cascade of different Atg proteins (Nishimura & Tooze, 2020). Among these, the scramblase Atg9 and the membrane-bending and ubiquitin-like Atg8 (Martens & Fracchiolla, 2020) play important roles in phagosome formation and maturation (Figure 2A). Zhang et al. estimate that an average 400 nm diameter autophagosome growing in about 10 min requires some 3 million lipids; this implies lipid transport rates of about 5,000 lipids/autophagosome/second *in vivo*. Although Atg2 is capable of binding about 20 glycerophospholipid molecules (Valverde et al., 2019), the apparent rates of transfer of about 0.02–1 lipid/molecule/second measured *in vitro* fall dramatically short (Maeda et al., 2019; Osawa et al., 2019). How bridge LTPs like Atg2 accomplish fast unidirectional lipid transfer to grow large membranous structures like the autophagosome on relatively short time scales thus remains a key problem to be solved.

To address this discrepancy Zhang et al. introduce and somehow formalize the contribution of physical forces (Sitarska & Diz-Munoz, 2020) such as membrane tension and osmotic membrane tension (OMT) linked to the concentration of membrane proteins (MPs) as forces driving transfer. Atg2 acts as a molecular sieve, transferring only glycerophospholipids but excluding MPs. Membrane tension is linked to MP concentration, lipid composition/packing and membrane curvature. As osmotic membrane tension increases with MP concentration and higher membrane curvature, the ensuing gradient of tension could drive lipid transport from ER to a nascent phagosome decorated with Atg8 (Figure 2B). This mechanism is very similar to the Marangoni flow observed and described in rigorous mathematical terms for lipid nanotubes connecting two vesicles (Dommersnes et al., 2005) where surprisingly, transfer will initially proceed towards the vesicle of highest surface tension (*i.e.*, the nascent phagosome) until Poiseuille flow counteracts it.

Based on these assumptions and for the range of OMTs estimated to prevail in living cells, the Authors now extrapolate rates of lipid transfer across the 200 Å-long Atg2 protein bridge

of about 34 to 3,400 lipids/bridge/second compatible with the fast and large dimensional expansion occurring during autophagosome biogenesis. Albeit, the contributions of MP concentration and OMT discussed by Zhang et al. remain to be further quantified and characterized in experimental detail. This aspect should prove challenging as both donor and acceptor membranes have complex lipidic and proteinaceous compositions. While the ER donor compartment, subdivided into sheet and tubular domains, contains a vast array of MPs performing architectural, signaling or catalytic functions, different effectors proteins such as Atg8, dynamically decorate the phagosomal acceptor membrane throughout its biogenesis and can thus affect its curvature and surface tension to generate a surface tension gradient that favors the vectorial transfer of lipids towards the phagosome. Nevertheless, proper experimental estimation of such OMT gradients is currently difficult. Last, other factors such as local viscosity or the number of Atg2 molecules recruited/needed for the biogenesis of one phagosome are unknowns.

Lipid gradients, membrane asymmetry and MPs such as scramblases (Maeda et al., 2020; Matoba et al., 2020) that counteract these gradients (Figure 1B) also contribute to bridge-mediated lipid transfer by creating a ‘lipid molecular sink’ favoring the fast transfer from donor to acceptor membranes through fast equilibration (up to ~10,000 lipids/molecule/second) of lipid concentrations between leaflets in both donor and acceptor membranes; in any case the identification of ER *and* phagosomal scramblases already constitutes a step forward towards a more holistic description of organelle biogenesis mediated by bridge LTPs (Ghanbarpour et al., 2021).

These tantalizing predictions should be tested both *in vitro* and in numerical simulations. In particular a better understanding of (*i*) the dynamics of lipid and solvent/ion molecules along the protein tunnel or groove and (*ii*) the passive and/or active roles played by other MPs, are required to validate these assumptions. The use of more sophisticated reconstituted liposomal systems including other effector proteins (Landajuela et al., 2016) and also fluorescent lipid tension reporters (Colom et al., 2018) to detect fluctuations in lipid packing/membrane curvature resulting from a combination of membrane tension (osmotic pressure) and lipid composition changes should help probing such assumptions. Better physical modeling of the influence of effector protein-induced membrane curvature should also improve our understanding of these processes (Sakai et al., 2020).

The recent progress in artificial intelligence-based protein structure prediction achieved by *AlphaFold* (Jumper et al., 2021; Tunyasuvunakool et al., 2021) together with the advent of other sophisticated computational tools enabling molecular dynamic simulations on complex macromolecular ensembles such as membranes and protein complexes (Baaden, 2019; Enkavi et al., 2019) could also allow testing the hypothesis and models posited by Zhang *et al.* These physicochemical rules might also apply to other recently described bacterial ‘nanotube’ LTPs such as the bacterial lipophilic envelope-spanning tunnel Let (Isom et al., 2020). In a broader perspective, the models described in this work pave the way to a flurry of experiments combining biophysical and computational methods to better understand the complex processes involved in membrane-bound organelle biogenesis.

Acknowledgments

P.F.E thanks Professor Jean-François Joanny from the Institute Curie in Paris for his comments and suggestions on the manuscript.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the National Institute of General Medical Sciences, (grant number GM120173).

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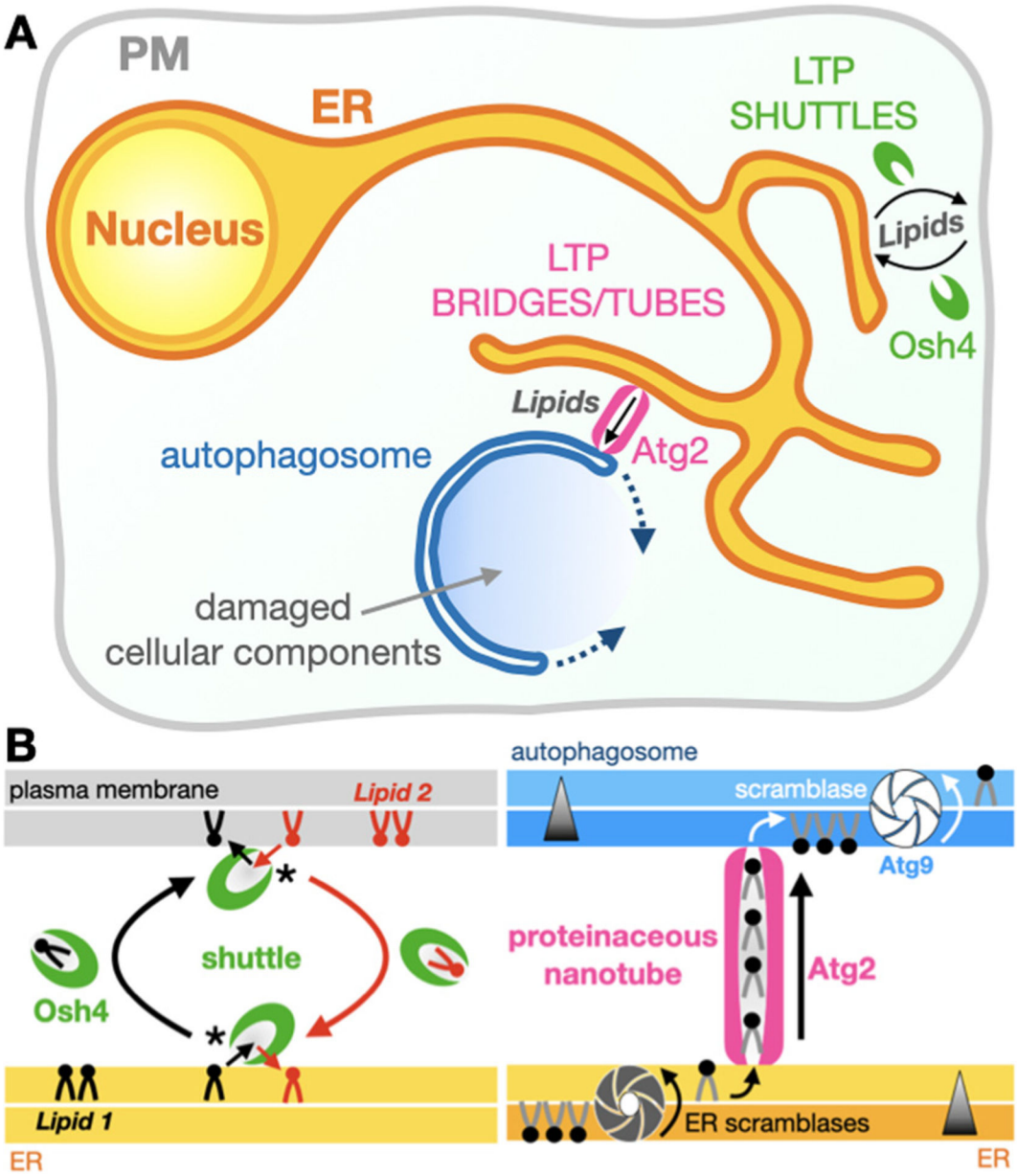

Figure 1.

Lipid shuttles and lipid bridges mediate different aspects of cellular membrane dynamics. A: Examples of non-vesicular lipid transfer by lipid shuttles and lipid bridge function at ER-to-PM and ER-toautophagosome contact sites, respectively. B: The Osh4 lipid shuttle can discretely counter-exchange two specific lipids between membranes thus finely tuning membrane composition and maintaining organelle membrane identity, asymmetry and polarity within cells. A protein bridge such as Atg2 functions as a proteinaceous nanotube transferring lipids in bulk with lesser specificity but at substantially faster rates to support the rapid expansion of large membrane-enclosed structures such as autophagosome and other organelles. *Note*. ER = endoplasmic reticulum; PM = plasma membrane.

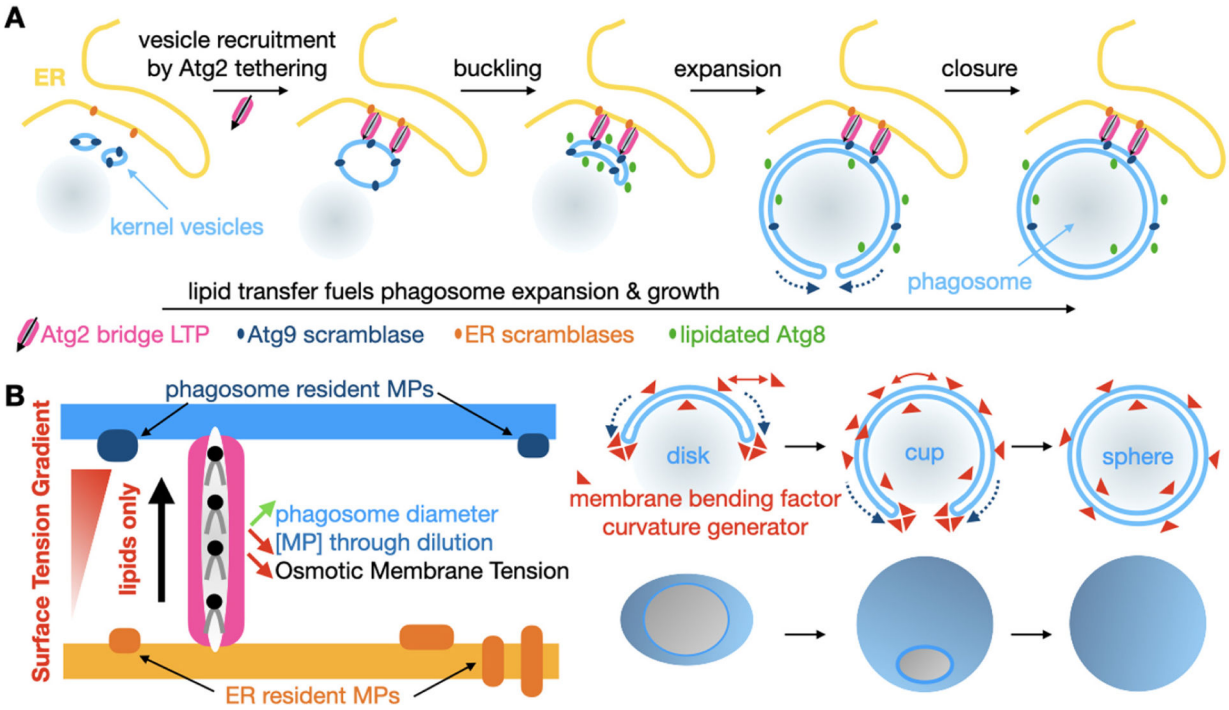

Figure 2.

Factors influencing fast bulk lipid transfer through bridge LTPs. A: Autophagosome formation and growth. A phagosome initiates from small vesicles containing scramblase Atg9. Following their Atg2-mediated tethering to the ER and acquisition of lipidated Atg8, bulk glycerophospholipid transfer through Atg2 fuels rapid expansion of these vesicles. B: Effects of vesicle growth on membrane protein (MP) concentration and osmotic membrane tension (OMT). A LTP bridge selectively transfers some lipids but excludes proteins. Local membrane protein concentration and membrane curvature affects surface tension. A gradient of surface tension between donor and acceptor membrane initially drives lipid transfer at rates compatible with phagosome expansion in vivo.