

SPOTLIGHT

Thank ORP9 for FFAT: With endosomal ORP10, it's fission accomplished!

 Louise H. Wong*, Andrea Martello*, and Emily R. Eden

Heterogeneity in endosomal membrane phospholipid content is emerging as a regulator of endocytic trafficking pathways. Kawasaki et al. (2021. *J. Cell. Biol.* <https://doi.org/10.1083/jcb.202103141>) demonstrate exchange of endosomal PI4P for PS by ORP10 at ER-endosome contact sites, with the consequent recruitment of endosomal fission factors.

Most cellular lipids are synthesized in the ER, often undergoing rapid redistribution to other cellular membranes, thereby maintaining low concentrations at the ER. Consequently, lipids exiting the ER may need to be transported against their concentration gradient. Lipid flow along a gradient to the ER can drive countertransport of ER-derived lipid to membranes with a higher lipid concentration. This nonvesicular lipid exchange occurs at membrane contact sites (MCS), where different organelles are closely apposed, providing a platform for lipid transport proteins including oxysterol-binding protein (OSBP)-related proteins (ORPs). Lipid specificity, which varies between ORPs, is defined by the OSBP-related domain (ORD). The ORD of ORP10 shares phosphatidylinositol-4-phosphate (PI4P) and phosphatidylserine (PS) binding residues with ORP5/8 and can bind and extract PS from liposomes (1), suggesting a potential role in PI4P-PS counter transport, analogous to that of ORP5/8 at ER-plasma membrane MCS (2). ORPs are targeted to specific organelles by interaction between their PH domain and membrane phospholipids. Most ORPs also possess a FFAT motif (two phenylalanines in an acidic tract), which simultaneously targets the ORP to ER-localized VAMP-associated proteins (VAPs) at MCS between the ER and other organelles. ORP10, however, lacks a FFAT motif, yet was found to stabilize ER-Golgi

MCSs (Fig. 1 A) for PI4P transport to the ER (2). Kawasaki et al. have now uncovered a novel function for ORP10 in PI4P-PS lipid exchange at the ER-endosome interface (Fig. 1 B), with downstream effects on endosomal fission and retrograde transport (3).

The PH domain of ORP10 selectively binds PI4P and is required for ORP10 recruitment to the TGN (2) and endosomes (3), both home to PI4KIIα, a PI4P-producing kinase. Rapid PI4P degradation at the ER by the phosphatase Sac1 generates a PI4P gradient at the ER-endosome or TGN interface, with PI4P flow to the ER driving countertransport of PS to the endosome (as also predicted for the Golgi). Activity of endosomal PI4P phosphatase Sac2 (4) may hamper formation of an endosome-ER PI4P gradient, but since ORP10 did not colocalise well with Sac2 (3), they likely function at different endosome populations.

PS synthesis at MCSs may also contribute to ORP10-mediated lipid exchange. Targeting PS synthase to ER:mitochondria contacts in yeast was found to promote PS transport out of the ER to mitochondria (5). Similarly, ER to endosome PS transport was increased when PS synthase was targeted to ER:endosome MCS. Localized PS gradients from PS synthesis in the ER at MCSs, coupled with rapid decarboxylation of PS to phosphatidylethanolamine (PE) in mitochondria/endosomes by yeast PS decarboxylases Psd1/Psd2, could contribute

to lipid exchange. In mammalian cells, though, since no endosomal decarboxylase has been identified, ORP10-mediated lipid exchange is likely to be primarily driven by the PI4P gradient. Whether this process is facilitated by localized activation of PS synthase at MCS has not yet been demonstrated. Since PS synthase activity is negatively regulated by PS, exit from the ER is a key factor in its biogenesis. Recruitment of specific ORPs to endosomes/TGN by PI4P for ER tethering and consequent lipid exchange provides an elegant regulatory pathway for PI4P-PS homeostasis in cellular membranes.

ORP10 shares functional similarities with ORP11: both proteins comprise an N-terminal PH domain and a C-terminal ORD, with a linker region in between harboring a coiled-coiled domain. Unlike other ORPs, ORP10 and ORP11 possess neither a FFAT motif nor a membrane spanning domain to enable ER interaction, but heterotypic interaction with ORP9, which does contain a FFAT motif, has been demonstrated for both proteins. Kawasaki et al. identified an ORP9-ORP10 interaction at ER-endosome MCSs that is dependent on the ORP10 linker region. ORP9 was also implicated in ORP10-mediated lipid exchange at the TGN, where it may play a redundant role with OSBP in maintaining ER contact. Similarly, ORP11 is also recruited to the TGN and, to a lesser extent, the endosome, by ORP9, with the interaction

University College London Institute of Ophthalmology, London, UK.

*L.H. Wong and A. Martello contributed equally to this paper; Correspondence to Emily R. Eden: e.eden@ucl.ac.uk.

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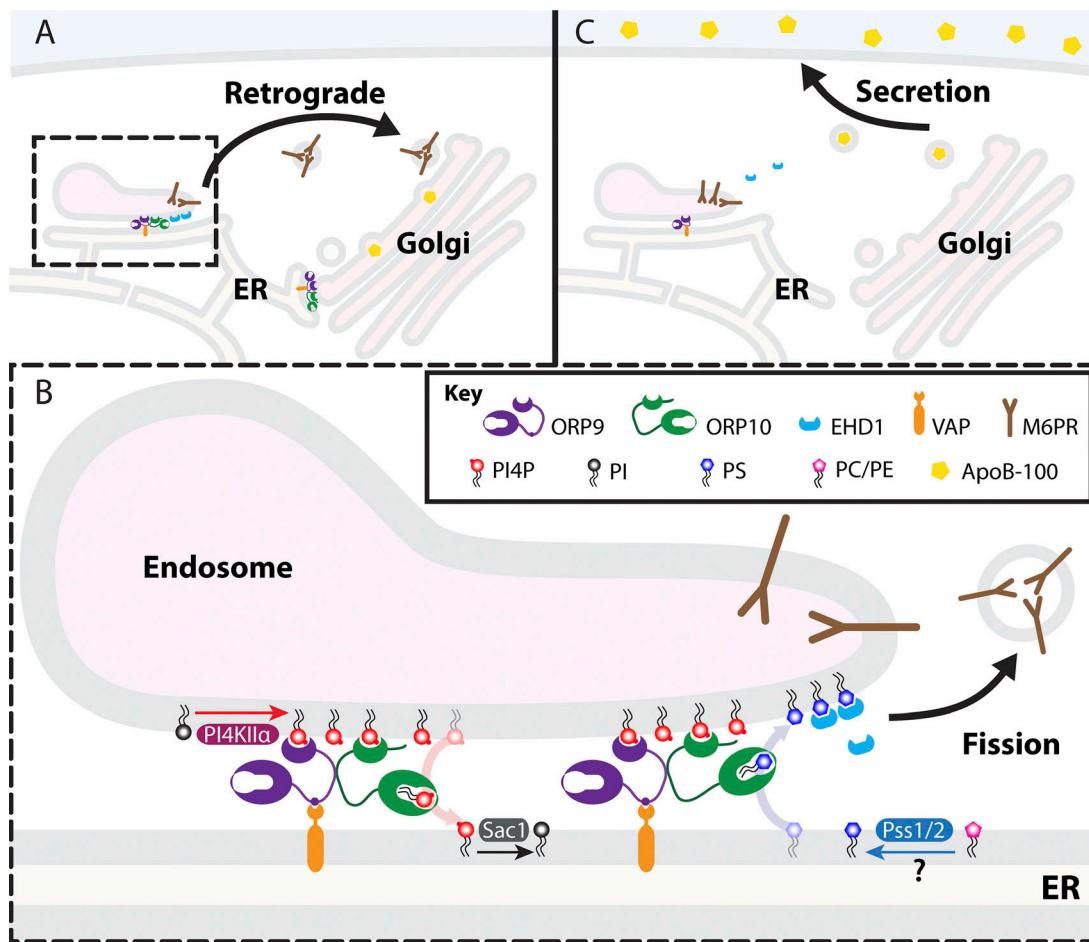


Figure 1. Regulation of retrograde and secretory traffic by ORP10-mediated lipid exchange. **(A)** ORP10 interacts with VAP-bound ORP9 at ER–endosome and ER–Golgi MCSs, with downstream effects on retrograde transport of mannose 6-phosphate receptor (M6PR). Boxed region (detailed in B) depicts ORP10 at the ER–endosome interface. **(B)** ORP10 functions in lipid exchange between the ER and endosomes, transporting endosomal PI4P to the ER in exchange for ER-derived PS. Production of PI4P in endosomes by PI4KIIa-dependent phosphorylation of phosphatidylinositol (PI), coupled with its consumption in the ER by ER-localized Sac1, generates a PI4P concentration gradient from the endosome to the ER. Low membrane PS concentrations in the ER are maintained by PS inhibition of PS synthesis from phosphatidylcholine (PC) by Pss1 or from PE by Pss2, with PS synthesis at ER–endosome contact sites promoting rapid PS export from the ER in yeast (not yet known if a similar mechanism operates in mammalian cells). ORP10 mediates PI4P transport along its gradient to the ER, driving countertransport of PS by ORP10 against its concentration gradient to the endosome. PS enrichment at the endosome leads to recruitment of the ATPase EHD1 to facilitate endosome fission for retrograde transport. **(C)** Depletion of ORP10 prevents lipid exchange at ER–endosome contact sites, resulting in a loss of retrograde transport of M6PR. Additionally, ER–Golgi MCSs are diminished, and secretion of ApoB-100 is increased.

depending on the linker region of both proteins (6).

The finely tuned regulation of PI4P/PS is emerging as an important determinant of endocytic traffic. Previous studies have shown that endosomal PI4P accumulation inhibits retrograde transport from endosomes to the TGN (7), while endosomal PS regulates endosome to Golgi retrograde traffic. As depicted in Fig. 1 B, Kawasaki et al. have built on this to show that through interaction with VAP-bound ORP9, ORP10 mediates lipid countertransport at ER–endosome MCSs, removing PI4P from, and

supplying PS to, the endosome, with consequent recruitment of the membrane scission protein EHD1 to control endosomal fission and retrograde transport (3). Spatial and temporal regulation of endosome fission by ER–endosome MCSs involves recruitment of the ER membrane protein TMCC1 to the budding endosome by the actin regulator Coronin 1C, stabilizing the MCS (7), but the mechanism by which MCS might effect scission has remained elusive. The findings of Kawasaki et al. present an explanation: by providing a platform for lipid exchange, MCSs promote the recruitment of EHD1, which

belongs to a conserved class of ATPases that can oligomerise in ring-like structures around tubules to mediate fission (8). VAP interaction with OSBP at ER–endosome MCSs is also required for retrograde transport (7), but potential redundancy between ORP9/OSBP in ORP10-mediated lipid exchange, or if ORP10 functions at Coronin 1C/TMCC1-regulated MCS is not yet established.

Interestingly, ORP10 function at the TGN has been implicated in regulating ApoB-100 secretion (Fig. 1 C), with hypersecretion reported in ORP10-depleted cells (9). FFAT1, which promotes PI4P consumption by Sac1

at ER:TGN contacts, also negatively regulates ApoB-100 exit from the TGN in a PI4KIII β -dependent manner, suggesting direct regulation of ApoB-100 secretion by PI4P at the TGN (9). Could PI4P coordinate nutrient sensing with cargo sorting and secretion at the TGN? PI4P has been described as lipid biosensor of cytosolic pH, with protonation of its head group regulating protein interactions (10). The influence of cytosolic pH on ORP10-PI4P interaction may provide an additional layer of regulation of lipoprotein secretion in response to changes in cellular energy/pH.

How ORP10 function is coordinated at Golgi and endosomal membranes and the significance of potential redundancy with

ORP11 remains unclear. The regulation of Sac2 activity and how it relates to ER-endosome lipid exchange is also intriguing. While questions still remain, an important role for ORP10 is emerging in maintaining homoeostasis between endosome maturation, retrograde traffic and secretory transport.

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