Regulation of Endosomal Sorting and Maturation by ER-Endosome Contact Sites

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Abstract
Endosomes are a heterogeneous population of intracellular organelles responsible for sorting, recycling, or transporting internalized materials for degradation. Endosomal sorting and maturation are controlled by a complex interplay of regulators, with RAB GTPases and phosphoinositides playing key roles. In this decade, another layer of regulation surfaced with the role played by membrane contact sites between the endoplasmic reticulum (ER) and endosomes. Specific regulators of ER-endosome contact sites or proteins localized at these sites are emerging as modulators of this complex endosomal ballet. In particular, lipid transfer or recruitment of various complexes and enzymes at ER-endosome contact sites play an active role in endosome sorting, scission, and maturation. In this short review, we focus on studies describing ER-endosome contact sites in these three endosomal processes.

Keywords
membrane contact sites, endosomes, endoplasmic reticulum, ORP10, TMEM16K, phosphoinositides, OSBP TMCC1, PIK4lla/β

Introduction
Cells constantly exchange molecules with the extracellular environment, and internalized macromolecules transit through sequential vesicular organelles called endosomes. Endosomes are often classified into three populations: early, late, and recycling (Huotari & Helenius, 2011). This classification is mostly related to the presence of specific RAB GTPases and phosphoinositides, as well as to endosomal morphology. Early endosomes receive the bulk of internalized materials (Cullen & Steinberg, 2018). These tubulovesicular organelles harbor heterogeneous subdomains (van der Beek et al., 2022) where various independent sorting events unfold (Figure 1A) (Naslavsky & Caplan, 2018). Sorted cargos are then directed towards the trans-Golgi network (TGN), plasma membrane, or recycling endosomes (Cullen & Steinberg, 2018). As sorting occurs, endosomal subdomains mature, resulting in a late endosome/multivesicular body (Podinovskaia et al., 2021) that ultimately fuses with a lysosome (Figure 1A) (Huotari & Helenius, 2011). Endosomal sorting, mainly regulated by four endosomal sorting complexes, as well as recycling, and maturation were mostly believed to be independent of other organelles. However, ER-endosome membrane contact sites (MCSs) are now emerging as platforms that modulate these events. In this short review, we discuss the roles and involvement of ER-endosome MCSs in endosomal trafficking and maturation (Figure 2A).

Role of Oxysterol-Binding Protein (OSBP)-Related Proteins (ORPs) at Membrane Contact Sites in Endosomal Scission
Membrane-contact sites are areas where independent organelles are close enough to allow direct communication between them (Prinz et al., 2020). The proximity between organelles permits directed non-vesicular lipid transport and modulation of signaling events, among other processes (Balla et al., 2019; Raiborg et al., 2015b; Wong et al., 2019). ORPs are a class of lipid transfer proteins (LTPs) that mediate lipid exchange at MCSs (Nakatsu & Kawasaki, 2021). They do so by binding and transferring lipids via the OSBP-related domain (ORD), which recognizes various ligands, including oxysterol, cholesterol, phosphoinositides, phosphatidylserine (PS) (Nakatsu & Kawasaki, 2021), and potentially phosphatidylcholine (PC)

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(D’souza et al., 2020; Tong et al., 2021). Interestingly, ORPs mediate the counter-directional transport of lipids; PtdIns(4)P, in particular, drives the transport of another lipid against a concentration gradient (Mesmin et al., 2013). ER-endosome MCSs are thus important regulators of PtdIns(4)P level in cells, since PtdIns(4)P at endosomes will be transported by ORPs to the ER. Here, the lipid phosphatase SacI will dephosphorylate PtdIns(4)P to yield phosphatidylinositol (PI), ensuring a PtdIns(4)P concentration gradient between endosomes and the ER (Nakatsu & Kawasaki, 2021). ORPs are associated with various ER-organelle MCSs through the concerted action of two determinants: 1) a PH domain that interacts with a specific phosphoinositide and 2) an FFAT motif that binds to the ER-localized (VAMP)-associated protein A (VAPA)/VAPB (Antonny et al., 2018); however, how ORP localization, activity, and functions are regulated is an ongoing question.

Intriguingly, a role in endosomal scission was demonstrated for ORP10, an unconventional ORP that lacks a canonical FFAT motif (Kawasaki et al., 2022). ORP10 was initially identified as a regulator of ER-TGN contact sites (Venditti et al., 2019). The authors demonstrated, through a FRET-FLIM assay measuring ER-TGN contact sites (Venditti et al., 2019), that ORP10 depletion resulted in a decreased occurrence of ER-TGN contact sites. The ability of ORP10 to bind both PtdIns(4)P and PS was required for the occurrence of ER-TGN contact sites (Venditti et al., 2019). An assessment of ORP10 localization through over-expressed fluorescently tagged proteins revealed a predominant Golgi localization in HeLa cells, consistent with a PtdIns(4)P preference for its PH domain (Venditti et al., 2019) and its role in ER-TGN MCS maintenance. However, using 3xHA-tag to generate ORP10-knockin Cos7 cells, Kawasaki et al. (2022) identified a more restricted localization pattern, where ORP10 accumulated in puncta associated with ER tubules. Interestingly, these puncta were colocalized with endosomal proteins, such as RAB5, RAB7, and PI4KIIα, suggesting a potential role for ORP10 in ER-endosome MCSs.

PtdIns(4)P is present in endosome subdomains and most abundant in late endosomes (Baba et al., 2019). This endosomal PtdIns(4)P pool is mostly generated by PI4KIIα/β. Endosomal ORP10 localization is dependent on its PH domain and requires endosomal PtdIns(4)P (Kawasaki et al., 2022). The ER association is contingent on the ORP10 interaction partner ORP9, through a VAPA/B-ORP9 association (Figure 2B). It is worth noting that ORP10 was previously identified to associate with VAPA at the ER by bimolecular fluorescence complementation (BiFC), through an indirect interaction (Weber-Boyvat et al., 2015), alluding to ORP9 as a general modulator of ORP10 ER recruitment. Both in vitro and in vivo experiments

**Figure 1.** Endosomal sorting complexes and maturation. An ER-endosome MCS independent view. (A) Following clathrin-dependent, clathrin-independent, or CLIC/GEEC endocytosis, endocytic vesicles reach Rab5-enriched early endosomes, where cargos are sorted by various sorting complexes (e.g., retromer; retriever, ccc complex and ESCPE) which cooperate with the WASH complex to direct cargos to a specific location (trans-Golgi network (TGN), plasma membrane (PM), or Rab11 recycling endosomes (RE)), adapted from (Simonetti & Cullen, 2019). Rab7-enriched maturation (degradative) subdomains are also formed on these endosomes, which ultimately mature to RAB7-positive late endosomes/multivesicular bodies (LE/MVB) and fuse with lysosomes.
Figure 2. Specific ER-endosome MCSs in cargo sorting and scission. (A) Various ER-endosome MCSs with early endosome (EE), recycling endosome (RE) and late endosome (LE). (B) ORP10 cooperates with ORP9-VAPA/B to mediate PtdIns(4)P and PS counter-transport to regulate endosomal PS level, EHD1 recruitment and tubule scission. Adapted from (Wong et al., 2022) (C) ATP8A1 at recycling endosomes, increases PS cytosolic-leaflet levels and EHD1 recruitment. The grey dotted arrow indicates hypothetical PS transfer between the ER and recycling endosomes. (D) Potential independent or cooperative roles played by PtdIns(4)P, WASH complex, and EHD1 in cargo sorting and tubule scission at ER-endosome MCSs. The lower tubule displays the situation described by Dong et al. (2016), where an OSBP-VAPA/ B-SNX2 complex regulates endosomal PtdIns(4)P to affect WASH activity, endosomal actin levels and potentially tubule scission. The upper tubule displays a hypothetical collaboration between ORP9-ORP10, EHD1 and WASH in coordinating cargo sorting (WASH) and endosomal tubule scission (EHD1). (E) TMCC1-coronin 1C interactions at ER-endosome MCSs promote endosomal scission. (F) TMEM16 K binds with its N-terminal domain to PtdIns(3)P, negatively affects endosomal PtdIns(3)P, and generates ER-endosome MCSs through interacting with the C-terminal region of RAB7. ER, endoplasmic reticulum.
show that ORP10 mediates PtdIns(4)P and PS counter-transport in the ER-endosome MCS to increase endosomal PS levels (Kawasaki et al., 2022). Deletion of ORP10 or ORP9 decreased endosomal PS, suggesting that the two proteins are both needed for PS transport. Given that cytoplasmic expression of lactadherin C2 domain detects cytosolic-facing PS (Lee et al., 2015), any observed PS decrease likely occurred on the endosome cytosolic leaflet. Contrary to expectations, ORP10 deletion did not affect PtdIns(4)P levels, whereas loss of ORP9 increased it. A possible hypothesis is that ORP9 may have provided some compensatory mechanism. Although not tested, ORP11, a close ORP10 paralogue could also have compensated for ORP10 loss.

Considering the endosomal localization of ORP10, Kawasaki et al. (2022) analyzed whether differences in endosomal PS levels affected trafficking events, revealing that retrograde transport of cation-independent mannose 6-phosphate receptor (CI-MPR) was reduced in ORP10-deleted cells. These authors also assessed EHD1 recruitment in ORP10 KO cells, given that PS recruits EHD1 (Lee et al., 2015) a well-known regulator of tubulovesicular scission (Deo et al., 2018; Naslavsky & Caplan, 2011). They found that endosomal EHD1 recruitment decreased with lower PS levels, leading to slower kinetics of SNX1-labelled tubule fission (Kawasaki et al., 2022). Additionally, they showed that ORP10 activity in retrograde trafficking required the ORD domain, implying that PtdIns(4)P and PS counter-transport was essential; while endosomal sorting complexes were unaffected. Altogether, this manuscript provided evidence that ORP10 at ER-endosome contact sites, is an important modulator of PS accumulation and endosomal fission (Figure 2B).

In line with the role of PS in EHD1 recruitment, Lee et al. (2015) demonstrated in Cos1 cells that ATP8A1, a P4-ATPase that translocates PS to the cytosolic leaflet of membranes is required for recycling endosome tubule scission. Importantly, this study showed that ATP8A1 is highly localized to recycling endosomes and is required to enrich PS on the cytosolic side of these endosomes, resulting in EHD1 recruitment and tubule scission (Figure 2C). Recycling endosomes are also far more enriched with PS than early or late endosomes. Together with findings from Kawasaki et al. (2022), these data suggest that PS is essential for EHD1 recruitment and cargo sorting across multiple endosome types. We can also speculate that ORP10 and ATP8A1 mediate endosomal scission through a shared pathway that allows high PS levels at specific endosomal subdomains. An intriguing experiment will be to investigate combined deletions of these regulators or whether overexpression of one could rescue the loss of the other. Moreover, these ER-endosome MCSs may allow for the recruitment of other endosomal sorting regulators, such as the WASH complex, to potentiate sorting of specific cargo classes.

### Phosphoinositides in ER-Endosome MCS

Phosphoinositides are well-described regulators of membrane trafficking (Jean & Kiger, 2012) and subtle changes in their levels affect a large array of cellular functions (Hammond & Burke, 2020). Further highlighting a link between PtdIns(4)P and other endosomal phosphoinositides in endosomal sorting, it had been observed that a transition between PtdIns(3)P and PtdIns(4)P governed by the myotubularin MTM and PI4KIIα was required for endocytic recycling of integrins and transferrin receptor (Ketel et al., 2016). Interestingly, this conversion mechanism requires PtdIns(4)P at RAB11 recycling endosomes to allow for appropriate exocyst recruitment. Because ER-endosome MCSs regulate PtdIns(4)P (Dong et al., 2016; Kawasaki et al., 2022; Petkovic et al., 2020) and recycling endosomes have high PS concentrations (Lee et al., 2015), it is tempting to speculate that ORP10-mediated counter-transport at PI4KIIα endosomes coordinates with other endosomal PIP phosphatases and kinases to fine-tune tubule scission with sorting events and tubule extensions. This process would likely affect a larger spectrum of cargos than only the CI-MPR previously described (Kawasaki et al., 2022).

Another interesting link between endosomal phosphoinositides and ER-endosome contact sites is the effect of OSBP- and VAPA/VAPB-mediated MCS on PtdIns(4)P regulation (Dong et al., 2016). The SNX-BAR protein SNX2 interacts with PtdIns(3)P in the early endosome (Dong et al., 2016), and SNX2 overexpression in COS-7 cells leads to VAPA/B accumulation at SNX2-positive endosomes. Importantly, the SNX2-mediated VAPA/B accumulation requires endosomal PtdIns(3)P, suggesting that SNX2 interacts with VAPA/B at ER-endosome MCSs (Dong et al., 2016). At these MCSs, OSBP transfers PtdIns(4)P to the ER, where it is dephosphorylated to PI by Sac1 phosphatase (Dong et al., 2016) (Figure 2D). In experiments with HeLa cells, OSBP or VAPA/B deletion increased endosomal PtdIns(4)P levels. In contrast to the ORP10 phenotype (Kawasaki et al., 2022), endosomal PtdIns(4)P accumulation leads to aberrant WASH recruitment and increased actin polymerization in endosomes (Dong et al., 2016). Aberrant WASH activity then causes defects in CI-MPR trafficking. Although the exact mechanism is unclear, increased WASH recruitment from PtdIns(4)P accumulation is dependent on PI4KIIα/β, meaning the latter are key regulators of endosomal sorting and fission. Notably, cortactin-binding PtdIns(3,5)P2 reduces actin in RAB7 endosomes (Hong et al., 2015). Moreover, WASH depletion leads to increased endosomal tubulation (Derivery et al., 2009). These findings highlight a potential parallel pathway to the one involving ORP10, given that OSBP-VAPA/B ER-endosome MCSs are more prevalent in RAB7-positive endosomes. In this scenario, phosphoinositide (PtdIns(3)P and PtdIns(4)P) regulation controls endosomal scission through WASH recruitment and activity.
whereas PS transport coupled with EHD1 recruitment influences endosomal fission of different cargos or towards different destinations (Figure 2D, lower tubule). Alternatively, WASH recruitment could occur at ORP10-regulated ER-endosome MCSs to regulate cargo sorting more than tubule fission (Figure 2D, higher tubule). In support of this possibility, WASH-induced actin polymerization reduces the diffusion of sequestered cargo for efficient scission by EHD1 (Simonetti & Cullen, 2019). Finally, it is worth mentioning that mutations in the spastin, strumpellin, or REEP1 genes cause hereditary spastic paraplegia (HSP). Spastin is present at ER-endosome contact sites in neurons and influences endosomal tubule fission (Allison et al., 2017), potentially acting together or in parallel with ORP10. These examples denote strong links between endosomal phosphoinositides and ER-endosome contact sites.

**Direct Regulation of ER-Endosome MCS Formation in Cargo Sorting**

The ER establishes numerous connections with other intracellular organelles through MCSs (Valm et al., 2017). These MCSs have profound implications for trafficking events, as already summarized; however, the direct effects of the tested proteins on MCS formation and maintenance were not investigated in previous studies. Modulation of ER-endosome contact sites was found to affect endosomal carrier fission through recruiting the WASH complex to endosomal tubules (Rowland et al., 2014). This process is dependent on the TMCC class of ER-anchored proteins (Hoyer et al., 2018). TMCC1 is recruited to tubule bud sites by coronin 1C, an actin regulatory protein present in Rab7 endosomes. Hence, when either TMCC1 or coronin 1C is reduced, both ER-endosome MCSs and CI-MPR trafficking decrease (Figure 2E). This change is likely independent of WASH-mediated actin dynamics, given that WASH/FAM21 deletion did not affect ER recruitment or fission of endosomal tubules. Therefore, the TMCC1/coronin 1C-regulated ER-endosome MCS would directly affect endosomal tubule scission, while WASH recruitment would most likely control cargo sorting, rather than the fission event.

The active sorting of signaling receptors into intraluminal vesicles (ILVs) occurs throughout the endosomal maturation stage, and relies, at least partly, on the ER-endosome contact sites. Specifically, EGFR downregulation and lysosomal degradation upon activation require EGFR incorporation into the ILVs of multivesicular bodies (Tomas et al., 2014). A specific subset of ER-endosome contact sites, scaffolded by Annexin A1, affects EGFR signaling (Eden et al., 2016). Interestingly, the overexpression of Annexin A1 promoted the occurrence of ER-endosome MCSs. This ability of Annexin A1 to modulate ER-endosome MCSs is directly influenced by the EGFR: a phosphomimetic mutant of Annexin A1 at the EGFR phosphorylation site, increases the extent of ER-endosome MCSs compared to wild-type cells, while a non-phosphorylatable mutant does not affect EGFR-driven MCSs (Eden et al., 2016). Moreover, the ER-localized tyrosine phosphatase, PTP1B, affects the EGFR at ER-endosome MCSs (Eden et al., 2010), and the EGFR-specific ER-endosome MCSs, scaffolded by Annexin A1, serve as action sites on the EGFR for PTP1B (Wong et al., 2018). These instances highlight that in addition to scission events, ER-endosome MCSs also affect receptor sorting into ILVs.

**TMEM16K in ER-Endosome MCS and Endosomal Sorting**

In addition to the above examples, a connection between phosphoinositides, Rab GTPases, and ER-endosome MCSs modulated by TMEM16 K, an ER-localized lipid scramblase, has been uncovered (Petkovic et al., 2020). Mutations in TMEM16 K are causative of spinocerebellar ataxia (SCAR10) (Vermeer et al., 2010), and TMEM16 K is localized in the ER of U2OS cells (Petkovic et al., 2020), in addition to other cells (Kramer & Hawley, 2003; Maass et al., 2009). Using a BioID approach, the authors identified multiple links between TMEM16 K, the ER, and endolysosomal proteins (Petkovic et al., 2020). Comparison of wild-type and TMEM16 K KO mouse embryonic fibroblasts highlighted deficient anterograde trafficking of CI-MPR and CtxB in TMEM16 K mutant cells, while the anterograde secretory pathway was unaffected. Significantly, TMEM16 K was in close proximity to VAPA/B, SNX1, SNX2, and RAB7A, and the RAB7A/TMEM16 K interaction was demonstrated to form endosome-ER contact sites. The RAB7A/TMEM16 K interaction was mediated by the C-terminal region of TMEM16 K. A PIP-strip overlay assay identified binding of the TMEM16 K N-terminus to endosomal phosphoinositides PtdIns(3)P, PtdIns(4)P, and PtdIns(3,5)P2, implying that TMEM16 K links ER to endosomes via multivalent interactions. However, TMEM16 K deletion did not affect the number of ER-endosome MCSs, nor did it affect endosomal PtdIns(4)P levels, suggesting that its effects on endosomal sorting differed from those of ORP10 (Kawasaki et al., 2022). Accordingly, TMEM16 K deletion was associated with increased PtdIns(3)P levels, indicating a potential problem in phosphoinositide conversion rather than PtdIns(4)P accumulation.

The need for TMEM16 K scramblase activity suggests that transfer between membrane leaflets of specific phospholipids is required for efficient endosomal sorting (Figure 2F). Because the ORP10 paralog ORP11 was identified in the TMEM16 K BioID dataset (Petkovic et al., 2020), TMEM16 K could potentially act in the same pathway as ORP10 and ATP8A1 to ensure an appropriate PS concentration on endosome cytosolic leaflets.
MCS in Endosomal Maturation

The number of ER-endosome contact sites increases as endosomes mature (Friedman et al., 2013), observable through the conversion of Rab5-positive endosomes to Rab7. This maturation-related switch is mediated in part by the MON1A/B-CCZ complex, which removes Rab5 GEF while concomitantly activating Rab7 (Langemeyer et al., 2020; Poteryaev et al., 2010; Rink et al., 2005). A study tested whether the increase in ER-late endosome contact sites is coincidental or plays a role in endosomal maturation (Wu & Voeltz, 2021). Mechanistically, it was demonstrated that the ER-resident protein Rtn3L was recruited to ER-endosome MCSs by Rab9a, which is found at late endosomes along with Rab7 (Wu & Voeltz, 2021). Depletion of either Rab9 or Rtn3L impaired endosomal maturation, with depleted cells showing a significant increase in the percentage of Rab5/Rab7 double-positive endosomes, suggesting defective endosomal conversion. Depletion also affected cargo sorting via reducing CI-MPR localization at the TGN and decreasing epidermal growth factor receptor (EGFR) degradation after stimulation. Altogether, these findings illustrate that Rtn3L and Rab9 affect endosome maturation and cargo sorting at ER-endosome MCSs (Figure 3A). It will be interesting to test if these MCSs could participate in the recruitment of known endosomal conversion regulators like the MON1A/B-CCZ complex.

The frequency and duration of ER-endosome contact were found to be independent of microtubules (Rowland et al., 2014). Nonetheless, in a different context, ER-endosome contact affects late endosome movement during neurite outgrowth or in response to cholesterol levels. Interestingly, in neurons, the ER-anchored protein protrudin can interact with late endosomal Rab7 and PtdIns(3)P to form ER-endosome MCSs (Raiborg et al., 2015a). Given that protrudin interacts with kinesin motors, these ER-endosome MCSs would allow the handoff of kinesin complexes to Rab7/FYCO1 complexes. This results in the directed displacement of late endosomes, facilitating neurite outgrowth. These protrudin/FYCO1 handoffs happen stochastically as late endosomes move.

The intracellular localization of late endosomes also appears to be regulated by ORP1L-controlled ER-endosome MCSs, in response to cholesterol levels (Rocha et al., 2009). At high levels, the ORD domain of ORP1L binds to lysosomal cholesterol and forms a complex with Rab7/RILP, which interacts with the dynein/dynactin complex to modulate minus-end transport. When cholesterol is low, however, ORP1L does not bind to it, allowing the ORP1L FFAT motif to interact with VAPA/B and decrease late endosomal movement.

Although ER-endosome contact sites affect multiple biological functions, in most cases, deletion of localized proteins does not affect the contact sites. Hence, defining the minimal or interacting factors regulating ER-endosome MCS is important. A study identified a novel ER-endosome regulator that mediates three-way contact sites between the ER endosome and mitochondria (Elbaz-Alon et al., 2020). The ER-transmembrane protein PDZD8 interacts with protrudin in the ER, and both proteins interact with endosomal Rab7 to scaffold ER-endosome-mitochondria contact sites. The functional role of these MCSs is unknown. However, overexpressing the Rab5-to-Rab7 conversion regulator WDR1 (Liu et al., 2016) with PDZD8 strongly increases the number of ER-late endosome contact sites, suggesting a potential role in endosomal maturation.

Conclusions

The various manuscripts highlighted illustrate a strong influence of ER-endosome MCSs on endosomal sorting and maturation. Significantly, many proteins visit these interaction...
platforms, with PtdIns(4)P and RABs being central players in various ER-endosome MCSs.

While yeasts have been instrumental in defining the role of various types of MCS in vivo, most studies to date on ER-endosome MCSs have been performed in only a few transformed cell lines. Hence, future research should aim to better define the impact of ER-endosome MCSs in multicellular organisms. Given the conservation of these genes in model organisms and the ease of performing knock-ins by CRISPR/Cas9, it will be interesting of generating point mutants unable to localize at or scaffold ER-endosome MCSs, instead of full knockdown, and to evaluate how these contact sites affect the organism. Future in vivo projects will prove very informative in defining the exact roles of ER-endosome MCSs in human disease.

Finally, the known roles of ER-endosome MCSs in endosomal sorting and maturation have largely been derived from studying CI-MPR or EGFR trafficking. Because endosomal sorting complexes regulate a large array of transmembrane proteins, we should expand studies to more cargo so that we can decipher how these various ER-endosome contacts overlap at the level of cargos. Again, using in vivo models might highlight phenotypes that will lead to specific cargos, thus expanding the role of ER-endosome MCSs. Many discoveries have provided insight that hints at this expanded role, clarifying the precise biological functions and organisal requirements of ER-endosome MCSs, as well as their interplay with other relevant endosomal processes, will be truly interesting.

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References
Allison, R., Edgar, J. R., Pearson, G., Rizo, T., Newton, T., Günther, S., Berner, F., Hague, J., Connell, J. W., Winkler, J., Lippincott-Schwartz, J., Beetz, C., Winner, B., & Reid, E. (2017). Defects in ER-endosome contacts impact lysosome function in hereditary spastic paraplegia. Journal of Cell Biology, 216(5), 1337–1355. https://doi.org/10.1083/jcb.201609033

Antony, B., Bigay, J., & Mesmin, B. (2018). The oxyosteryl-binding protein cycle: Burning off PI(4)P to transport cholesterol. Annual Review of Biochemistry, 87(4), 809–837. https://doi.org/10.1146/annurev-biochem-061516-044924

Baba, T., Toth, D. J., Sengupta, N., Kim, Y. J., & Balla, T. (2019). Phosphatidylinositol 4,5-bisphosphate controls Rab7 and PLEKHM1 membrane cycling during autophagosome–lysosome fusion. The EMBO Journal, 38(8), e100312. https://doi.org/10.15252/embj.2019102837

Balla, T., Kim, Y. J., Alvarez-Prats, A., & Pemberton, J. (2019). Lipid dynamics at contact sites between the endoplasmic reticulum and other organelles. Annual Review of Cell and Developmental Biology, 35, 85–109. https://doi.org/10.1146/annurev-cellbio-100818-125251

Cullen, P. J., & Steinberg, F. (2018). To degrade or not to degrade: Mechanisms and significance of endocytic recycling. Nature Reviews Molecular Cell Biology, 19(11), 679–696. https://doi.org/10.1038/s41580-018-0053-7

Deo, R., Kushwaha, M. S., Kamerkar, S. C., Kadam, N. Y., Dar, S., Babu, K., Srivastava, A., & Pacaud, T. J. (2018). ATP-dependent membrane remodeling links EHD1 functions to endocytic recycling. Nature Communications, 9(1). https://doi.org/10.1038/s41467-018-07586-z

Derivery, E., Sousa, C., Gautier, J. J., Lombard, B., Loew, D., & Gauthreau, A. (2009). The Arp2/3 activator WASH controls the fission of endosomes through a large multiprotein Complex. Developmental Cell, 17(5), 712–723. https://doi.org/10.1016/j.devcel.2009.09.010

Dong, R., Saheki, Y., Swarup, S., Lucas, L., Harper, J. W., De Camilli, P., & de Camilli, P. (2016). Endosome-ER contacts control actin nucleation and retromer function through VAP-dependent regulation of PI4P. Cell, 166(2), 408–423. https://doi.org/10.1016/j.cell.2016.06.037

D’Souza, R. S., Lim, J. Y., Turgut, A., Servage, K., Zhang, J., Orth, K., Sosale, N. G., Lazzara, M. J., Allegood, J., & Casanova, J. E. (2020). Calcium-stimulated disassembly of focal adhesions mediated by an orp3/iqsec1 complex. Elife, 9, 1–33. https://doi.org/10.7554/eLife.54113

Eden, E. R., Sanchez-Heras, E., Tsapara, A., Sobota, A., Levine, T. P., & Futter, C. E. (2016). Annexin A1 tethers membrane contact sites that mediate ER to endosome cholesterol transport. Developmental Cell, 37(5), 473–483. https://doi.org/10.1016/j.devcel.2016.05.005

Eden, E. R., White, A. J., Tsapara, A., & Futter, C. E. (2010). Membrane contacts between endosomes and ER provide sites for PTP1B-epidermal growth factor receptor interaction. Natural Cell Biology, 12(3), 267–272.

Elbaz-Alon, Y., Guo, Y., Segev, N., Harel, M., Quinnell, D. E., Geiger, T., Avinoam, O., Li, D., & Nunnari, J. (2020). PDZD8 Interacts with protrudin and Rab7 at ER-late endosome membrane contact sites associated with mitochondria. Nature Communications, 11(1), 1–14. https://doi.org/10.1038/s41467-020-17451-7

Friedman, J. R., Dibenedetto, J. R., West, M., Rowland, A. A., & Voeltz, G. K. (2013). Endoplasmic reticulum-endosome contact increases as endosomes traffic and mature. Molecular Biology of the Cell, 24(7), 1030–1040. https://doi.org/10.1091/mbc.e12-10-0733

Hammond, G. R. V., & Burke, J. E. (2020). Novel roles of phosphoinositides in signaling, lipid transport, and disease. Current Opinion in Cell Biology, 63, 57–67. https://doi.org/10.1016/j.celb.2019.12.007
Hiragi, S., Matsui, T., Sakamaki, Y., & Fukuda, M. (2021). TBC1D18, A novel Rab5-GAP, coordinates endosome maturation together with Mon1. *BioRxiv*. https://doi.org/10.1101/2021.11.11.468194.

Hong, N. H., Qi, A., & Weaver, A. M. (2015). PI(3,5)P2 controls endosomal branched actin dynamics by regulating cortactin-actin interactions. *Journal of Cell Biology*, 210(5), 753–769. https://doi.org/10.1083/jcb.201412127.

Hoyer, M. J., Chitwood, P. J., Ebbemeier, C. C., Striepen, J. F., Qi, R. Z., Old, W. M., & Voeltz, G. K. (2018). A novel class of ER membrane proteins regulates ER-associated endosome fission. *Cell*, 175(1), 254–265.e14. https://doi.org/10.1016/j.cell.2018.08.030.

Huotari, J., & Helenius, A. (2011). Endosome maturation. *The EMBO Journal*, 30(17), 3481–3500. https://doi.org/10.1038/embj.2011.286.

Jean, S., & Kiger, A. A. (2012). Coordination between RAB GTPase functions. *Nature Reviews Molecular Cell Biology*, 13(7), 463–470. https://doi.org/10.1038/nrm3379.

Kawasaki, A., Sakai, A., Nakanishi, H., Hasegawa, J., Taguchi, T., Sasaki, J., Arai, H., Sasaki, T., Igarashi, M., & Nakatsu, F. (2022). PI(4)P/PS Countertransport by ORP10 at ER-endosome membrane contact sites regulates endosome fission. *Journal of Cell Biology*, 221(1), e202103141. https://doi.org/10.1083/jcb.202103141.

Ketel, K., Krauss, M., Nicot, A. S., Puchkov, D., Wieffer, M., Müller, R., Subramanian, D., Schultz, C., Laporte, J., & Haucke, V. (2016). A phosphoinositide conversion mechanism for exit from endosomes. *Nature*, 529(7586), 408–412. https://doi.org/10.1038/nature16516.

Kramer, J., & Hawley, R. S. (2003). The spindle-associated transmembrane protein Axin identifies a new family of transmembrane proteins in eukaryotes. *Cell Cycle*, 2(3), 173–175. https://doi.org/10.4161/cc.2.3.368.

Langemeyer, L., Borchers, A. C., Herrmann, E., Füllbrunn, N., Han, Y., Perz, A., Auffarth, K., Kümmel, D., & Ungermann, C. (2020). A conserved and regulated mechanism drives endosomal rab transition. *Elife*, 9, 1–20. https://doi.org/10.7554/elif.e.56090.

Lee, S., Uchida, Y., Sakamaki, Y., Nakagawa, T., Kishimoto, T., Mukai, K., Inaba, T., Kobayashi, T., Molday, R. S., Taguchi, T., & Arai, H. (2015). Transport through recycling endosomes requires EHD1 recruitment by a phosphatidylserine translocase. *The EMBO Journal*, 34(5), 669–688. https://doi.org/10.15252/embj.20149703.

Liu, K., Jian, Y., Sun, X., Yang, C., Gao, Z., Zhang, Z., Liu, X., Li, Y., Xu, J., Jing, Y., Mitani, S., He, S., & Yang, C. (2016). Negative regulation of phosphatidylinositol-3-phosphate levels in early-to-late endosome conversion. *Journal of Cell Biology*, 212(2), 181–198. https://doi.org/10.1083/jcb.201506081.

Maass, K., Fischer, M. A., Seiler, M., Temmerman, K., Nickel, W., & Seedorf, M. (2009). A signal comprising a basic cluster and an amphipathic α-helix interacts with lipids and is required for the transport of Ist2 to the yeast cortical ER. *Journal of Cell Science*, 122(5), 625–635. https://doi.org/10.1242/jcs.036012.

Mesmin, B., Bigay, J., von Filleck, J. M., Lacas-Gervais, S., Drin, G., & Antony, B. (2013). A four-step cycle driven by PI(4)P hydrolysis directs Sterol/PI(4)P exchange by the ER-Golgi tether OSBP. *Cell*, 155(4), 830–843. https://doi.org/10.1016/j.cell.2013.09.056.

Nakatsu, F., & Kawasaki, A. (2021). Functions of oxysterol-binding proteins at membrane contact sites and their control by phosphoinositide metabolism. *Frontiers in Cell and Developmental Biology*, 9(June), 1–18. https://doi.org/10.3389/fcell.2021.664788.

Naslavsky, N., & Caplan, S. (2011). EHD Proteins: Key conductors of endocytic transport. *Trends in Cell Biology*, 21(2), 122–131. https://doi.org/10.1016/j.tcb.2010.09.003.

Naslavsky, N., & Caplan, S. (2018). The enigmatic endosome – sorting the ins and outs of endocytic trafficking. *Journal of Cell Science*, 131(13), jcs216499. https://doi.org/10.1242/jcs.216499.

Petkovic, M., Oses-Prieto, J., Burlingame, A., Jan, L. Y., & Jan, Y. N. (2020). TMEM61K Is an interorganelle regulator of endosomal sorting. *Nature Communications*, 11(1). https://doi.org/10.1038/s41467-020-17016-8.

Podinovskiaia, M., Pescianotto-Baschong, C., Buser, D. P., & Spang, A. (2021). A novel live-cell imaging assay reveals regulation of endosome maturation. *Elife*, 10. https://doi.org/10.7554/elif.e.70982.

Poteryaev, D., Dutta, S., Ackema, K., Zerial, M., & Spang, A. (2010). Identification of the switch in early-to-late endosome transition. *Cell*, 141(3), 497–508. https://doi.org/10.1016/j.cell.2010.03.011.

Prinz, W. A., Toulmay, A., & Balla, T. (2020). The functional universe of membrane contact sites. *Nature Reviews Molecular Cell Biology*, 21(1), 7–24. https://doi.org/10.1038/s41580-019-0180-9.

Raiborg, C., Wenzel, E. M., Pedersen, N. M., Olsvik, H., Schink, K. O., Schultz, S. W., Vietri, M., Nisi, V.,ucci, B., Brech, A., Johansen, T., & Stenmark, H. (2015a). Repeated ER-endosome contacts promote endosome translocation and neurite outgrowth. *Nature*, 520(7546), 234–238. https://doi.org/10.1038/nature14359.

Raiborg, C., Wenzel, E. M., & Stenmark, H. (2015b). ER-endosome contact sites: Molecular compositions and functions. *The EMBO Journal*, 34(14), 1848–1858. https://doi.org/10.15252/embj.201591481.

Rink, J., Ghigo, E., Kalaizidizis, Y., & Zerial, M. (2005). Rab conversion as a mechanism of progression from early to late endosomes. *Cell*, 122(5), 735–749. https://doi.org/10.1016/j.cell.2005.06.043.

Rocha, N., Kuijl, C., van der Kant, R., Janssen, L., Houben, D., Janssen, H., Zwart, W., & Neefjes, J. (2009). Cholesterol sensor ORP1L contacts the ER protein VAP to control Rab7-RILP:p150Glued and late endosome positioning. *Journal of Cell Biology*, 185(7), 1209–1225. https://doi.org/10.1083/jcb.200811005.

Rowland, A. A., Chitwood, P. J., Phillips, M. J., & Voeltz, G. K. (2014). ER Contact sites define the position and timing of endosome fission. *Cell*, 159(5), 1027–1041. https://doi.org/10.1016/j.cell.2014.09.023.

Simonetti, B., & Cullen, P. J. (2019). Actin-dependent endosomal receptor recycling. *Current Opinion in Cell Biology*, 56, 22–33. https://doi.org/10.1016/j.ceb.2018.08.006.

Tomas, A., Futter, C. E., & Eden, E. R. (2014). EGF Receptor trafficking: Consequences for signaling and cancer. *Trends in Cell Biology*, 24(1), 26–34. https://doi.org/10.1016/j.tcb.2013.11.002.

Tong, J., Tan, L., & Im, Y. J. (2021). Structure of human ORP3 ORD reveals conservation of a key function and ligand...
specificity in OSBP-related proteins. *PLoS ONE, 16*(4 April), 1–15. https://doi.org/10.1371/journal.pone.0248781
Tu, H., Wang, Z., Yuan, Y., Miao, X., Li, D., Guo, H., Yang, Y., & Cai, H. (2022). The PripA-TbcrA complex-centered Rab GAP cascade facilitates macropinosome maturation in Dictyostelium. *Nature Communications, 13*(1), 1787. https://doi.org/10.1038/s41467-022-29503-1
Valm, A. M., Cohen, S., Legant, W. R., Melunis, J., Hershberg, U., Wait, E., Cohen, A. R., Davidson, M. W., Betzig, E., & Lippincott-Schwartz, J. (2017). Applying systems-level spectral imaging and analysis to reveal the organelle interactome. *Nature, 546*(7656), 162–167. https://doi.org/10.1038/nature22369
van der Beek, J., de Heus, C., Liv, N., & Klumperman, J. (2022). Quantitative correlative microscopy reveals the ultrastructural distribution of endogenous endosomal proteins. *Journal of Cell Biology, 221*(1), e202106044. https://doi.org/10.1083/jcb.202106044
Venditti, R., Rita Rega, L., Chiara Masone, M., Santoro, M., Polishchuk, E., Sarnataro, D., Paladino, S., D’Auria, S., Varriale, A., Olkkonen, V. M., Di Tullio, G., Polishchuk, R., & De Matteis, M. A. (2019). Molecular determinants of ER–Golgi contacts identified through a new FRET–FLIM system. *Journal of Cell Biology, 218*(3), 1055–1065. https://doi.org/10.1083/jcb.201812020
Vermeer, S., Hoischen, A., Meijer, R. P. P., Gilissen, C., Neveling, K., Wieskamp, N., de Brouwer, A., Koenig, M., Anheim, M., Assoum, M., Drouot, N., Todorovic, S., Milic-Rasic, V., Lochmuller, L., Stevanin, G., Goizet, C., David, A., Durr, A., Brice, A., & Knoers, K.. (2010). Targeted next-generation sequencing of a 12.5 Mb homozygous region reveals ANO10 mutations in patients with autosomal-recessive cerebellar ataxia. *American Journal of Human Genetics, 87*(6), 813–819. https://doi.org/10.1016/j.ajhg.2010.10.015
Weber-Boyvat, M., Kentala, H., Peränen, J., & Olkkonen, V. M. (2015). Ligand-dependent localization and function of ORP-VAP complexes at membrane contact sites. *Cellular and Molecular Life Sciences, 72*(10), 1967–1987. https://doi.org/10.1007/s00018-014-1786-x
Wong, L. H., Eden, E. R., & Futter, C. E. (2018). Roles for ER: Endosome membrane contact sites in ligand-stimulated intraluminal vesicle formation. *Biochemical Society Transactions, 46*(5), 1055–1062. https://doi.org/10.1042/BST20170432
Wong, L. H., Gatta, A. T., & Levine, T. P. (2019). Lipid transfer proteins: The lipid commute via shuttles, bridges and tubes. *Nature Reviews Molecular Cell Biology, 20*(2), 85–101. https://doi.org/10.1038/s41580-018-0071-5
Wong, L. H., Martello, A., & Eden, E. R. (2022). Thank ORP9 for FFAT: With endosomal ORP10, it’s fission accomplished!. *Journal of Cell Biology, 221*(1), 9–11. https://doi.org/10.1083/jcb.202112057
Wu, H., & Voeltz, G. K. (2021). Reticulon-3 promotes endosome maturation at ER membrane contact sites. *Developmental Cell, 56*(1), 52–66.e7. https://doi.org/10.1016/j.devcel.2020.12.014