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Coordination of inter-organelle communication and lipid fluxes by OSBP-related proteins

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Contents:

Abbreviations: ER, endoplasmic reticulum; FFAT, two phenylalanines in an acidic tract; LE, late endosome; LTP, lipid-binding/transfer protein; Lys, lysosome; MCS, membrane contact site; ORD, OSBP-related ligand-binding domain; ORP, OSBP-related protein; OSBP, oxysterol-binding protein; OSBPL, oxysterol-binding protein-like; PIP, phosphatidylinositol phosphate; PI4P,

phosphatidylinositol-4-phosphate; PM, plasma membrane; PS, phosphatidylserine; VAP, VAMP-associated protein

ABSTRACT

Oxysterol-binding protein (OSBP) and OSBP-related proteins (ORPs) constitute one of the largest families of lipid-binding/transfer proteins (LTPs) in eukaryotes. The current view is that many of them mediate inter-organelle lipid transfer over membrane contact sites (MCS). The transfer occurs in several cases in a 'counter-current' fashion: A lipid such as cholesterol or phosphatidylserine (PS) is transferred against its concentration gradient driven by transport of a phosphoinositide in the opposite direction. In this way ORPs are envisioned to maintain the distinct organelle lipid compositions, with impacts on multiple organelle functions. However, the functions of ORPs extend beyond lipid homeostasis to regulation of processes such as cell survival, proliferation and migration. Important expanding areas of mammalian ORP research include their roles in viral and bacterial infections, cancers, and neuronal function.

The yeast OSBP homologue (Osh) proteins execute multifaceted functions in sterol and glycerophospholipid homeostasis, post-Golgi vesicle transport, phosphatidylinositol-4-phosphate, sphingolipid and target of rapamycin (TOR) signalling, and cell cycle control. These observations identify ORPs as lipid transporters and coordinators of signals with an unforeseen variety of cellular processes. Understanding their activities not only enlightens the biology of the living cell but also allows their employment as targets of new therapeutic approaches for disease.

Key words: Cell signalling**;** Lipid metabolism**;** Lipid transport**;** Membrane contact site**;** ORP**;** OSBPL

1. Introduction

Lipids are essential components of cellular membranes, each organelle having a distinct membrane lipid composition providing it a unique identity and function. This requires precise spatio-temporal regulation of lipid distribution between the organelles. Most lipids or their precursor forms are synthesised in the endoplasmic reticulum (ER) and transferred to their organelle destinations by both vesicle-independent and vesicle-mediated mechanisms. A major portion of the vesicle-independent lipid transfer is mediated by lipid-binding/transfer proteins (LTPs)[1, 2] that are in many cases shown to operate over membrane contact sites (MCS), zones of close apposition between organelle limiting membranes [3, 4]. Oxysterol binding protein (OSBP) and OSBP-related proteins (ORP1-11) are among the largest families of LTPs, encoded by 12 genes in mammals and categorized into six subfamilies (I-VI; Fig. 1) based on sequence similarity and gene structure [5-7].

The early findings that oxysterols, 27-carbon oxygenated derivatives of cholesterol, act as potent suppressors of cholesterol biosynthesis, prompted a search for proteins mediating the effects of oxysterols on lipid metabolism. Protein fractions with oxysterol-binding activity were isolated for various biological sources [8-13], and Taylor, Shown and Kandutsch et al. eventually identified OSBP as an oxysterol receptor whose affinity for different sterols correlated with their potenty to repress hydroxymethylglutaryl coenzyme A reductase, a rate-limiting enzyme of cholesterol biosynthesis [14, 15]. Later study by the group of M.S. Brown and J.L. Goldstein revealed that OSBP is not a major regulator of cholesterol homeostasis. The protein was not found to localize within the nucleus but rather in the cytoplasm, and 25-hydroxycholesterol treatment enigmatically induced its shift to the Golgi complex [16]. Discovery of the sterol regulatory element binding proteins (SREBPs)[17-19] and liver X receptors (LXRs)[20-22] as transcriptional regulators of sterol metabolism turned the attention away from OSBP. However, the study of OSBP continued in the group of N. Ridgway [23-27]. In the late 1990s and soon post millenium, families of OSBP homologues were identified in yeast, mammals and other taxa. In the yeast *Saccharomyces cerevisiae* 7 OSBP homologue (Osh) proteins, Osh1-7p, categorized into four subfamilies (I-IV) were identified [28-30](Fig. 1); They have played crucial roles in deciphering the mechanistic principles of ORP action (see section 8). In humans and other mammals, there are 12 *OSBP*/*OSBPL* genes encoding >19 different OSBP-related proteins (ORPs; Fig. 1), alternatively called OSBP-like (OSBPL) proteins. These proteins are ubiquitously present in cells and tissues, each of which expresses a large repertoire of ORPs, with typically only 1-3 family members absent in any single cell type. The

tissue expression patterns of ORPs have, as a rule, been systematically addressed only at the level of a gene [5, 7], not at splice variant or transcription start site precision. However, variant-specific expression analyses have been reported for individual ORPs such as ORP1 [31] and ORP3 [32].

It is increasingly appreciated that a number of ORPs act as lipid transporters over MCS. However, the functions of ORPs extend beyond lipid homeostasis to regulation of processes such as cell proliferation, survival and migration. They have an increasing number of identified protein interaction partners and play key roles in signalling functions such as focal adhesion kinase (FAK) activation for cell migration [33], mTORC1 signalling for cell proliferation [34], and vascular endothelial growth factor (VEGF) signalling in endothelial cells [35]. The focus of this review is to discuss in detail the diverse functions of mammalian ORPs not only from the viewpoint of basic biology but also considering their potential as possible future therapy targets for human disease. We begin with an introduction of the major structural features of ORPs and the functions of these proteins as lipid transporters over MCS, then focus for a while on ORPs with established or proposed functions in cholesterol and PIP trafficking in the endocytic pathway, and then move on to the postulated roles of ORPs in cardiometabolic diseases, cancers and infectious disease. Finally, we present an overview of yeast *Saccharomyces cerevisiae* OSBP homologues (Osh), the study of which has yielded crucial insight into the structure and function of ORPs.

2. Major structural features of ORPs

All ORPs in various taxa share a conserved carboxy-terminal lipid-binding domain known as OSBP-related ligand-binding domain (ORD; Fig. 2), which contains the so-called 'OSBP fingerprint' sequence EQVSHHPP forming part of the PIP-binding determinants within the ORD. The initial hallmark study of Im et al. [36] reported a high-resolution structure of the yeast *S. cerevisiae* ORP Osh4p with five different sterols bound within the ORD. This study revealed a β-barrel-like fold containing a lipid-binding cavity, in which the bound sterol is oriented with its 3β-hydroxyl group facing the bottom of the cavity. The cavity is large enough to host a sterol and several water molecules, explaining why Osh4p can bind different sterols. The bound sterol stabilizes a closed conformation of a lid structure consisting of a two-stranded β-sheet and three α-helices. The lid-open conformation of Osh4p was suggested to expose basic amino acid residues near the mouth of the ligand cavity that interact with phosphate groups at membrane surfaces, thus facilitating sterol extraction from the bilayer. Importantly, the group of G. Drin found in 2011 that the Osh4p ORD

binds, in addition to sterols, phosphatidylinositol 4-phosphate (PI4P; Fig. 2), the sterol and PI4P binding being mutually exclusive, and exchanges sterol for PI4P between membranes [37]. The authors discovered that charged amino acid residues defining a shallow pocket under the lid recognize the PI4P head group, and the PI4P fatty acyl chains loosely interact with the sterol binding cavity in a rather non-specific manner. The histidines in the conserved OSBP fingerprint motif EQVSHHPP were found to interact with the polar head group of PI4P (Fig. 2), suggesting that all ORPs might bind PI4P (or possibly other PIPs). Since the initial Osh4p structure, high-resolution structures of the ORDs of yeast Osh1p [38], Osh3p [39] and Osh6p [40, 41], as well as of mammalian ORP1 (Fig. 2)[42], ORP2 [43], and ORP3 [44] have been reported. These structural analyses have revealed interesting differences in the mode of ligand binding to distinct ORP family members: The PI(4,5)P_2 binding mode in ORP1 and -2 differs from that of PI4P in the other ORDs. The polar head of PI(4,5)P_2 binds in a different orientation than that of PI4P in the other ORDs. Moreover, in ORP1 and -2 one of the PI(4,5)P_2 acyl chains is within the ORD cavity while the 2nd chain is curled up at the top of the cavity [42, 43]. In contrast, in Osh4p, Osh6p and ORP3 both acyl chains of the bound PI4P are inserted into the ORD ligand cavity [37, 40, 41, 44](Fig. 2). Of note, the structural analyses of yeast Osh3p and mammalian ORP3 showed that the lipid-binding cavity of these ORDs is too narrow to accommodate sterols [39, 44], consistent with the view that phosphoinositide rather than sterol binding is a common denominator of the ORPs.

Besides the ORD domain, most ORPs possess a two phenylalanines in an acidic tract (FFAT) motif for interaction with VAMP-associated proteins (VAPs) of the ER [45], and a pleckstrin homology (PH) domain for interaction with phosphoinositide head groups at the adjoining organelle membranes [5-7](Fig. 1). ORPs carrying a PH domain in their amino-terminal half are generally designated as long (L), and ones without a PH domain short (S) subtype proteins; Of several mammalian ORPs, both L and S variants exist. Of the 7 yeast Osh proteins, three (Osh1-3p) represent the L subtype, while four (Osh4-7p) belong to the S category [28](Fig. 1). Among the mammalian ORPs, ORP2 is unique being present only as S variant [5-7]. The variant forms of ORPs typically arise through differential mRNA splicing [32](https://www.ncbi.nlm.nih.gov/nuccore). However, use of alternative promoters is suggested to generate the ORP1S and -L variants [6].

The PH domains of ORPs differ in their PIP binding specificity and affinity that essentially contributes to their subcellular localization. As examples, the PH domain of OSBP is selective for PI4P [46, 47] and additionally associates with the small GTPase ARF in *trans*-Golgi membranes [46, 48], while the PH domains of ORP5 and ORP8 interact with PI(4,5)P_2 and PI4P at the plasma

membrane (PM)[49-51]. Importantly, most ORPs carry two PIP-binding domains: The PH domain that interacts with the head groups of PIPs, and the ORD which appears in all studied cases capable of binding and extracting PIPs from membranes [52-54]. As examples, the ORD of OSBP transfers cholesterol and PI4P in an exchange-type fashion at MCS between ER and *trans*-Golgi [55], the ORDs of ORP5 and -8 mediate PS/PI(4,5)P_2 and PS/PI4P exchange at ER-PM MCS [49, 50], and the ORD of ORP2 transfers cholesterol from late to recycling endosomes or the plasma membrane in exchange for PI(4,5)P_2 [33, 43]. The distribution of ORP5 and -8 between the reticular ER and ER-PM MCS is, in the context of the negatively charged PM inner leaflet surface, additionally regulated by a polybasic segment adjacent to their PH domain and an anionic stretch at the amino-terminus of ORP8L [51, 56](Fig. 1).

In addition to the above functional domains, mammalian ORP1L and yeast Osh1p and Osh2p have close to their amino-terminus an ankyrin repeat (ANK) domain mediating protein-protein interactions (Fig. 1): The ANK domain of ORP1L binds the small GTPase Rab7 on late endosomes, while that of Osh1p binds the nucleus-vacuole junction (MCS) component Nvj1p [57, 58]; The functional role of the Osh2p ANK domain remains unknown. In the amino-terminal lid region of the yeast Osh4p ORD (also in Osh5p), an amphipathic lipid packing sensor (ALPS) motif mediates association of the protein with highly curved membrane domains [59]. Moreover, ORPs contain coiled coil-forming domains which are in the case of OSBP and ORP4 shown to mediate homo- or heterodimerization [16, 60, 61].

3. Function of ORPs in counter-current lipid transfer over membrane contact sites

Lipids are in several cases transported from one organelle membrane to another against their concentration gradients. A good example of this is the transport of cholesterol from its site of synthesis in ER membranes with a low cholesterol content, to the *trans*-Golgi and the PM, compartments with a high cholesterol concentration [62]. Another example is PS; it needs to be transported from the ER to the PM, which has PS enriched in its cytoplasmic leaflet [63]. Such lipid transport obviously requires the expenditure of metabolic energy. The mechanisms supplying this energy remained for long poorly understood. A hallmark observation in the LTP field offering crucial hints to such mechanisms was made by de Saint-Jean et al. [37], who found that the ORD of yeast Osh4p can accommodate two different types of lipids, sterols and PI4P, and presumably transfer them in opposite directions in an exchange-type fashion. Structural analyses and sequence comparisons between the ORP family members [37, 39, 64] suggested that all ORPs may be able to accommodate PI4P with their ORD ligand pocket, and that the fully conserved 'OSBP fingerprint' sequence EQVSHHPP in fact forms part of the inositol-phosphate binding cleft in the protein structure.

The mammalian OSBP targets the ER via its FFAT motif binding to VAPs, and the *trans*-Golgi network (TGN) through PH domain interactions with PI4P and the GTPase ARF [46, 48, 55, 60](Fig. 3). Consistent with the proposed sterol/PI4P exchange model, the group of B. Antonny provided in 2013 convincing evidence that OSBP transfers cholesterol from the ER to TGN over MCS, and PI4P in the opposite direction [55]. The PI4P is synthesized in TGN membranes via the PI 4-kinases PI4KIIα and PI4KIIIβ, and after its arrival at the ER, hydrolysed by the PIP phosphatase Sac1. This cycle of PI4P synthesis and hydrolysis has two crucial functions: (i) It energizes the transfer of cholesterol against its concentration gradient and (ii) The removal of PI4P from TGN by OSBP acts as a means of negative feedback regulation of OSBP function. When a large amount of PI4P has been transferred, the PH domain of OSBP detaches from the TGN membrane, switching off the activity of OSBP. A follow-up study based on the use of OSW-1, a small-molecular inhibitor of OSBP, suggested that this OSBP-driven bidirectional lipid transport cycle consumes approximately 50% of the total cellular PI4P pool, the consumption depending on the amount of cholesterol to be transported [65]. Interestingly, OSBP carries in its amino-terminal part an intrinsically disordered region shown to act as an entropic barrier that regulates protein dynamics and orientation at the ER-TGN MCS [66].

ORPs constitute a large family of proteins, which were initially all assumed to bind sterols. However, the group of Y.J. Im, who reported the structure of yeast Osh3p and recently of human ORP3 ORDs, observed that their lipid-binding cavities are too narrow to accommodate the bulky 4-ringed sterol structure [39, 44]. At the same time, the group of A.-C. Gavin [40] documented a function of yeast Osh6p and Osh7p in PS transport from the ER to the PM, suggesting that these ORPs bind PS and PI4P as their two ligands and transport them in opposite directions. Of note, phylogenetic analysis by Maeda et al. [40] suggested that mammalian ORP5 and ORP8 belong to the same clade as Osh6p and -7p. Consistent with this sequence relatedness, the group of P. De Camilli provided two years later convincing evidence that ORP5 and -8 transport PS from the ER to the PM, in exchange for PI4P [49](Fig. 3). The nature of the counter-transport substrate was subsequently questioned by the group of R.H. Yang, whose analyses suggested that the PH domains of ORP5/8 rather target the PM through $PI(4,5)P_2$ than PI4P [50]. Notably, the ORD of ORP8 was shown to transport $PI(4,5)P_2$ much more efficiently than PI4P, putting forward the hypothesis that PIPs other than PI4P may be responsible for the membrane targeting of specific ORPs and also act as counter-transfer substrates. Interestingly, the groups of G. Fairn and T. Balla dissected the protein sequence upstream of the PH domain of ORP8, identifying negatively and positively charged segments that through electrostatic repulsion/attraction regulate the PH domain-dependent association of the protein with the negatively charged surface of the PM cytoplasmic leaflet [51, 56]. Sohn et al. [51] showed evidence that ORP5/8 recruitment to the PM occurs through interactions of the PH domains and adjacent basic residues with both PI4P and $PI(4,5)P_2$. Although ORP5 activity requires normal levels of these PIPs, ORP8 is called on only when $PI(4,5)P_2$ levels are increased. The authors concluded that the regulation of ORP5/8 attachment to the PM by both PIPs provides a powerful means to determine the relative flux of PI4P toward the ER for PS transport/Sac1-mediated dephosphorylation and PIP 5-kinase-mediated conversion to $PI(4,5)P_2$. Interestingly, the group of F. Giordano demonstrated that ORP5 and -8 also localize at ER-mitochondrial junctions (MAM) and their knockdown compromises mitochondrial structure and function [67]. It remains to be determined whether ORP5/8 might transfer PS also from the ER to mitochondria. Notably, in this case the transfer would take place down and not up a concentration gradient, and thus follow different mechanistic principles than at the ER-PM junctions. Moreover, ORP5 was shown to function at ER-lipid droplet (LD) junctions, the data suggesting that it mediates there the removal of PI4P from the LD in exchange for PS [68, 69]. In addition to ORP5 and -8, also ORP10 (lacking a FFAT or other ER-targeting motif) was implicated as a PS transporter, which supplies this lipid from the

ER to the TGN in HeLa cells [70]. However, the latest study, also carried out in HeLa cells, found ORP10 to localize at ER-endosome MCS via dimerization with ORP9, which carries a FFAT motif and binds to VAPs at these MCS [71]. ORP10 was shown to mediate PS/PI4P countertransport, apparently transferring PS from the ER to endosomes in exchange for PI4P, with impacts on the retrograde trafficking of mannose-6-phosphate receptor and endosome fission. We find it possible that ORP10 may act as a PS/PI4P exchanger at both ER-TGN and ER-endosome MCS.

The mechanistic principles of ORP-mediated lipid counter-current transport cycles of Osh4p [72] and Osh6p [41] were further confirmed by the group of G. Drin in two elegant studies published in 2015. In these papers, Moser von Filseck et al. (i) employed quantitative, real-time lipid transport assays to demonstrate that Osh4p transports sterol against its gradient between two membranes by dissipating the energy of a PI4P gradient, and (ii) demonstrated, by using a high-resolution structure determined for Osh6p:PI4P complex and site-specific mutant proteins, that Osh6p follows the same principle in PS-PI4P counter-current transport. Of note, like Osh4p, Osh1p was found to bind ergosterol and PI4P within its ORD cavity, and suggested to counter-transport these lipids at ER-Golgi MCS and/or at the nucleus-vacuole junction (NVJ)[38, 52].

To conclude, a number of ORPs in both mammalian cells and yeast are shown to execute lipid counter-current transport. Here, a lipid such as a sterol or PS, is moved over MCS against its concentration gradient in exchange for PI4P or another PIP, which is transferred down its concentration gradient. The transport cycles are energized and the PIP gradient maintained by organelle-specific cycles of PIP synthesis and hydrolysis. Thus far there is evidence for the functionality of this principle in sterol and PS transport, and a recent report suggests that mammalian ORP3 may mediate the counter-current transport of phosphatidylcholine (PC) vs. PI4P at ER-PM MCS [73]. How extensively this model applies to the ORP family and to various lipid classes remains to be determined. Moreover, the mechanistic principles of putative ORP-mediated lipid transfer down a concentration gradient may become an interesting topic of future study. The MCS locations at which the distinct ORPs are envisioned to function are summarized in Table 1.

Table 1. Membrane contact site localizations of human and yeast ORPs[1]

Species	Protein	MCS	Reference(s)
Human	OSBP	ER-TGN[2] ER-endosome Endosome-TGN	[55, 74] [75] [76]
	ORP4L/OSBP2	ER-TGN	[77]

	ORP1L	ER-LE/Lys ER-Lys-Mitochondria ER-Phagosome	[78] [79] [80]
	ORP2	ER-LD	[81]
	ORP3	ER-PM	[73, 82, 83]
	ORP6	ER-PM	[84]
	ORP5A	ER-PM ER-Mitochondria	[49-51] [67]
	ORP8L	ER-PM ER-Mitochondria	[49-51] [67]
	ORP8(S)	ER-PM	[56]
	ORP9L	ER-Golgi	[85]
	ORP10	ER-TGN	[70]
		ER-endosome	[71]
Yeast	Osh1p	NVJ, ER-Golgi	[58, 86]
	Osh2p	ER-PM ER-endocytic site	[87] [88, 89]
	Osh3p	ER-PM ER-endocytic site	[87] [89]
	Osh6p	ER-PM	[40, 87, 90, 91]
	Osh7p	ER-PM	[87, 91]

[1]Note: ORPs without a demonstrated MCS localization are omitted form the table.
[2]Abbreviations: ER, endoplasmic reticulum; TGN, trans-Golgi network; LE, late endosome; Lys, lysosome; LD, lipid droplet; PM, plasma membrane; NVJ, nucleus-vacuole junction

4. ORPs with functional roles in in the endocytic pathway

Cells acquire cholesterol through endogenous synthesis or endocytic uptake of low-density lipoproteins (LDL) from the extracellular milieu. Cholesterol esters contained by the internalized lipoproteins are hydrolyzed by lysosomal acid lipase (LAL) in endosomes/lysosomes with a reduced pH, followed by transport of the unesterified cholesterol to the limiting membrane of LE/Lys by the luminal Niemann-Pick C2 protein (NPC2) and the integral membrane protein NPC1 [92, 93]. After this, a lot of the LE/Lys cholesterol arrives at the plasma membrane (PM) and is thereafter forwarded to the ER, where the enzymes esterifying the excess cholesterol [93, 94], and the sterol homeostatic sensor machinery [95, 96] are located. In addition to transport via the PM, LDL-cholesterol can reach the ER via retrograde membrane trafficking from LE to the *trans*-Golgi network (TGN)[97], or it can move from LE/Lys to the ER membranes directly [93, 98, 99], or indirectly via peroxisomes [100, 101], these modes of transport apparently occurring via MCS.

Upon depletion of exogenous lipoprotein-derived cholesterol, cells meet their need of cholesterol by enhanced biosynthesis, which occurs predominantly in ER membranes. Under these circumstances cholesterol transport from the ER to endosomes becomes necessary, as formation of

the cholesterol-rich intraluminal vesicles (ILV) of LE requires direct cholesterol transfer from the ER [102].

Transport of cholesterol to and from the various endocytic compartments is mediated via a multitude of protein machineries [93, 94], among them the ORPs. The most convincing evidence in this context has been presented for ORP1L, ORP2 and OSBP, However, also ORP1S, ORP5, ORP6, and ORP11 have been implicated in endosomal cholesterol trafficking [103-106].

A further, emerging aspect of endocytic pathway lipid transport that involves the ORPs is the distribution of phosphoinositides on endosomes [33, 75]. PIPs are synthesized at distinct membrane compartments, including endosomes, by lipid kinases with specific subcellular localizations, and turned over by specific phosphatases [107-109]. They can also move between organelles along with vesicle transport or be transferred by LTPs such as the ORPs (see below paragraphs). Of the PIPs, the 3-phosphorylated species PI3P, PI(3,4)P_2 and PI(3,5)P_2, as well as PI(4,5)P_2, execute crucial functions within the endocytic pathway. PI3P synthesized by VPS34 is a critical component of sorting endosomes, while the generation of PI(3,4)P_2 and the dephosphorylation of PI(4,5)P_2 regulate clathrin-coated pit (CCP) maturation and vesicle uncoating [110, 111]. PI(3,5)P_2 generated by the lipid kinase Fab1/PIKfyve is critical for cellular homeostasis and adaptation to stimuli. Its deficiency are linked to human diseases, especially those of the nervous system, and result in defects in autophagy and the degradative function of lysosomes [112, 113]. In addition to controlling plasma membrane events including exocytosis and CCP formation in endocytosis, PI(4,5)P_2 is also critical in many other membrane trafficking events such as endosomal trafficking, sorting of hydrolases to lysosomes, initiation of autophagy, and formation of autophagic lysosomes [114-117]. The reports on roles of ORPs in endocytic pathway PIP trafficking or metabolism involve the transfer of PI4P from phagolysosomes to ER by ORP1L (see 4.1), the capacity of ORP2 to facilitate the synthesis of PI(4,5)P_2 on recycling endosomes and its transport to LE (see 4.2), and the removal of PI4P from endosomes by OSBP (see 4.4). Moreover, cholesterol transport function of the late endosomal ORP1L was found to be allosterically regulated by PI(3,4)P_2 and PI(4,5)P_2 (see 4.1).

4.1. ORP1L and ORP1S

OSBP-related protein 1 (ORP1) also called OSBP-like protein 1 (OSBPL1) is present as two major variants, a ’short’ one (ORP1S) that constitutes essentially an OSBP-related ligand-binding domain (ORD) only, and a ’long’ one (ORP1L) with an additional FFAT motif, a PH domain, and four ankyrin

repeats near its amino-terminus [31](Fig. 1). The function of ORP1L in organelle dynamics and cholesterol transport in the late endocytic pathway is well established. ORP1L targets NPC1-positive LE/Lys via interaction of its ankyrin repeats with the small GTPase Rab7 [57, 118, 119] and the ER through VAP proteins. A tripartite complex of ORP1L with Rab7 and RILP (Rab7-Interacting Lysosomal Protein) recruits the dynein-dynactin motor complex on LE/Lys to drive microtubule (−) end directed organelle movement [120](Fig. 4). Under low-cholesterol conditions the affinity of ORP1L for VAP increases, resulting in enhanced ER-LE MCS and detachment of the dynein-dynactin complex, thus preventing the (−) end directed transport of LE [78, 118, 121]. RILP also binds the LE tethering homotypic fusion and vacuole protein-sorting (HOPS) complex and the dynactin subunit $p150^{Glued}$, and ORP1L regulates in a cholesterol-dependent fashion the interactions between RILP, HOPS, and $p150^{Glued}$ [122]. Besides ER-LE MCS, ORP1L mediates contacts between the ER and autophagic vacuoles, which slow down the maturation of autophagosomes. Moreover, the ORP1L-mediated MCS suppress the binding of Pleckstrin Homology and RUN Domain Containing M1 (PLEKHM1) to Rab7. PLEKHM1 and RILP together recruit the HOPS complex for fusion of autophagosomes with LE/Lys. ORP1L-mediated ER-autophagosome MCS thus control the final stages of autophagy [123].

While the function of ORP1L in LE/Lys motility and autophagosome maturation is well established, its function in intracellular lipid transport is more controversial. Initially, Kobuna et al. [124] found that ORP1L depletion in HeLa cells induced enlarged LE/MVB lacking a normal ILV content, resembling the LE generated upon cholesterol restriction of *Caenorhabditis elegans*. The implication of this observation was that ORP1L may mediate cholesterol transport from the ER to LE (Fig. 4). Consistently, Eden et al. [125] showed a function of ORP1L in the transfer of cholesterol from the ER to LE via Annexin A1-regulated MCS. These MCSs occurred between the ER and LE/MVB at which the protein tyrosine phosphatase PTP1B downregulates *in trans* the activity of epidermal growth factor receptor (EGFR). The above papers suggested that the ER-LE cholesterol trafficking route plays an important role in the formation of ILV within the LE/MVB and in spatial regulation of EGFR signaling. Could ORP1L also mediate cholesterol trafficking in the opposite direction, from LE to the ER (Fig. 4)? The group of N. Ridgway devised CRISPR-Cas9 to show that knockout of ORP1L in cultured cells inhibited cholesterol esterification by 60-80%, consistent with a defect in cholesterol transport to the ER [126]. Moreover, the ORP1L knockout increased *de novo* cholesterol synthesis in the ER, thus adding further evidence for a defect in cholesterol transport towards to ER. Consistently, overexpression of ORP1L was reported to increase ER-Lys MCS, reducing the lysosomal

cholesterol accumulation in NPC1 null cells [98]. However, puzzlingly, the cholesterol accumulation was not only rescued by overexpression of full-length ORP1L but also by a deletion construct devoid of the ORD, suggesting that the observed functional rescue may rather be due to expansion of ER-LE/Lys MCS than a direct role of ORP1L as a cholesterol transporter.

Interestingly, the adenoviral protein RIDα can rescue the cholesterol storage phenotype of *NPC1* mutant fibroblasts and reconstitutes LE/Lys to ER transport of cholesterol; This RIDα-mediated pathway was shown to depend on ORP1L [127]. Of note, the RIDα-induced lipid transport attenuated proinflammatory signaling by Toll-like receptor 4 (TLR4), which plays an important role in adenovirus pathogenesis and depends on lipid raft domains [128]. Thus, it appears that adenovirus takes advantage of ORP1L to modify lipid raft cholesterol in order to suppress innate immunity attacking the virus. Also in this case the ORD of ORP1L appeared dispensable for the RIDα-induced cholesterol transfer [127], indicating that ORP1L may act as a membrane-tethering component rather than an a genuine sterol transporter. If this indeed were the case, the actual cholesterol transfer should take place via other carrier protein(s) or possibly spontaneously over the narrow MCS gap. However, one is left wondering how well the rescue achieved by overexpression of ORP1L may reflect the physiologic transport process. Could artificial expansion of the LE/Lys-ER MCS induce a mode of transport that is not physiologically significant under normal conditions?

The ORP1L-mediated cholesterol transport was found to be allosterically enhanced by PI(4,5)P_2 and PI(3,4)P_2, but ORP1L was unable to transport these PIPs [42]. However, in contrast to this data, ORP1L was reported to mediate the transfer of phagolysosomal PI4P to the ER during the resolution of macrophage phagolysosomes, and ORP1L depletion slowed down resorption of the phagolysosomes [80]. Analogously, ORP1L was recently found to transport PI4P from lysosomes to mitochondria at three-way MCS of the ER, lysosomes and mitochondria [79]; Such contacts were reported to play a crucial role in mitochondrial division. Of note, the authors of the above two studies did not address a possible role of ORP1L in cholesterol transport at the MCS under study. Thus, it remains unclear whether ORP1L has lipid counter-current transporter activity.

The function of ORP1S, which lacks the membrane targeting determinants present in the amino-terminal half of ORP1L, is poorly known, with only few studies on it published. The group of N. Ridgway generated ORP1L/ORP1S double knockout HeLa cells and found that loss of ORP1S rescues the defect in cholesterol esterification observed in ORP1L KO cells. Further results indicated that ORP1S opposes the function of ORP1L by transferring cholesterol from LE to the PM, making it inaccessible for direct transfer to the ER by ORP1L [106]. However, data by the group of R.H. Yang

employing a similar double knockout model contradicted the above findings, suggesting that both ORP1S and ORP1L mediate cholesterol transfer to the ER where it is esterified [42]. Interestingly, ORP1S was also reported to enter the nucleus and modulate liver X receptor (LXR)-mediated apolipoprotein E (*APOE*) gene expression, reflecting an intranuclear aspect of its function [129]. To conclude, the function of ORP1S is thus far poorly understood and warrants further study.

4.2. ORP2

OSBP-related protein 2 (ORP2), also known as OSBP-like protein 2 (OSBPL2), is a close homologue of ORP1. It is unique among the mammalian ORPs in existing only as a short form lacking a PH domain. ORP2 is largely cytosolic but can peripherally target LD, ER-lipid droplet MCS, and PI(4,5)P_2-rich endosomes. We initially demonstrated that ORP2 overexpression results in enhanced cellular cholesterol efflux and transport of newly synthesized cholesterol to the PM [130, 131]. Although not necessarily providing information on physiologic function of the endogenous ORP2, these observations suggested that ORP2 has the capacity transport cholesterol intracellularly. CRISPR-Cas9 knockout of *OSBPL2* in hepatocytes resulted in alterations of the actin cytoskeleton and suppression of cell proliferation, adhesion, migration and AKT signaling, defects in glucose uptake and metabolism, and reduced triglyceride synthesis [132, 133]. As AKT activity depends on the synthesis of PI(3,4,5)P_3 [134] and F-actin dynamics is crucially regulated by membrane phosphoinositides, especially PI(4,5)P_2 [135], we reasoned that genetic manipulations of ORP2 could disturb phosphoinositide homeostasis at the PM. Consistent with this notion, we found that ORP2 not only binds cholesterol but also PI(4,5)P_2, PI(3,4,5)P_3 and PI4P [136]. Experiments with the cholesterol probe perfringolysin O domain D4H suggested that ORP2 facilitates the transfer of cholesterol towards the PM. The tentative donor compartment was LE, where D4H-accessible cholesterol accumulated upon ORP2 knockdown or overexpression of mutants incapable of binding cholesterol or PIP [136]. The group of R.H. Yang [43] simultaneously reported a high-resolution structure of ORP2, a tetramer with bound PI(4,5)P_2, while the cholesterol-bound protein was found to be monomeric. Importantly, the authors produced evidence that ORP2 transports these two lipids in a counter-current fashion, depletion of ORP2 reducing cholesterol and increasing PI(4,5)P_2 at the PM. We detected in primary human umbilical vein endothelial cells (HUVECs) subjected to ORP2 knockdown a similar increase of PM PI(4,5)P_2, but failed to detect a significant reduction of PM cholesterol [35], indicating that depletion of ORP2 by different methods (knock-out or knock-

down) in different cell types may result in different compensatory responses modifying the phenotype. Since a number of vesicular and non-vesicular routes of cholesterol transport between the PM and cellular endomembranes have been identified (reviewed in [62]) and distinct cell types may express and employ these pathways to a different extent, HeLa cells [43] and HUVECs [35] could display differential capacity to compensate for a reduction of PM cholesterol upon depletion of ORP2. Interestingly the HUVECs subjected to ORP2 KD displayed a defect in vascular endothelial growth factor (VEGF) signaling and a shift of both VEGFR2 and cholesterol from a light lipid raft membrane fraction to heavier membranes. ORP2 knockout mice were viable and superficially healthy, but exhibited a mild abnormality in retinal angiogenesis, consistent with a function of ORP2 in endothelial signaling that drives angiogenesis.

The most recent work by E. Ikonen's group provides insight into the specific cholesterol transport step mediated by ORP2. They tagged ORP2 in the endogenous locus of A431 cells with GFP and a degron tag allowing acute depletion of the protein upon treatment with auxin [33]. The experiments employing this tool suggested that ORP2 mediates the transport of LDL-derived cholesterol from LE/Lys to recycling endosomes (RE) containing integrin β1 and focal adhesion kinase (FAK), thus affecting focal adhesion dynamics and cell adhesion. This study suggested that cholesterol transferred to RE facilitates the recruitment of FAK on the organelles. FAK in turn activates PIP-kinase I-γ on endosomes thus enhancing the synthesis of PI(4,5)P_2 [137]. ORP2 apparently transports this PI(4,5)P_2 to LE/Lys, where it regulates transport tubule formation (Fig. 4). Whether this two-way lipid transporter activity of ORP2 may involve MCS is not known. Although it is possible that the ORP2-mediated transfer of cholesterol to RE may indirectly affect the flux to the PM, the data does not exclude a second function of the protein in direct cholesterol/PI(4,5)P_2 transport between LE/Lys and the PM.

Escajadillo et al. [138] demonstrated that ORP2 knockdown modified steroid hormone synthesis in adrenocortical cells. The depletion of ORP2 suppressed the expression of several enzymes responsible for cortisol production and of the transcriptional regulator steroidogenic factor 1 (SF-1). Moreover, it resulted in cellular cholesterol accumulation and reduction of the oxysterols 22-hydroxycholesterol and 7-ketocholesterol. Furthermore, ORP2 was suggested to interact physically with LXR and to promote nuclear LXR expression, reminiscent of the previously mentioned observations on ORP1S [129]. Although the above effects of ORP2 knockdown on steroid hormone synthesis and oxysterols could at least in part reflect a defect in intracellular cholesterol

trafficking, the data indicates that ORP2 may also regulate sterol homeostasis by other mechanisms including transcriptional regulation.

4.3. Functions of ORP1 and ORP2 in vivo

We demonstrated some years ago that a heterozygous loss-of-function variant of *OSBPL1A* resulting in an early truncation (C39X) of the encoded ORP1L protein associates with very low circulating HDL-cholesterol [139]. Moreover, fibroblasts from the variant carriers showed a defect in cholesterol efflux to apolipoprotein A-I, which could reflect a LE/Lys cholesterol trafficking defect. We had earlier reported that overexpression of human ORP1L in macrophages enhanced atherogenesis in LDL-receptor deficient mice and inhibited macrophage cholesterol efflux [140], consistent with a function of ORP1L in cholesterol trafficking *in vivo*. Carlin and Manor [141] suggested the intriguing scenario that ORP1L could represent an alternative, under normal conditions tightly regulated cholesterol transport machinery, co-opted upon specific pathologic situations. Examples of this are the endo-lysosomal lipid accumulation upon adenoviral infection that is mitigated by the RIDα-ORP1L route [142], and foam cell formation characterized by excessive cholesterol ester synthesis and storage e.g. in atherosclerotic plaques [143]. To conclude, the physiologic functions of ORP1L and their pathologic utilization in disease warrant detailed future study.

Human mutations in *OSBPL2* encoding ORP2 result in autosomal dominant non-syndromic hearing loss due to cochlear hair cell damage [144-146]. Can this relate to the cholesterol transport function of ORP2? In the OC1 auditory cell line and *Osbpl2b* mutant zebrafish, depletion of ORP2 was found to result in elevated cellular cholesterol biosynthesis and reactive oxygen species (ROS) production, reduced AMP kinase (AMPK) activity, and mitochondrial damage [147]. Whether these dysregulations play a role in the cochlear cell dysfunction in humans is as yet unclear. The cochlear cells of *OSBPL2*/ORP2 knockout Bama miniature pigs were shown to display defective stereocilia [148], and the ORP2-deficient cochlear cells exhibited reduced FAK activity [149]. These observations lend support to the hypothesis that the etiology of the hearing loss may involve defective cholesterol transport and FAK activity. In addition to the hearing loss, the knockout pigs presented with hypercholesterolemia, representing the first *in vivo* evidence for a role of ORP2 in controlling serum cholesterol levels. However, no direct evidence for a dependency of this phenotype on defective cholesterol egress from LE/Lys was presented.

Many of the ORPs are abundantly expressed in the central nervous system (CNS) [150]. Despite this fact, the functions of ORPs in neurons or glial cells have been addressed hardly at all. The sporadic observations thus far published involve ORP2, ORP6 and OSBP. Weber-Boyvat et al. [151] reported that knockdown of ORP2 in young mouse hippocampal neurons reduced the length of dendrites and synapse number, and eventually induced cell death. These seminal findings suggested a function of ORP2 in neurite outgrowth and neuronal viability. Mochizuki et al. [84] reported that ORP6, a family member enriched in the CNS, is in mouse cerebellar granular neurons found in the ER and the ER-PM MCS, and regulates there the turnover of PI4P. Furthermore, Gu et al. [152] identified OSBP as a target and downstream effector of miR-124 suggested to regulate neurite outgrowth and elongation through an as yet poorly understood mechanism. We find it plausible that lipid transport functions in the extremely lipid-rich CNS are vital for processes such as neurite outgrowth and neurotransmission [153-155]. Detailed study of the thus far neglected neuronal functions of ORPs is therefore warranted.

4.4. OSBP

Studies in the recent years have revealed that the cholesterol/PI4P transfer functions of OSBP are not limited to the ER-TGN counter-current trafficking of cholesterol and PI4P (see section 3), but also involve endosomal lipid transport. Initially, the group of P. De Camilli provided evidence that OSBP removes PI4P from LE/Lys over MCS, thus controlling actin nucleation on the endosomes and function of the retromer complex that mediates retrograde membrane trafficking from endosomes to Golgi [75]. Later on, Lim et al. [156] discovered that OSBP regulates a lysosomal cholesterol pool important for mechanistic target of rapamycin complex 1 (mTORC1) signaling. In Niemann-Pick C (NPC) cells with lysosomal cholesterol and sphingolipid accumulation mTORC1 is hyperactive, inhibits autophagy, and cannot be inactivated by cholesterol depletion. Lim et al. [156] found that depletion or inhibition of OSBP in NPC cells reduces mTORC1 recruitment onto Lys, dampens the aberrant mTORC1 signaling, and restores autophagy. This function of OSBP depended on its ability to bind both cholesterol and PI4P as well as its targeting to both ER and Lys, suggesting that OSBP transfers the two lipids in a counter-current fashion at ER-Lys MCS. Additionally, OSBP was recently found to transfer cholesterol from recycling endosomes to the TGN [76]. In this study, the authors identified a protein named RELCH that interacts with OSBP at the TGN and the small GTPase Rab11 on RE, thus mediating tethering of the two organelles. Depletion of any of these three proteins

resulted in endo-lysosomal cholesterol accumulation. Moreover, the authors provided *in vitro* evidence that OSBP mediates cholesterol transfer from the Rab11-positive RE to the TGN.

4.5. ORP5, -6 and -11

ORP5 was initially reported to interact with NPC1 and to remove cholesterol from the limiting membrane of LE/Lys [104]. However, the physiologic significance of this finding has remained enigmatic as later studies reported a function of ORP5 in PS-PIP exchange at ER-PM MCS [49, 50], and as a controller of PI4P concentration on lipid droplets [68, 69]. Since plasma membrane PS strongly impacts the transport of cholesterol [157], we find it possible that the reported effect of ORP5 on LE/Lys limiting membrane cholesterol could be indirect and mediated by the function of ORP5 in PS transport to the PM. On the other hand, the recently reported capacity of ORP5 to modulate the function of mTORC1 on LE/Lys [34] can be considered as support for its direct role in LE/Lys cholesterol trafficking.

Knockdown of ORP6 was reported to result in a clustering of endosomes and cholesterol accumulation in these organelles, as well as reduced cholesterol esterification [105]. Consistently, ORP6 overexpression enhanced cholesterol trafficking and efflux in hepatocytes and macrophages. Furthermore, the authors found that hepatic ORP6 expression correlated positively with plasma high-density lipoprotein (HDL)-cholesterol in human subjects and was reduced in atherosclerotic plaques. These observations suggested that ORP6 may play a role in intracellular cholesterol trafficking, tentatively from endo-lysosomes, required for the efflux of cholesterol to plasma HDL.

ORP11 is one of the least studied family members. It was localized to both TGN and endosomes and reported to dimerize with ORP9 [158], which could provide a means of peripheral membrane association for this protein lacking an ER-targeting FFAT motif or trans-membrane segment. When macrophages are supplied with phosphatidylglycerol (PG), they synthesize abundant bis(monoacylglycero)phosphate (BMP), a unique lipid of the LE/MVB intraluminal vesicles. This protects the cells from the cytotoxic effect of oxidized LDL (oxLDL) by facilitating the egress of oxLDL-derived cholesterol from the LE/Lys. Removal of cholesterol from these compartments prevents the synthesis of cytotoxic oxysterols synthesized non-enzymatically within the late endocytic organelles, such as 7β-hydroxycholesterol and 7-ketocholesterol [159]. Arnal-Levron et al. [103] found that knockdown of ORP11 attenuated the cytoprotective effect of PG and resulted in macrophage accumulation of free cholesterol, reduced cholesterol efflux, and elevated

synthesis of the non-enzymatic oxysterols. These findings indicated that ORP11 may act to mediate the egress of oxLDL-derived cholesterol form LE/Lys. However, no evidence for a function of ORP11 as a direct cholesterol transporter was reported. Of note, polymorphisms in the gene *OSBPL11* encoding ORP11 were reported to associate with a number of cardiovascular risk factors, including plasma LDL-cholesterol levels, in severely obese Canadian subjects [160], supporting a connection of ORP11 function with cholesterol metabolism *in vivo*.

To conclude, there are an increasing number of proteins with established or implicated roles in lipid trafficking in the endocytic pathway, including seven members of the ORP/OSBPL protein family. The physiologic coordination of cholesterol trafficking by these proteins and the other endo-lysosomal cholesterol transport machineries is as yet poorly understood and warrants detailed study. A thorough understanding of the complex endosomal cholesterol trafficking apparatus will help us comprehend the molecular pathogenesis of diseases such as lysosomal storage, neurodegenerative and cardiovascular diseases, promoting the discovery of new therapeutic approaches.

5. ORPs, plasma lipids and cardiometabolic diseases

Considering that a number of ORPs have established roles in intracellular lipid trafficking, one would expect their function to be strongly reflected in the composition and concentrations of circulating lipoproteins. Accordingly, *OSBP/OSBPL* gene alleles and expression levels could be anticipated to associate with diseases connected with dyslipidemia, such as atherosclerotic cardiovascular diseases and type 2 diabetes mellitus. However, these genes are not hot spots for polymorphisms or mutations associated with circulating lipids or cardiometabolic diseases. There are only a limited number of such genetic associations of *OSBP/OSBPL* single nucleotide polymorphisms reported in GWAS studies. The first was found by Teslovich et al. [161], who observed an association of *OSBPL7* (encoding ORP7) single nucleotide polymorphisms (SNPs) with low-density-lipoprotein (LDL) and total cholesterol (TC) in >100,000 individuals of European ancestry. Since we detected highest ORP7 expression in the epithelia of the gastrointestinal tract [162], we envisioned that this protein could play a role in intestinal lipid adsorption, which could potentially explain the observed genetic associations. Interestingly, a recent study reported that small-molecular compounds targeting ORP7 stabilized ABCA1 and increased the ABCA1-dependent cholesterol efflux from kidney podocytes to

lipid-poor high-density lipoprotein (HDL), assigning ORP7 a novel function as a regulator of cholesterol homeostasis and kidney function [163].

Further associations of ORP function with circulating lipids were reported for ORP10 and -11 encoded by *OSBPL10* and *-11*, respectively. We observed that knock-down of ORP10 in human hepatoma cells resulted in an increase of apolipoprotein B-100 (apoB-100) secretion as well as triglyceride (TG) synthesis and secretion [164]. Consistently, polymorphisms in the *OSBPL10* gene were found to be associated with high TG [165] and LDL-cholesterol levels [166] and peripheral artery disease [167]. Later on, the role of ORP10 in hepatocyte apoB lipoprotein secretion was confirmed in the study of Venditti et al. [70], who implicated that ORP10 transfers PS from the ER to the TGN. This PS transfer apparently modifies the lipid composition and function of *trans*-Golgi membranes in a way that limits the secretion of apoB-containing particles. The mechanism of ORP10 action discovered provides a plausible explanation to the genetic association of *OSBPL10* with circulating LDL- and total cholesterol. ORP10 lacks the ER-targeting FFAT motif, but was shown to dimerize with ORP9L anchored at ER membranes via the VAP proteins [164]. This dimerization could provide it the capacity to associate with ER-TGN MCS, similarly as it is reported to localize at ER-endosome MCS [71].

The function of ORP11, a paralogue of ORP10, is poorly understood. This protein, which is expressed at highest levels in adipose tissue [168], localizes at the interface of the TGN and endosomal compartments, and, like ORP10, dimerizes with ORP9L [158]. ORP11 overexpression was shown to induce the accumulation of lamellar lipid bodies in the vicinity of the Golgi complex and endosomes, suggesting a disturbance of lipid trafficking. Interestingly, *OSBPL11* polymorphisms were associated with several cardiovascular risk factors in obese Canadians with the metabolic syndrome, including diastolic blood pressure, LDL-cholesterol hyperglycemia/diabetes and metabolic syndrome *per se* [160]. These observations suggest that the functions of both ORP10 and -11 are connected with the etiology of dyslipidemia and metabolic disease.

Ma et al. [169] carried out analysis of the Framingham Heart Study data, identifying the *OSBPL8-ZDHHC17* genomic region in chr 12 as one associated with HDL-cholesterol levels. This finding was nicely consistent with our observations that *OSBPL8* knockout in mice resulted in elevated circulating HDL-cholesterol levels [170]. Furthermore, OSBPL8 knock-down stimulated macrophage cholesterol efflux [171], and hematopoietic loss of ORP8 resulted in a reduced progression of atherosclerotic lesions in LDL-receptor deficient mice [172]. How these observations

could relate to the established role of ORP8 as a PS transporter (see section 3) remains to be elucidated.

Yao et al. [148] characterized knockout Bama miniature pigs lacking ORP2/OSBPL2, an established intracellular cholesterol/PI(4,5)P_2 transporter [33, 35, 43, 136]. Similar to humans carrying *OSBPL2* mutations [144-146], the knockout pigs presented with progressive hearing loss with degeneration of cochlear hair cells and morphological abnormalities of hair cell stereocilia. Importantly, they also displayed hypercholesterolemia seen in the total, LDL- and HDL-cholesterol fractions, which was aggravated upon high-fat feeding [148]. Although OSBPL2 mutations have in human studies associated with non-syndromic hearing loss, and no connection with dyslipidemia has been reported, this animal model observation reveals a distinct impact of ORP2 on lipoprotein metabolism.

To conclude, ORPs have not appeared as major regulators of circulating lipoproteins or cardiometabolic diseases in human studies. However, there are genetic observations suggesting links of *OSBPL7*, *-10* and *-11* with circulating TG, LDL and total cholesterol, as well as of *OSBPL8* with HDL-cholesterol concentrations. Thus, the functions of ORPs in intracellular lipid transfer and signalling do reflect on systemic lipid metabolism and, putatively, the associated diseases. Therefore, further study on a possible role of ORPs as therapy targets for cardiometabolic diseases in warranted.

6. ORPs and cancer

The vigorous proliferation and migration of cancer cells necessitate an ample supply of lipids for membrane biogenesis. This lipid supply is provided through either enhanced synthesis or uptake of lipids from the extracellular milieu [173]. Therefore, reprogramming of lipid metabolism is considered one of the emerging future approaches to combat cancer. ORPs mediate inter-organelle lipid transport [52-54] and, even though they do not act as major regulators of bulk lipid synthesis, storage and oxidation, their manipulations do modulate lipid homeostatic responses [23, 24, 106, 126, 133] or the mode of cellular energy metabolism [174, 175]. One can thus envision that ORPs may impact the survival, growth and migration of cancer cells by modifying cellular lipid homeostasis, lipid fluxes, or bioenergetics.

Another central aspect of lipid metabolism highly relevant for cancer is the synthesis and turnover of PIPs. Here, the key processes targeted in drug development are the synthesis and the

degradation of the crucial signaling lipid phosphatidylinositol 3,4,5-trisphosphate [PI(3,4,5)P_3 or PIP_3], which is generated from PI(4,5)P_2 through the activity of phosphoinositide 3-kinases (PI3Ks). PIP_3 in turn activates phosphoinositide-dependent kinase 1 (PDK1) required for the activation of AKT/protein kinase B, a key component driving cell proliferation, migration, and survival [176]. Importantly, PI3K/AKT signaling also modulates activity of the mTOR, a central hub in the metabolic control during tumorigenesis [177]. In cancers the PI3K signaling can be abnormally activated upon modification of upstream regulators such as *HER2* (epidermal growth factor receptor, EGFR), activating mutations in PI3K itself, or in PI3K effectors such as AKT [178]. Oncogenic mutations may also occur in components that catalyze the hydrolysis of PIP_3, such as Phosphatase and tensin homologue (PTEN), a PIP_3 3-phosphatase and tumor suppressor depleted or inactivated in several types of cancers [179].

PI4P, a ligand of many if not all ORPs and an immediate precursor of PI(4,5)P_2, is a key substrate in two PIP-dependent signaling pathways. In addition to PI3K/AKT signaling, it plays a central role in phospholipase C signaling. PLCs hydrolyze PI(4,5)P_2 to release the 2nd messengers diacylglycerol (DAG) and inositol 1,4,5-trisphosphate (IP_3), the latter of which triggers Ca^{2+} release from the ER stores and a wide variety of Ca^{2+}-dependent signaling cascades [180].

The ORPs, the first identified PIP ligand of which was PI4P [37], are also shown to bind other PIPs, including PI(4,5)P_2, PI(3,4)P_2, and PI(3,4,5)P_3, either through their PH domain or their ORD [42, 43, 50, 130, 136, 174]. Therefore, modulation of PIP-dependent signaling is one of the mechanisms through which they are believed to affect malignant growth [174, 175, 181]. However, the involvement of ORPs in cancers is likely not mediated by lipid interactions alone: ORPs are also shown to interact physically with proteins playing important roles in oncogenic signaling, as discussed below.

6.1. ORP3 in cancers

ORP3 is highly expressed in neuronal, epithelial and hematopoietic cells [162]. This protein and its close homologue ORP7 were shown to interact with the small GTPase R-Ras [182], a Ras paralogue with a somewhat controversial role in cancer [183]. We identified ORP3 as a phosphoprotein that regulates the formation of polarized cell surface protrusions, cell spreading on substratum and β1-integrin activity [184]. The interaction of ORP3 with VAPA was found to be regulated by phosphorylation of ORP3, and co-expression of the two proteins resulted in an activation of R-Ras

signaling [83]. Importantly, the group of T. Balla recently showed that protein kinase C (PKC) activates the PM association of ORP3. Full activation of ORP3 decreased plasma membrane PI4P levels and inhibited store-operated Ca^{2+} entry [82]. Furthermore, ORP3 was recently found to interact with IQSec1, a GTP exchange factor for Arf5, and the ORP3-IQSec1 complex was translocated upon PKC activation and Ca^{2+} elevation to ER-PM MCS adjacent to focal adhesions [73]. Interestingly, ORP3 was found to extract PI4P from the plasma membrane, apparently in exchange for phosphatidylcholine. This lipid exchange was demonstrated to play an important role in focal adhesion turnover pivotal for cell migration, tumorigenesis and metastasis [185, 186].

ORP3 expression is enhanced in testicular cancers [187-189] as well as B-cell malignancies [190-192]. Furthermore, the *OSBPL3* region in chromosome 7 is frequently gained in osteosarcomas and tumors of the colon [193-195]. Considering that constitutively active R-Ras mutations occur in a number of invasive cancers [196-198], excessive ORP3 could enhance tumorigenesis e.g. by facilitating the migration of the malignant cells through altered integrin activity [199, 200] or by enhancing survival signals [201, 202]. In support of a functional link between ORP3 and skewed Ras signalling in cancer, the study of Jiao et al. [203] suggested that induction of *OSBPL3* by hypoxia inducible factor 1 (HIF-1A) promotes colorectal cancer progression through activation of the Ras pathway. Additionally, ORP3 was identified as a putative target of BCR-ABL up-regulated in bone marrow cells from subjects with chronic myeloid leukemia [204]. On the other hand, reduced ORP3 mRNA levels were reported to correlate with a shorter survival of colon cancer patients with advanced nodal metastasis and subjects with grade 3 colon cancer, while the low ORP3 mRNA levels appeared advantageous in patients with a T2 tumor size [205], indicating that ORP3 might represent a new prognostic marker for stratification of colon cancer patients. Puzzlingly, also a tumor suppressor role of ORP3 has been suggested: Knockdown of ORP3 enhanced the malignant transformation of human fibroblasts and its knockout in mice resulted in aberrant expansion of lymphoid progenitors and formation of pauci-clonal B-cell lymphoma [206]. Furthermore, ORP3 was reported to predict a good treatment response in patients with glioblastoma [207]

To conclude, elevated expression of ORP3 is frequently found associated with cancers. However, also roles as a tumor suppressor or a predictor of a good treatment response have been suggested. The controversial observations most likely reflect diverse functions of ORP3 in different malignant cell contexts. E.g., the effect of ORP3 could differ depending on whether a Ras pathway is activated in the tumor cells. Moreover, the plasma membrane association of ORP3 depends on protein kinase C (PKC) activation, especially when combined with Ca^{2+} increase [82], and plays an

important role in focal adhesion turnover during cell migration [73]. Therefore, the impact of ORP3 on tumorigenesis may depend on the status of PKC signalling and Ca^{2+} homeostasis within the malignant cells.

6.2. ORP4 in hematologic malignancies

ORP4 is a closely related paralogue of OSBP and is therefore also known as OSBP2. ORP4 is expressed as three variants, a full-length protein carrying a PH domain (ORP4L), a variant with part of the PH domain truncated (ORP4M), and a short variant lacking a PH domain (ORP4S; Fig. 1)[208]. While OSBP is expressed ubiquitously, ORP4 displays a limited expression pattern obviously reflecting a higher degree of functional specialization [209, 210].

ORP4 mRNA was initially found upregulated in HeLa cells, in metastatic breast cancer tumors, circulating cancerous cells of patients with small cell lung cancer, and in human epithelial cells expressing the human papillomavirus 16 E6 and E7 proteins. Therefore, it was initially called *HLM* (HeLa metastatic gene)[211]. The protein was also found induced in the leukocytes of patients with chronic myeloid leukemia [212]. In a hallmark study, the group of N. Shair [213] characterized natural products (cephalostatin 1, OSW-1, ritterazine B and schweinfurthin A) inhibiting the growth of a number of human cancer cell lines. These compounds were found to target OSBP and ORP4L, and were hence designated ORPphilins. This study revealed the functional involvement of ORPs in cancer cell survival and proliferation, and its conclusions were soon endorsed by the studies of Garcia-Prieto et al. [214] and Charman et al. [208]. Garcia-Prieto et al. [214] reported that OSW-1 kills leukemia cells at subnanomolar concentrations through a mechanism that involves elevation of cytosolic and mitochondrial [Ca^{2+}]. Charman et al. [208] demonstrated that ORP4 knockdown in HEK293 or HeLa cells resulted in a growth arrest, while its depletion in non-transformed intestinal epithelial cells induced apoptotic cell death. Moreover, Bensen et al. [215] recently showed a high antiproliferative capacity of OSW-1 in both monolayer and 3D ovarian cancer spheroid models, which was markedly potentiated by the depletion of extracellular lipids. The above observations suggested that ORP4 is mandatory for the growth or survival of rapidly proliferating and malignant cells.

Importantly, the group of D. Yan observed that ORP4L is essential for the survival of T-cell acute lymphoblastic leukemia (T-ALL) cells [175]. In contrast to many tumor cells that rely on aerobic glycolysis, T-ALL cells acquire energy through robust oxidative phosphorylation. ORP4L was found

to be absent in healthy T-cells but abundant in T-ALL cells, and has the capacity to scaffold in T-ALL cells a signaling complex consisting of CD3ε, Gαq/11, and phospholipase C β3 (PLCβ3). This G-protein coupled signaling complex activates PLCβ3 to produce IP_3, a function executed in normal T-cells by PLCγ1. ORP4L thus switches T-ALL cells to robust PLCβ3-mediated IP_3 production, ER Ca^{2+} release and respiration. Consistently, ORP4L knockdown resulted in suboptimal bioenergetics, T-ALL cell death and abrogation of T-ALL cell engraftment *in vivo* in a NOD/SCID mice model. The authors also demonstrated that ORP4L knock-down inhibited the proliferation of cervical cancer cell lines C33A, HeLa and CaSki, its overexpression resulting in an opposite effect [216]. Also in this study the underlying mechanism was tracked down to cellular Ca^{2+} homeostasis. Further data by D. Yan's group suggested that ORP4L also promotes the translocation of PLCβ3 from the nucleus, where it resides in resting Jurkat T-cells, to the PM [217]. Interestingly, ORP4L was additionally shown to interact with the carboxy-terminus of IP_3 receptor 1 (ITPR1)[181]. Truncated ORP4L lacking the ITPR1-binding region was capable of increasing IP_3 production but failed to induce Ca^{2+} release from the ER, consistent with a model in which ORP4L not only activates PLCβ3 but could also couple the generation of IP_3 to its interaction with ITPR1.

Importantly, D. Yan's group also investigated the function of ORP4L in $CD34^{+}CD38^{-}$ leukemia stem cells (LSCs) isolated from patients suffering from acute myeloid leukemia (AML) [174]. Moreover, they developed a derivative of OSW-1, LYZ-81, which showed increased selectivity for ORP4L vs. OSBP and reduced general cytotoxicity. ORP4L was found to be essential for LSC bioenergetics and survival, similar to the T-ALL cells. Mechanistic experiments suggested that ORP4L can pull $PI(4,5)P_2$ out of the PM and present it to PLCβ3 for hydrolysis. This intriguing observation awaits further validation and more detailed investigation. Of note, the OSW-1 derivative LYZ-81 was shown to significantly inhibit the engraftment of human LCSs in NOD/SCID mice.

To conclude, ORP4L has the capacity to maintain the survival and proliferation of rapidly growing cells both *in vitro* and *in vivo*. It is present only in a limited number of tissues and cell types, and its complete knockout causes in mice a mild phenotype characterized by male sterility [209]. ORP4L could thus represent a potential therapy target for specific forms of cancer including leukemias.

6.3. ORP5 in pancreatic cancer

The association of ORP5 with pancreatic cancer first emerged when Koga et al. [218] studied the expression and function of ORP5 in pancreatic cancer cells, observing abundant expression of the ORP5 mRNA in a cell line with high potential for invasion and metastasis, and ORP5 knockdown reduced the invasion rate of the cells. Importantly, markedly higher one-year and overall survival rates of patients with ORP5-negative pancreatic adenocarcinoma as compared to ORP5-positive tumors were observed. Ishikawa et al. [219] subsequently found that ORP5 can induce SREBP2, a key transcriptional regulator of cholesterol biosynthesis. The authors suggested that the association of high ORP5 expression with the poor prognosis could be attributed to an ability of ORP5 to drive robust cholesterol synthesis to facilitate vigorous proliferation and invasion of the cancer cells. Furthermore, the observed induction of histone deacetylase 5 (HDAC5), a target of SREBP2, could further aggravate the malignant phenotype.

According to the present perception, ORP5 is not a major regulator of cholesterol homeostasis. The current view is that it transports PS from the ER to the plasma membrane over MCS in exchange for PI4P [49] or other PIPs, mainly PI(4,5)P_2, which bind to both its PH domain and its ORD [50, 51](see section 3). In addition to ER-plasma membrane MCS, ORP5 also localizes to ER-mitochondria MCS and its knockdown compromises mitochondrial morphology and function [67]. In our opinion, the ER-plasma membrane PS transport function of ORP5 is most likely relevant for its cancer association: The PS content of the plasma membrane is crucial for the activation of AKT, a kinase that drives cell survival, proliferation and migration [220]. Depletion of plasma membrane PS through knockdown of ORP5 or -8 inhibited cell proliferation and anchorage-independent growth [221], which might in part be explained by mislocalization of the oncoprotein KRAS to cellular endomembranes upon the PM PS depletion. We find it possible that also the reported effect of ORP5 knockdown on LE cholesterol trafficking [104] could represent a secondary effect of a disturbance of PS transport to the PM, as PS was reported to play an important role in cholesterol trafficking from the PM to the ER mediated by GRAMD/Aster proteins [157].

Adding another interesting mechanistic aspect to the function of ORP5 in cancer, the group of R.H. Yang observed that ORP5 depletion in HeLa cells or disruption of its lipid binding interfered with cell proliferation, migration and invasion [34]. The authors found that ORP5 interacts with mTOR and activates mTORC1. This observation is obviously consistent with the earlier findings on ORP5 function at LE/lysosomes [104]. mTORC1 represents a point of convergence between the PI3K/AKT and mitogen-activated protein kinase (MAPK)/MEK/ERK signalling pathways which are typically hyperactivated in cancers [222]. Therefore, the association of ORP5 with cancer cell

invasion capacity and prognosis of pancreatic cancer could also be related to its ability to modify mTORC1 activity. In addition to the pancreatic cancer association reviewed above, elevated ORP5 expression was also observed in metastasis-positive lung tissue. ORP5 overexpression was found to enhance while its knock down reduced the invasiveness of lung cancer cells [223].

6.4. Role of ORP8 in tumor cell apoptosis

ORP8 is, like ORP5, associated with ER-PM and ER-mitochondria MCS [49, 67]. ORP8 was found downregulated in hepatocellular carcinoma (HCC) as compared to healthy liver tissue, concomitant with an elevation of miR-143, which directly targets ORP8 [224]. Overexpression of ORP8 induced apoptosis in primary HCC cells and cell lines, coinciding with the translocation of the death receptor Fas to the PM and induction of Fas ligand (FasL), apparently due to an ER stress response and activation of NF-κB. Consistent with the capacity of ORP8 to suppress tumorigenesis *in vivo*, intratumoral administration of ORP8 in a nude mice HepG2 xenograft model significantly inhibited tumor growth. The dampening of ORP8 expression in HCC could provide one putative explanation for the resistance of HCC to apoptosis; ORP8 might thus act as a tumor suppressor in certain cancers. This notion is corroborated by the reduced expression of ORP8 expression in gastric cancer (GC) tissues and cells, as well as the observation that ORP8 overexpression in GC cells inhibited tumor growth in xenografted nude mice [225]. Consistent with the HCC study, the induction of apoptosis in GC cells was attributed to ER stress and inhibition of Wnt signaling. Moreover, reduced ORP8 expression was observed in non-small cell lung cancer (NSCLC) tissues and cells, and ORP8 overexpression induced apoptosis of the NSCLC cells [226]. As a putative mechanism underlying the downregulation of ORP8 in NSCLC, the authors identified an elevation of miR-421, which suppresses ORP8 expression.

Most studies are consistent with the idea that ORP8 suppresses tumor growth through its capacity to induce apoptosis. However, the detailed mechanism(s) through which ORP8 induces an ER stress response have remained somewhat unclear. Moreover, future investigations based on reducing or knocking out the expression of ORP8 in non-cancerous cells and *in vivo* in animal models of tumorigenesis are warranted to determine whether the endogenous levels of ORP8 are sufficient to physiologically sensitize cells to ER stress and subsequent apoptosis.

To conclude, ORPs constitute a family of proteins with the potential to regulate the growth and invasion capacity of cancerous cells, as summarized in Table 2. The most studied family

members in the context of cancer are ORP3, -4, -5 and -8. For the other eight ORP family members, no functionally substantiated major associations with malignant growth have been reported. However, there are a number of published observations suggesting altered expression of distinct ORP mRNAs in cancers. The cancer associations of ORPs are most likely due to the impacts of ORPs on PIP metabolism and PIP-mediated signaling. However, also protein-protein interactions with signaling components involved in tumorigenesis most likely play a role. Detailed study of ORP functions in the context of cell survival, proliferation and motility will promote the discovery of new target molecules and processes for the development of novel cancer therapies.

Table 2. Functions of ORPs with established cancer connections

Protein	**Type of cancer (+, up-regulated; –, down-regulated)**	**References**	**Functions**	**References**
ORP3	Lymphoid malignancies (+) Chronic myeloid leukemia (+) Testicular cancers (+) Implicated also as tumor suppressor	[190-192] [204] [187-189] [205, 206]	Regulation of R-Ras signaling Control of integrin activity, cell adhesion and focal adhesion dynamics, interaction with IQSec1 Regulation of PM PI4P and Ca^{2+} dynamics Protection from aneuploidy Exchange of PM PI4P for PC at the PM	[83, 184, 203] [73, 83, 184] [82] [206] [73]
ORP4	T-cell acute lymphoblastic leukemia (+) Acute myeloid leukemia (+) Chronic myeloid leukemia (+) Metastatic breast cancer (+) Small cell lung cancer (+)	 [175] [174] [212] [211] [211]	Activation of PLCβ3, resulting in IP_3 and Ca^{2+} release Coupling IP_3 to Ca^{2+} release from the ER via ITPR1 Enhancement of NFAT activity and ITPR1 expression	 [174, 175, 217] [181] [216]
ORP5	Pancreatic adenocarcinoma (+ predicts poor prognosis) Metastasis-positive lung (+)	[218, 219] [223]	Transport of PS from the ER to the PM Regulation of the distribution of KRAS Regulation of PM PI4P and $PI(4,5)P_2$ levels Promotion of $PI(4,5)P_2$ hydrolysis by PLCγ1 Regulation of mitochondrial function Regulation of mTORC1 activity Impacts on cholesterol transport and synthesis	 [49-51] [221] [49-51] [227] [67] [34] [104, 219]
ORP8	Hepatocellular carcinoma (**–**) Gastric cancer (**–**) Non-small cell lung cancer (**–**)	[224] [225] [226]	Transport of PS from the ER to the PM Regulation of the distribution of KRAS	 [49] [221]

			Regulation of mitochondrial function Regulation of PM PI4P and $PI(4,5)P_2$ levels Sensitization of cells to ER stress and apoptosis	[67] [49-51] [224-226]

7. ORPs in viral and bacterial infections

Viruses typically depend on host cell membranes and their lipids in several stages of their life cycle: binding and entry into cells, replication, particle assembly and release from cells (Fig. 5). Enveloped viruses also incorporate a lipid membrane onto the progeny viral particles released. Therefore, viruses modify host cell lipid metabolism, trafficking and signalling to create conditions optimally supporting the viral life cycle. Here, they exploit the cellular lipid transfer proteins including a number of the ORP family members [228]. Analogously, also bacterial pathogens can make use of the ORP machinery to secure their intracellular survival and proliferation, as shown for *Salmonella* [229, 230].

7.1. Flaviviridae: Hepatitis C virus, Dengue virus, and West Nile virus

The first observations on OSBP function in viral replication were reported for HCV, a positive-strand ssRNA virus belonging to the enveloped *Flaviviridae* [231]. The HCV replication compartment within cells comprises a highly convoluted ER-derived membrane structure designated 'membraneous web', which remains partially contiguous with the ER [232](Fig. 5). This modified intracellular compartment depends on PI4KIIIα, which is recruited and activated by the HCV nonstructural protein NS5A [233]. The PI4P synthesized is necessary for the integrity of the replication compartment. It recruits OSBP to the web; OSBP interacts at a MCS with the ER through the VAPA proteins and mediates the exchange of PI4P and cholesterol between the ER and the replication compartment, enriching the latter with cholesterol [234](Fig. 5). The HCV NS5A was also shown to interact with OSBP at the Golgi apparatus, and this interaction seems essential for the egress of HCV particles from the infected cells [231](Fig. 5). Protein kinase D (PKD) regulates the activity of OSBP and the ceramide transporter CERT at the *trans*-Golgi [235, 236], both of these proteins playing

important roles in Golgi secretory function. Depletion or inhibition of PKD was reported to enhance HCV release from cells, lending further support to a key role of OSBP and CERT in HCV particle egress [235]. Of note, itraconazole (ITZ), an antifungal agent with additional anti-cancer and anti-viral properties, was shown to target OSBP and to inhibit HCV replication [237]. Similarly, OSBP and ORP4L were found to be targets of so-called minor enviroxime-like compounds, which inhibit HCV replication [238]. In apparent contrast with the function of OSBP in HCV particle production, its close relative ORP4 was found to interact with HCV NS5B and to inhibit its activity, resulting in a reduction of HCV replication [239].

Another member of the *Flaviviridae* connected with OSBP function is Dengue virus (DENV), the genome of which is replicated at viral replication complexes (VRC) consisting of convoluted membranes, tubules, and double-membrane vesicles [240, 241], analogous to the HCV membraneous web. In contrast to HCV, pharmacologic inhibitors of PI4KIIIα (AL-9) and OSBP (OSW-1) had only minor inhibitory effect on DENV replication [242]. However, in a high-throughput screen, ITZ and its derivative posaconazole did effectively inhibit DENV replication. Moreover, knockdown of OSBP further sensitized cells to posaconazole, consistent with the notion that OSBP is the host protein responsible for the antiviral effect of the drug [243]. In addition to OSBP, silencing of ORP2 and ORP11 were reported to inhibit DENV replication, suggesting that also other ORP family members than OSBP play roles in this process [243]. Of note, the authors failed to observe localization of OSBP to the DENC VRCs. This suggests that, unlike in the case of HCV, the function of OSBP in DENV replication may be indirect, putatively reflecting alterations in the cellular lipid homeostasis rather than a direct lipid transporter activity at MCS involving the VRC. Moreover, ORP1L was also shown to interact with the West Nile virus Oas1b protein, and ORP1L knockdown decreased the viral replication [244].

7.2. Enteroviruses

Enteroviruses are non-enveloped positive-strand ssRNA viruses belonging to *Picornaviridae*. A number of important pathogens belong to the polio-, rhino-, coxsackie- and echovirus genuses classified as enteroviruses. Poliovirus rearranges cellular membranes via its 2BC and 3A proteins to generate a PI4P-rich replication organelle (RO)[245]. Arita et al. [246] first discovered that OSBP, recruited by the PI4P generated by PI4KIIIβ, accumulates cholesterol in the poliovirus RO. The group of F. van Kuppeveld soon observed that the OSBP/ORP4 antagonist OSW-1 suppressed the

replication of a range of enteroviruses including polio-, coxsackie- and rhinoviruses [247]. Moreover, ITZ was shown to inhibit enterovirus replication by targeting OSBP: OSBP or ORP4 overexpression were found to counteract the antiviral effects of ITZ, and OSBP knockdown suppressed the viral replication [237]. OSBP and ORP4L were also identified as targets of so-called minor enviroxime-like compounds, which, similar to the natural OSBP/ORP4 oxysterol ligand 25-hydroxycholesterol, inhibit enterovirus replication without impairing the activity of PI4KIIIβ [238, 248]. Roulin et al. [249] found that also rhinovirus replication depends on host cell factors driving PI4P-cholesterol counter-currents at the viral replication membranes. The replication required OSBP and PI4KIIIβ, the phosphatase Sac1 and the PI transfer protein (PITP) β. In addition, depending on the virus type, the replication required ORP1, -2, -5, -9 or -11, emphasizing the role of multiple OSBP homologues in the intracellular lipid fluxes instrumental for enterovirus replication.

7.3. Other viruses

The other viruses whose replication is thus far reported to depend on the OSBP/ORP machinery include the Aichi, African swine fever and adenoviruses. Similar to the enteroviruses, Aichi virus (AiV), a picornavirus of the genus *Kobuvirus*, hijacks OSBP-mediated cholesterol transport to enrich cholesterol at the viral replication membranes in exchange for PI4P [250]. OSBP, VAPA/B and Sac1 co-localize at the replication organelles and serve as host factors essential for AiV replication [251].

African swine fever virus (ASFV) is a large enveloped dsDNA virus belonging to *Asfarviridae*. ASFV remodels the cellular endomembranes into viral replication factories. It redistributes the intracellular membranes and redirects endocytic traffic in order to accumulate endosomal membranes providing a membrane and cholesterol supply necessary for establishing the viral factories [252]. The flux of cholesterol to the viral factories is mediated by multiple host proteins including PI4KIIIβ, acyl-coenzyme A binding domain containing 3 (ACBD3), and OSBP. ACBD3 recruits PI4KIIIβ to the viral factories, thus promoting the recruitment of OSBP that transfers cholesterol to the replication organelles. The viral replication is inhibited by the OSBP antagonists ITZ and 25-hydroxycholesterol [253].

Adenoviruses are non-enveloped dsDNA viruses of the family *Adenoviridae*, genus *Mastadenovirus*. The point in the adenovirus life cycle where ORP function has been discovered involves the adenoviral protein RIDα encoded by the early region E3 transcript. This protein

attenuates EGFR signalling and dampens pro-inflammatory NF-κB activity induced by the stress-activated EGFR signalling during viral entry [141]. In cells infected with RIDα mutant virus, an NPC-like endo-lysosomal lipid storage phenotype arises [142], suggesting that RIDα is required to maintain a normal cholesterol distribution in the infected cells. The RIDα-mediated egress of cholesterol from the LE/Lys depends on its interaction partner ORP1L [127, 128, 254], a function discussed in more detail in 4.1.

Interestingly, Amini-Bavil-Olyaee et al. [255] demonstrated that the antiviral effector protein interferon-inducible transmembrane protein 3 (IFITM3) interacts with VAPA and prevents its association with OSBP, thereby disrupting intracellular cholesterol homeostasis and inhibiting the entry of influenza A and vesicular stomatitis viruses. By disrupting VAPA-OSBP function, IFITM3 induces accumulation of cholesterol in multivesicular bodies and late endosomes, which inhibits the fusion of intraluminal virion-containing vesicles with endosomal membranes and thereby blocks virus release into the cytosol. Moreover, ORP1L was shown to control Ebola glycoprotein mediated viral penetration, a process which pertains endosomal fusion [122].

7.4. Salmonella

Besides the viral pathogens, a function of OSBP in the life cycle of Gram-negative bacterial species of the genus *Salmonella* has been discovered. Auweter et al. [229] first reported that OSBP enhances the intracellular replication of *Salmonella enterica* serovar Typhimurium. *Salmonella* encodes three secretion systems located in pathogenicity islands 1 and 2 (SPI-1 and -2). Type 3 secretion systems allow bacteria to inject needle-shaped molecular assemblies into the host cell. These effectors trigger reorganization of the host cell cytoskeleton, inducing membrane ruffles that engulf the invading bacteria. They also initiate the biogenesis of the *Salmonella*-containing vacuole (SCV), the intracellular compartment in which the bacteria replicate [256]. Auweter et al. [229] demonstrated that OSBP binds to the SPI-2 effector protein SseL, resulting in a redistribution of OSBP to the *Salmonella*-containing structures, and knocking down OSBP markedly inhibited the bacterial replication. Later on, Kolodziejek et al. [230] demonstrated that OSBP also binds to the *Salmonella* SseJ protein, which directs OSBP to the SCV membranes in concert with SseL. Deletion of the Sse proteins or OSBP knockdown increased bacterial release into the cytoplasm, suggesting that they are necessary for the stability of the SCV. The underlying mechanism was hypothesized to involve

control of the SCV limiting membrane lipid composition, but the OSBP-based machinery could also execute signaling functions on the SCV surface.

To conclude, a spectrum of viruses as well as bacterial pathogens modify host cell lipid metabolism, trafficking and signalling to create conditions optimally supporting the microbe's life cycle. To achieve this, they exploit the cellular LTPs including a number of the ORP family members. ORPs therefore represent potential targets for the development of new antiviral or –bacterial drugs.

8. Functions of *S.cerevisiae* OSBP homologs (Osh) in lipid metabolism, secretory vesicle transport, and metabolic regulation

The yeast *Saccharomyces cerevisiae* ORPs, designated Osh1-7p, have played central roles in gaining understanding of ORP structure and function in lipid metabolism, vesicle transport, and nutritional signaling. Of the yeast Osh proteins, three (Osh1-3p) represent the long and four (Osh4-7p) the short subtype of ORPs. Any of the seven yeast Osh proteins is sufficient to maintain viability, but disruption of all seven genes is lethal, demonstrating that the yeast Osh collectively execute an essential function [28].

8.1. Osh function in vesicle transport

The group of V. Bankaitis discovered in 1996 ORP function in secretory vesicle transport. They found that disruption of the yeast ORP *OSH4* also called *KES1* resulted in a by-pass of the temperature-sensitivity of mutants in *SEC14*, which encodes a phosphatidylinositol transfer protein (Sec14p) essential for secretory vesicle biogenesis [29, 257]. These findings indicated that Osh4p/Kes1p negatively regulates the Golgi secretory function. The study of Fairn et al. [258] further suggested that a major function of Osh4p is to regulate vesicular transport from the Golgi apparatus through modulation of Golgi apparatus PI4P level and availability. Adding a further level of complexity to the function of Osh4p, the group of C. Beh [259] reported a positive role for Osh4p in polarized exocytic vesicle transport. The authors detected Osh4p on exocytic vesicles on their way from the mother cell into the bud, where the protein was suggested to play a role in vesicle docking on the plasma membrane via the exocyst complex. Contrary to what is predicted for a sterol transfer protein, inhibition of sterol binding by the Osh4p Y97F mutation did not cause its inactivation. Rather,

OSH4(Y97F) was found to represent a gain-of-function mutation that causes dominant lethality (see 8.3).

The group of P. Novick further demonstrated that Osh4p reduces PI4P on secretory vesicles as they mature [260]. This reduction is needed for a switch in the regulation of Sec4p GDP/GTP exchange protein, Sec2p, from an interaction with an upstream Rab GTPase Ypt31/32p to that with the exocyst component Sec15p required for vesicle tethering at the PM. Of note, Osh4p was in 2005 crystallized with a number of different sterols by the group of J. Hurley. Its structure was the first high-resolution crystallographic structure of an ORP solved [36].

8.2. Function of Osh in intracellular lipid transport

Another line of study concentrated on the function of Osh proteins in non-vesicular sterol transport from the PM to the ER. The group of W. Prinz provided evidence that several Osh proteins have the capacity to transfer sterol *in vitro* and to induce stable contacts between donor and acceptor vesicles. Moreover, disruption of the *OSH* was reported to result in a marked, 80% inhibition of PM to ER sterol transport [87, 261]. Deletion of all Osh proteins also suppressed the transfer of newly synthesized ergosterol from ER to PM by 5- [262] or 20-fold [263]. However, contradicting findings were presented by the group of A. Menon, suggesting that Osh proteins rather regulate the lateral organization of PM ergosterol than act as intracellular sterol transporters [264].

A key observation concerning the function of ORPs as counter-current lipid transporters (see section 3) was made by the group of G. Drin [37], who demonstrated that Osh4p binds both sterols and PI4P in the same pocket within its ORD, suggesting that the protein could transport the two types of lipids in opposite directions via an exchange-type activity (Fig. 6A). The capacity of Osh4p to remove PI4P from the *trans*-Golgi provided a plausible explanation to the earlier finding that *osh4/kes1* mutation by-passed the *sec14* ts-mutant phenotype [29]. In the absence of PI4P removal from *trans*-Golgi network (TGN) a reduced generation of PI4P from a limiting amount of PI available could be tolerated. Moser von Filseck et al. [72] employed quantitative, real-time lipid transport assays to demonstrate that Osh4p indeed transports sterol against its gradient between two membranes by dissipating the energy of a PI4P gradient, the sterol transport being sustained through the maintenance of the PI4P gradient by the PI4P-phosphatase Sac1p (Fig. 6A). In addition to sterol transport between the ER and *trans*-Golgi, a more recent study provided evidence that the Osh proteins also mediate sterol transfer from the ER to mitochondria [265]. The sterol

concentration of mitochondrial membranes is lower than of the ER [266]. Thus, this transport would not occur against a concentration gradient, so a counter-transport ligand might in this case not be necessary. The function of Osh5p, a close relative of Osh4p, has remained elusive. It is unable to by-pass the *sec14* mutant phenotype [29]. However, there is evidence that it can transfer sterol [87] and extract PI4P *in vitro* [52], suggesting that it may acts as a sterol/PI4P exchanger. Both Osh4p and Osh5p carry in the lid of their ORD an amphipathic lipid packing sensor (ALPS) sequence, which is in the case of Osh4p shown to mediate association with membrane regions with packing defects, such as tubules, vesicles or edges of Golgi cisternae especially amenable for lipid extraction and transfer [59, 267].

Importantly, the group of A.-C. Gavin demonstrated in 2013 that Osh6p and Osh7p transport PS from the ER to the PM in yeast cells [40]. Osh6p had previously been implicated in yeast sterol metabolism but also shown to bind PIPs [268]. Moser von Filseck et al. [41] then reported in an excellent mechanistic study that Osh6p extracted PI4P and exchanged PS for PI4P between two membranes (Fig. 6B)(see section 3). They also solved the crystal structure of an Osh6p:PI4P complex and demonstrated that the transport of PS by Osh6p *in vivo* depends on PI4P recognition. Finally, the authors showed that the PI4P phosphatase Sac1p drives the PS transport by maintaining a PI4P gradient at the ER-PM interface. Recently, Osh6p was shown to be recruited to yeast ER-PM MCS by the tethering factor Ist2 [90, 91](Fig. 6B). The interaction is required for the synthesis of phosphatidylethanolamine (PE) by the PS decarboxylase Psd2p, after the PS transferred to the PM by Osh6p is endocytosed and reaches the sites of Psd2p activity in endosomes/Golgi/vacuoles [91].

8.3. Osh function in signaling and metabolic regulation

Mousley et al. [269] demonstrated that Osh4p mutants defective in sterol binding (such as Y97F, see 8.1) are deleterious for cell proliferation, apparently due to their enhanced PI4P-dependent TGN and endosome association. The growth arrest upon Osh4p gain-of-function (defect in sterol binding) was shown to be due to amino acid deficiencies caused by defective trafficking of amino acid permeases, dampened gene expression driven by Gcn4, a transcriptional activator of the general amino acid control (GAAC) regulon, and disturbed TORC1 activation. The nature of the signals generated by Osh4p on TGN and endosomes remained incompletely understood. However, the authors suggested that these could involve sphingolipid enrichment in the TGN and endosomal membranes. This view is consistent with data by the group of C. McMaster [270], who demonstrated

sphingolipid deficiencies in Osh4p/Kes1p-depleted yeast cells. The model emerging from these studies is that Osh4p acts as a sterol-regulated rheostat for TGN/endosomal PI4P/sphingolipid signaling, which coordinates lipid metabolism with TORC1 signaling and nitrogen sensing in yeast cells. Interestingly, Gebre et al. [271] reported a genetic interaction of another yeast ORP, *OSH6*, with *TOR1*, bringing up the possibility that a functional interplay with TOR1 may be a more general property among yeast ORPs. In a highly interesting study, Huang et al. [272] found that Osh4p and select other yeast ORPs execute cell cycle control activity as inhibitors of the G_1/S transition in nutrient-poor environments. The data imply that Osh4p, together with Sec14p, executes dual membrane trafficking and cell cycle control functions, and identify a regulatory axis that coordinates TGN/endosomal lipid signaling with cell cycle progression. The above studies provide a glimpse to the complex functions of ORPs in cellular physiology, involving membrane lipid compositions, read-out and integration of lipid signals with multiple aspects of cell regulation.

8.4. Osh function at membrane contact sites

Research carried out on the yeast Osh proteins has played an important role in formulating the MCS hypothesis of ORP function. Osh1p was first shown to localize at both the Golgi complex and the nucleus-vacuole junction (NVJ)[58], although it is not required for NVJ formation [86]. It interacts via its FFAT motif with Scs2p at the ER/nuclear envelope and via its it ankyrin repeat domain with the NVJ protein component Nvj1p, which is connected to the vacuole via Vac8p [86, 273]. Osh1p likely acts as an ergosterol/PI4P exchanger at the Golgi and the NVJ [38](Fig. 6C, D). At *trans*-Golgi it regulates post-Golgi vesicle transport [274], while at the NVJ it may transfer sterol from the outer nuclear envelope to the vacuole [38], where ergosterol is required for vacuole fusion [275, 276]. Interestingly, the group of C. Loewen showed that pH regulates via protonation of PI4P the recruitment of effector proteins to the TGN, including Osh1p, with impacts of cargo sorting and post-Golgi vesicle trafficking [274].

Of the seven yeast Osh, four (Osh2p, Osh3p, Osh6p, and Osh7p) were demonstrated to localize at the (MCS) zones of ER associated with the PM, designated cortical ER [87]. The authors also provided evidence that the Osh are able to tether ER and PM vesicles, thus generating structures resembling a MCS. The group of S. Emr demonstrated that Osh3p controls the activity of the PIP phosphatase Sac1p anchored at the ER, *in trans* towards its PM substrate PI4P (Fig. 6E), thus modulating PIP signaling and transport at the PM [277]. Analogously, Tavassoli et al. [278] provided

evidence that Osh3p facilitates the activity of a phosphatidylethanolamine N-methyltransferase, the ER-anchored Opi3p, at PM-ER MCS *in trans* on its substrate PE at the PM (Fig. 6E). One is tempted to speculate that also other ORP family members, including mammalian ones, may execute similar functions at specific organelle interfaces through sterol/phosphoinositide regulated assembly of effector protein complexes or distinct membrane domains.

Of note, analysis of Osh3p structure revealed that it recognizes PI4P by highly conserved residues at the entrance of the hydrophobic tunnel which is too narrow to accommodate sterols [39], consistent with the view that sterol binding is not a unifying property of ORPs. Interestingly, a recent report found that Osh3p forms aggregates under heat stress, resulting in the impairment of the polar localization of PI4P and the exocyst component Exo70 [279], adding a new twist to our perception of Osh functions. Furthermore, Del Dedo et al. [89] demonstrated that the yeast endocytic invaginations associate with the ER and that this association specifically requires Osh2 and Osh3p, which bridge the endocytic myosin-I Myo5 to the ER VAP protein Scs2p. Disruption of the ER MCS with endocytic sites delayed and weakened actin polymerization and interfered with endocytic vesicle scission. Moreover, the authors provided evidence that ORP-dependent sterol transfer facilitates the actin polymerization at endocytic sites [88, 89](Fig. 6F). Related to this study, we recently found evidence for interactions of the Osh with the exocytotic SNARE machinery driving secretory vesicle docking and fusion with the PM [151].

To conclude, *S. cerevisiae*, a workhorse of genetic and molecular studies in cell physiology, has been instrumental for our current understanding of ORP structure and function. Many of the conceptual advances achieved with yeast have paved the way for break-throughs in the study of ORPs in higher eukaryotes.

9. Concluding remarks

It is more than 20 years since the ORP families in *S. cerevisiae*, human, mouse were identified [5-7, 28-30, 280]. The first implications on their function at organelle junctions, later named membrane contact sites, were initially found in yeast [58], but the discovery of the FFAT motif mediating the ER association of many ORPs [45] was the spark that really focused our attention on ORP function at MCS. Another seminal finding was the realization that an ORP (yeast Osh4p) can bind two types of lipids within its ORD cavity and transfer them in an exchange-like fashion [37], resulting in a break-through in our understanding of their counter-current lipid transfer activity [33, 41, 43, 49, 55, 72].

Apparently, a relatively high number of ORP proteins are needed for specific functions at the distinct MCS, the number of which is constantly increasing. However, it is quite obvious that the function of these multi-modular proteins is not limited to inter-organelle lipid exchange. The information on their protein interactome is rapidly increasing [67, 132, 133, 175, 281], and a number of studies have evidenced the actions of ORPs as regulators of lipid-modifying enzymes and signaling components [83, 175, 277, 278, 282]. Moreover, through their impacts on organelle membrane lipid compositions, ORPs are indirectly involved in tuning a wide spectrum of cellular functions, such as lipid homeostatic responses [23, 133], vesicle transport [29, 260], cell adhesion [33, 88, 89, 132], and angiogenesis [35]. One can envision that the multiplicity of functions associated with signaling and metabolic control further increase the number of ORPs and their variant forms required. The high number of human ORP splice variants present at https://www.ncbi.nlm.nih.gov/nuccore is consistent with the view that extensive expression control and splice variation in humans accounts for the functional complexity which is at odds with the relatively low number of genes present [283]. Importantly, increasing evidence points at functions of ORPs as host factors with key roles in the life cycles of pathogenic viruses and bacteria [228-230], making them candidate targets for new antiviral or -bacterial drugs. The same holds true for cancers: With an increasing number of reports on functions of ORPs in the survival and malignant proliferation of cancer cells [174, 175, 208, 213, 219, 284-286], one can envision ORPs as potential targets for future anticancer therapies. Moreover, although the central nervous system is a hot-spot of ORP expression [150], we have thus far seen only the first few publications on neural ORP function [84, 151] – this field of research warrants extensive investigation. A next level of physiologic insight into ORP function necessitates (i) increasing knowledge on the structure of ORPs and the lipid ligands that these proteins bind to their PH and ORD domains, as well as structural insight on how ORPs bind their small-molecular antagonists, (ii) new *in vitro* assays allowing reconstitution of ORP-mediated lipid transfer and signaling processes, (iii) advanced genetically modified cultured cell models for pinpointing ORP functions at a new level of precision, and (iv) tissue-specific and inducible ORP knockout animal models to understand their physiology. Integrating data from all these four levels of study will make it possible to place the ORPs into their full functional context in the living organism and to evaluate their potential as therapy targets for human disease.

To conclude, the current evidence identifies ORPs as lipid transporters and integrators of signals involved in an unforeseen variety of cellular processes. Intense efforts to elucidate in detail their functional roles in eukaryotic, mammalian and human physiology, as well as their potential as

putative new therapy targets for cardiometabolic, infectious and neurologic diseases as well as cancers, are therefore warranted.

10. Disclosure

The authors have no conflicts of interest to disclose.

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Figure captions

Fig. 1. The human and yeast *Saccharomyces cerevisiae* ORP families. The human ORPs are presented as grouped into the six and the yeast Osh proteins into four subfamilies indicated with Roman numerals. Ligands identified for the ORDs of the family members are listed on the right. Abbreviations: ID, intrinsically disordered region; PH, pleckstrin homology domain; CC, coiled coil-forming sequence (mediating dimerization); FFAT, two phenylalanines in an acidic tract; OF, 'OSBP fingerprint' sequence EQVSHHPP; ORD, OSBP-related ligand-binding domain; ANK, ankyrin repeat domain; PB(+), polybasic segment; TM, trans-membrane segment; A(-), anionic region; GOLD, Golgi dynamics domain; ALPS, amphipathic lipid packing sensor; Chol, cholesterol; PI4P, phosphatidylinositol 4-phosphate; HC, hydroxycholesterol; PC, phosphatidylcholine; PS, phosphatidylserine. Note: The shorter ORP8 variant does have a PH domain and thus does not belong to the S category proper as defined in this and many other articles – we therefore call it ORP8(S).

Fig. 2. Structures of the human ORP1 and yeast Osh4p ORDs. As examples of OSBP-related ligand-binding domain structures, the 3D structures of the ORP1 (at the top; PDB 5zm7, 5zm6) and Osh4p (at the bottom; 3SPW) ORDs are displayed. Bound ligands [cholesterol (Chol), yellow; PI(4,5)P_2 or PI4P, green, indicated with arrows] are shown in context with lipid-binding hotspot regions. The loop closely apposed to the bound PI(4,5)P_2 or PI4P, formed by the OSBP fingerprint sequence (OF), and the lid of the ligand cavity are indicated with arrows. In the right-most top panel the ORP1 ORD structure with both ligands superimposed is shown, illustrating how the bound cholesterol is buried deeper in the ligand cavity than the PI(4,5)P_2, one fatty acyl chain of which is largely curled up outside the cavity. Note that in the Osh4p structure the fatty acyl chains of PI4P are arranged in a different manner, with both chains inserted in the lipid-binding cavity. Also the orientation of the phosphoinositide head group located in a cleft at the mouth of the lipid-binding cavity differs between the PI(4,5)P_2 bound to ORP1 and the PI4P bound to Osh4p (not visible in the images). In the amino acid sequences displayed the major regions associated with the bound ligands are highlighted with color coding that corresponds to the colors in the 3D structure images.

Fig. 3. The counter-current lipid transport model of ORPs. *Left:* OSBP mediates at ER-*trans*-Golgi network (TGN) MCS the transfer of cholesterol (Chol) from ER to TGN against its concentration

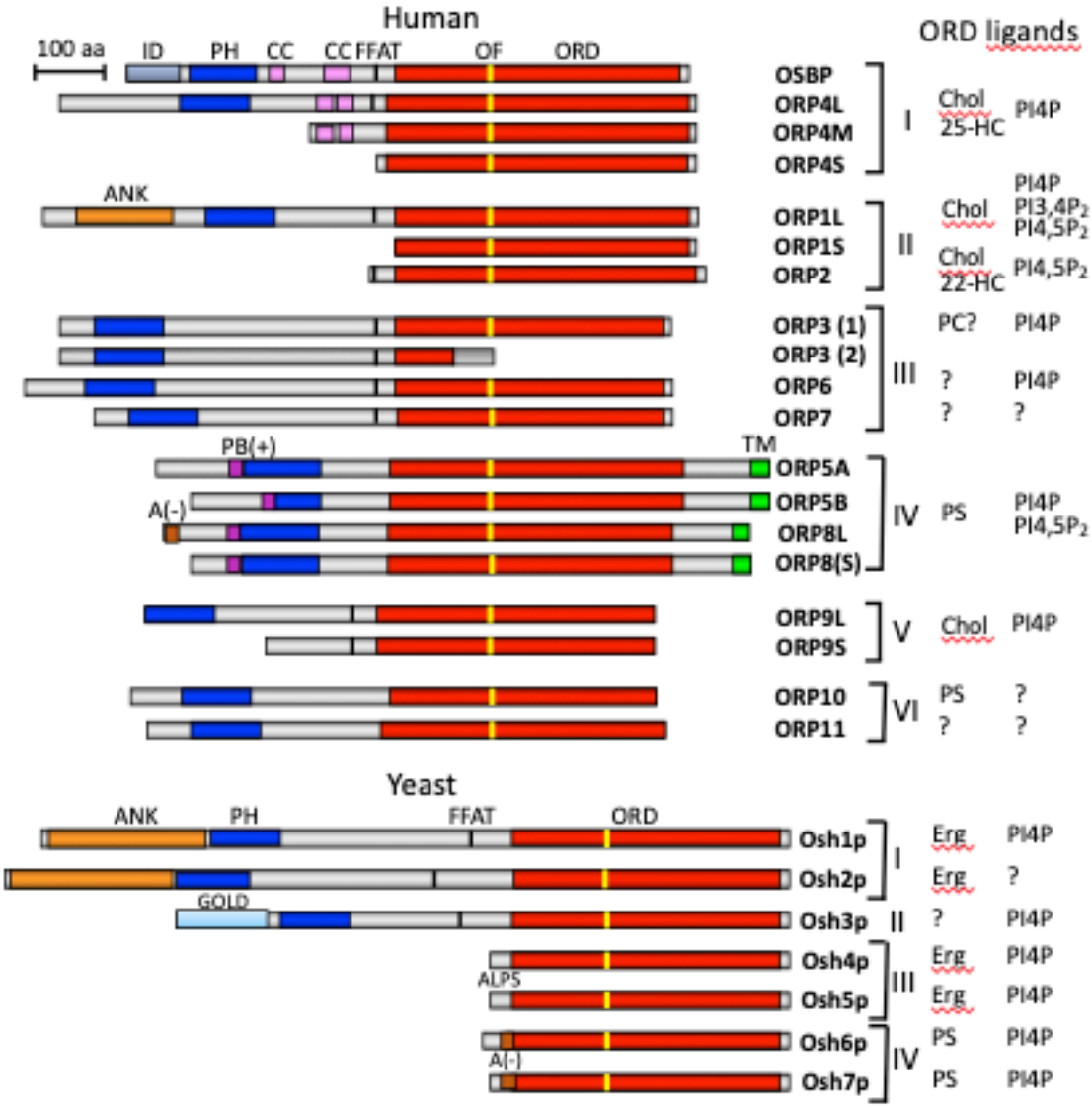
gradient. OSBP associates with ER via its two phenylalanines in an acidic tract (FFAT) domain that binds to VAMP-associated proteins (VAP), and with TGN through interaction of its pleckstrin homology domain (PH) with phosphatidylinositol 4-phosphate (PI4P) and the small GTPase ARF. *Right:* ORP5 and -8 transfer phosphatidylserine (PS) from the ER to the plasma membrane (PM) against the PS concentration gradient. In both cases the proteins transfer PI4P or PI(4,5)P_2 in the opposite direction, down its concentration gradient, which is maintained by local synthesis of PI4P or PI(4,5)P_2 by kinases (PI4K or PI4P5K, respectively) at the TGN or the PM, and hydrolysis of the PI4P in the ER by the phosphatase Sac1. The PI(4,5)P_2 may be hydrolysed by a sequential action of INPP5E and Sac1. ORP5 and -8 are anchored in the ER by a carboxy-terminal *trans*-membrane segment. When the concentration of the substrate PIP in the TGN/PM drops, the PH domain is detached from these compartments is, providing a means of feedback regulation of the transport process.

Fig. 4. The functions of ORP1L and ORP2 in endocytic pathway cholesterol trafficking. A, top panel. Under conditions of high late endosomal (LE)/lysosomal cholesterol (Chol), ORP1L is suggested to transfer Chol from the LE limiting membrane to the ER over LE-ER MCS. ORP1L binds to the ER via interaction of its FFAT motif VAP proteins and to the LE through interaction of its ANK domain with the small GTPase Rab7, the PH domain of ORP1L having an accessory role. A, bottom panel. Under these conditions, ORP1L can also be detached from the ER and participate in a complex with Rab7-interacting lysosomal protein (RILP) and dynein-dynactin, which drive the microtubule minus end-directed motility of the LE and autophagosome maturation. These proteins also associate with the homotypic fusion and protein sorting (HOPS) complex mediating LE/lysosome/autophagosome tethering and fusion. B. When LE-lysosomal Chol content is low, ORP1L mediates the transfer of cholesterol in the opposite direction, from the ER to LE, where the Chol is required for the formation of intralumenal vesicles and normal LE/lysosome degradative function. C. Monomeric ORP2 transfers Chol from LE to recycling endosomes (RE), where the Chol facilitates recruitment of focal adhesion kinase (FAK). FAK stimulates the activity of phosphoinositide kinase I (PIPKI), which synthesizes phosphatidylinositol 4,5-bisphosphate [PI(4,5)P_2]. The formed PI(4,5)P_2 is transported in the opposite direction, from RE to LE, by tetrameric ORP2. The LE PI(4,5)P_2 plays an important regulatory role in transport tubule formation at the LE. ORP2 also mediates, either indirectly or directly, the transport of Chol from endosomes to the plasma membrane

Fig. 5. Roles of OSBP in the hepatitis C virus (HCV) life cycle. The virus binds to receptors at the plasma membrane (PM) and enters cells via endocytosis, followed by viral RNA release and translation of viral polyprotein on ER-associated ribosomes. Viral RNA replication takes place at a modified, virally induced replication compartment called the membraneous web (MW). Although there are continuities between the ER and the MW, formation of a functional MW requires lipid transfer over ER-MW MCS, at which Nir2 transfers phosphatidylinositol (PI) from the ER to the MW for the synthesis of PI4P by PI-4-kinase IIIα. The formed PI4P recruits OSBP to the MW membrane, followed by OSBP-mediated cholesterol (Chol) transfer to the MW. At these MCS, also transfer of ceramides mediated by yet another LTP, ceramide transporter (CERT), takes place (not shown). Moreover, OSBP interacts with the HCV protein NS5A at the Golgi apparatus, and this interaction seems essential for the egress of HCV particles from the infected cells.

Fig. 6. Functions of the yeast *S. cerevisiae* Osh proteins at membrane contact sites. In the middle, a schematic image of a yeast cell with Osh localizations is shown. E, endosomes; SV, secretory vesicles; ER, endoplasmic reticulum; G, Golgi complex; N, nucleus; V, vacuole; NVJ, nucleus-vacuole junction; PM, plasma membrane. The panels A-F at the top and bottom depict six distinct functions of the Osh proteins at different MCS: A. Transport of ergosterol by Osh4p from the ER to other membrane organelles in exchange for PI4P. B. PS transfer from the ER to PM in exchange for PI4P by Osh6 and Osh7p, which bind to the ER-PM MCS tethering protein Ist2p. C. Osh1p-mediated ergosterol transfer from ER to *trans*-Golgi. D. Osh1p-mediated ergosterol transfer from outer nuclear membrane (ONM) to vacuole over the nucleus-vacuole junction (NVJ). E. Stimulation of the *in trans* activities of Opi3p, a phosphatidylethanolamine N-methyltransferase, and the phosphoinositide phosphatase Sac1 by Osh3p. F. Interaction of Osh2p with the myosin-I Myo5p at endocytosis sites, which regulates actin polymerization at these sites required for vesicle scission. Additional abbreviations: Scs2/22, yeast orthologues of VAP; Erg, ergosterol; Pro, proline-rich sequence.

Figure 1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PH
CC
CC
FFAT
OF
ORD
ORD ligands
OSBP
ORP4L
ORP4M
ORP4S
I
Chol
25-HC
PI4P
ANK
ORP1L
ORP1S
ORP2
II
Chol
22-HC
PI4P
PI3,4P2
PI4,5P2
PI4,5P2
ORP3 (1)
ORP3 (2)
ORP6
ORP7
III
PC?
?
?
PI4P
PI4P
?
PB(+)
TM
A(-)
ORP5A
ORP5B
ORP8L
ORP8(S)
IV
PS
PI4P
PI4,5P2
ORP9L
ORP9S
V
Chol
PI4P
ORP10
ORP11
VI
PS
?
?
?
Yeast
ANK
PH
FFAT
ORD
Osh1p
Osh2p
I
Erg
Erg
PI4P
?
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II
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Osh5p
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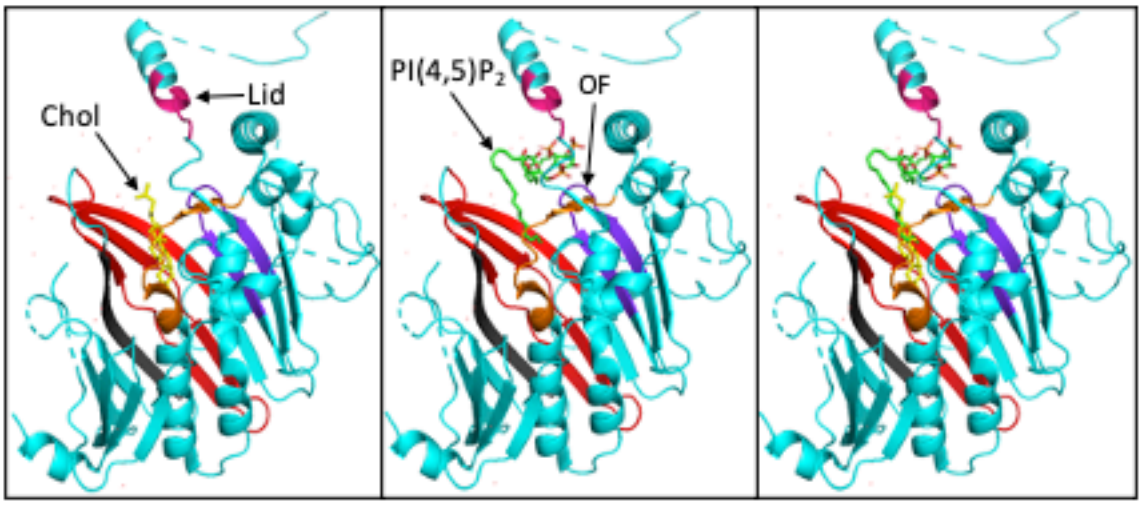
PS
PI4P
PI4P

Figure 2



ORP1 with cholesterol (5zm7) | ORP1 with $PI(4,5)P_2$ (5zm6) | Both ligands superimposed

```
/5zm7 534     541         551   556   561   566   571   576   581   586   591
      RTSLPSPM----------SILRKCIGMELSKITMPVIFNEPLSFLQRLTEYMEHTYLIHKASS

596   601   606   611   616   621   626   631   636   641   646   651   656   661   666
LSDPVERMQCVAAFAVSAVASQWERTGKPFNPLLGETYELVRDDLGFRLISEQVSHHPPISAFHAEGLNNDFIFH

671   676   681   686   691   696   701   706   711   716   721   726   731   736
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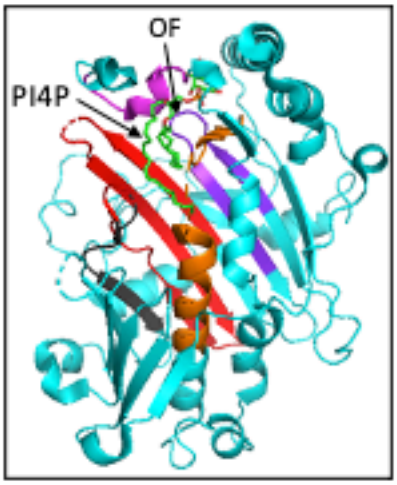

Osh4p with PI4P (3SPW)

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3spw   26    31    36    41    46    51    56    61    66
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     71    76    81    86    91    96   101   106   111
ICLIDPEVESPELARMLAVTKWFISTLKSQYCSRNESLGSEKKPLNPFI

 116   121   126   131   136   141   146   151   156   16
LGELFVGKWENKEHPEFGETVLLSEQVSHHPPVTAFSIFNDKNKVKLQ

 1   166   171   176   181   186   191   196   201
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       206   211   216   221   226
EGILVASPFVELEGKSYIQSSTGLLC
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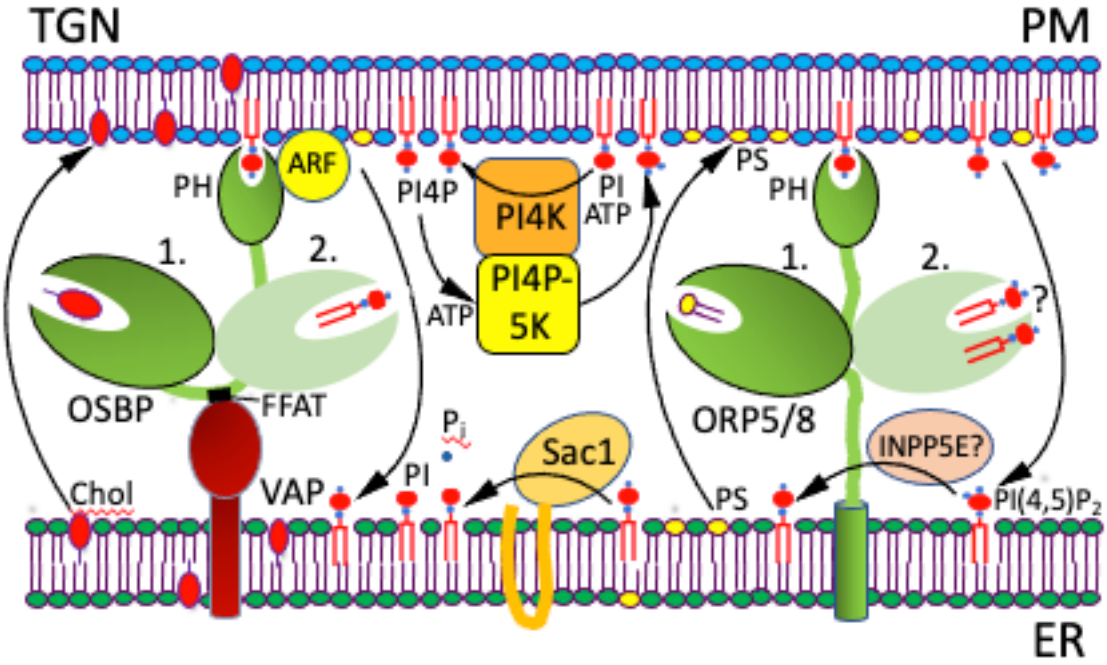
Figure 3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PH
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PI4K
PI
ATP
ATP
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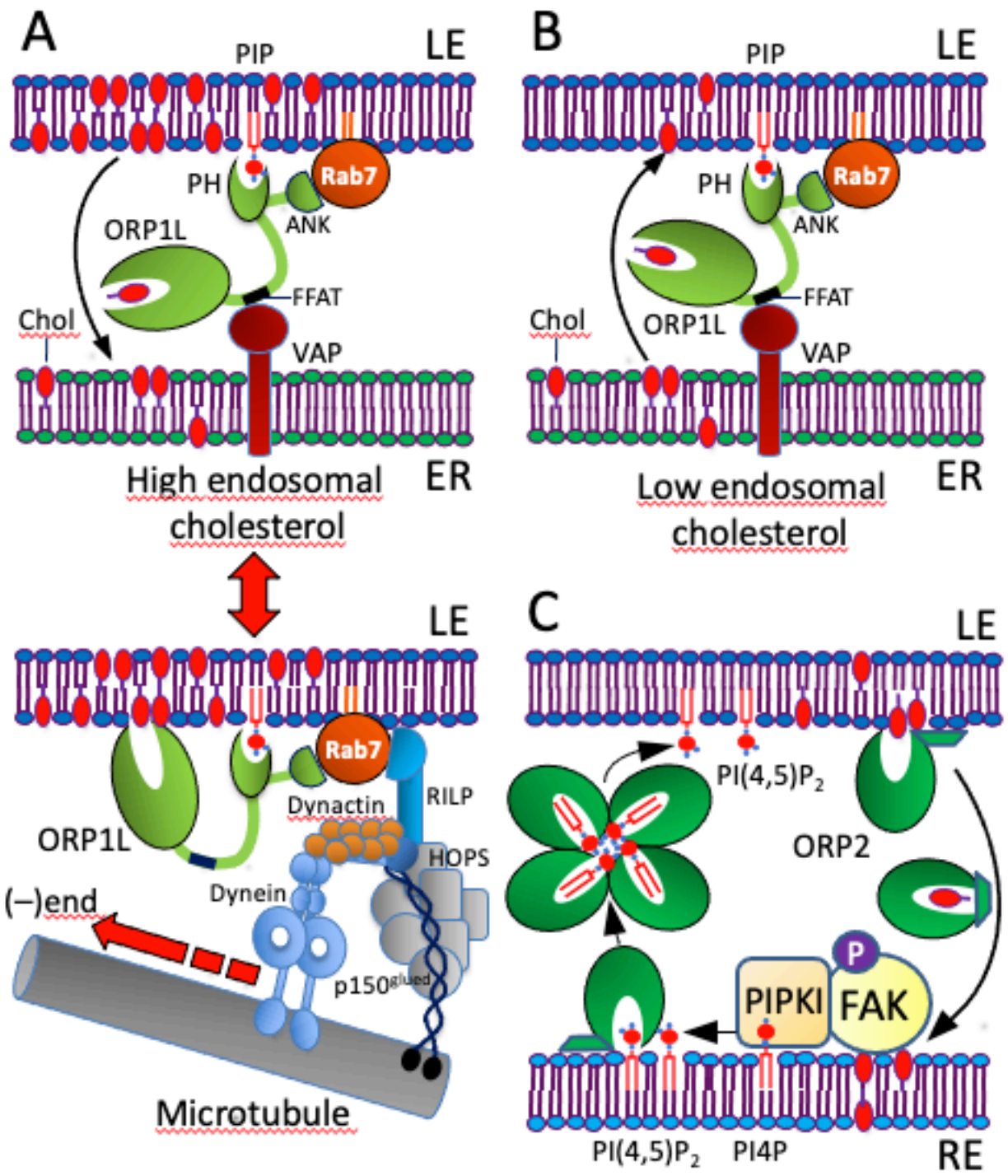
2.
OSBP
FFAT
VAP
Chol
PI
Pi
Sac1
PS
PH
1.
2.
?
ORP5/8
INPP5E?
PS
PI(4,5)P2
ER

Figure 4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PIP
LE
PH
Rab7
ANK
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FFAT
Chol
VAP
ER
High endosomal cholesterol
B
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PH
Rab7
ANK
FFAT
Chol
ORP1L
VAP
ER
Low endosomal cholesterol
LE
Rab7
Dynactin
RILP
ORP1L
HOPS
Dynein
(–)end
p150glued
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FAK
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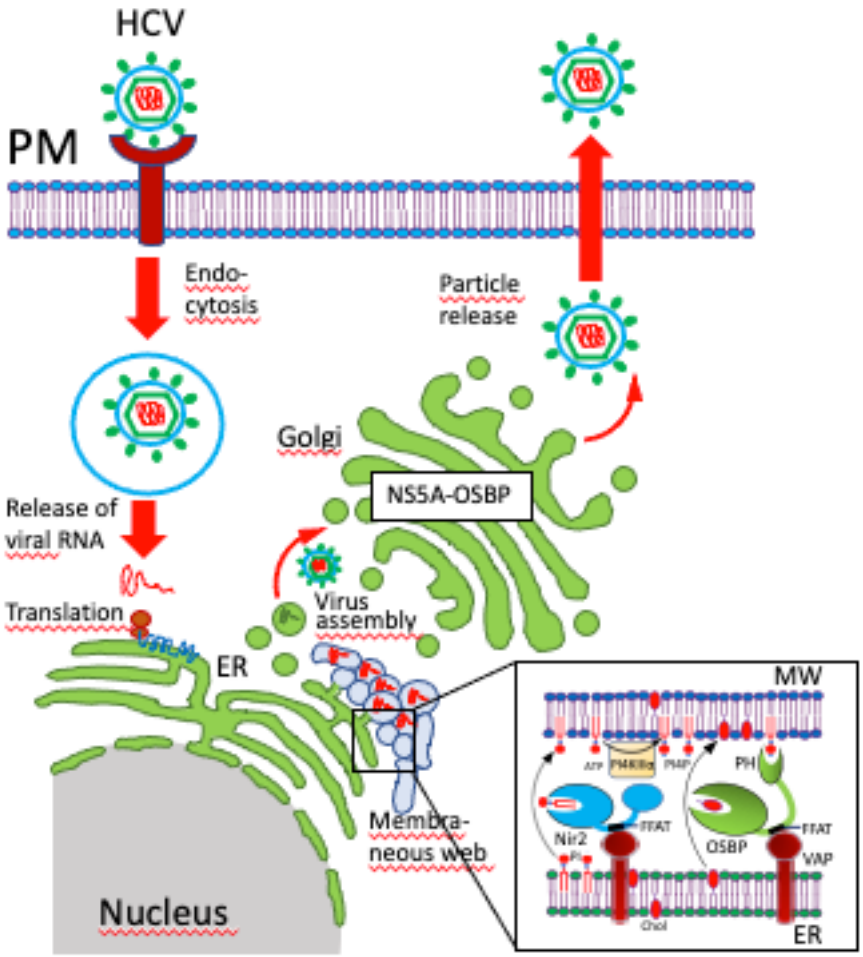
PI4P
RE

Figure 5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PM
Endo-
cytosis
Particle
release
Golgi
NS5A-OSBP
Release of
viral RNA
Translation
Virus
assembly
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Membra-
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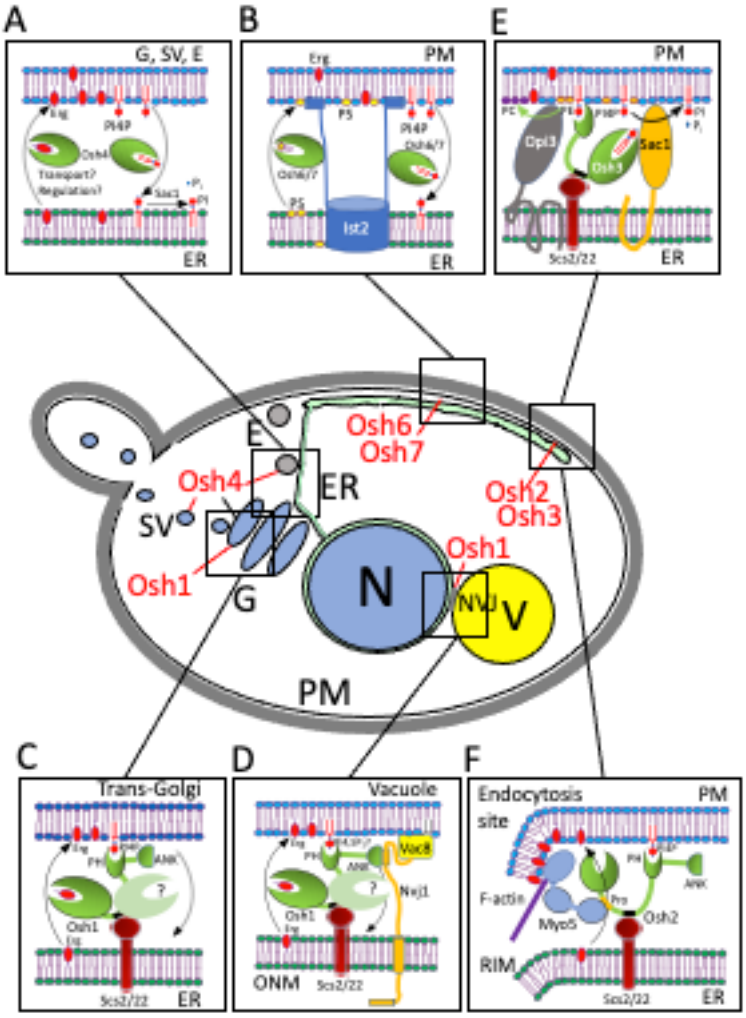
Nucleus
MW
PH
Nir2
FFAT
OSBP
VAP
ER

Figure 6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G, SV, E
Erg
PI4P
Osh4
Transport?
Regulation?
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ER
B
PM
Erg
PS
PI4P
Osh6/7
Ist2
ER
E
PM
Opi3
Osh3
Sac1
Scs2/22
ER
Osh6
Osh7
E
ER
Osh4
SV
Osh1
G
N
NVJ
V
Osh2
Osh3
Osh1
PM
C
Trans-Golgi
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Scs2/22
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D
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Vac8
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ONM
Scs2/22
F
Endocytosis site
PM
F-actin
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Pro
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RIM
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