Cell. Mol. Life Sci. 65 (2008) 228 – 236
1420-682X/08/020228-9
DOI 10.1007/s00018-007-7325-2

Cellular and Molecular Life Sciences

Review

Emerging roles of the oxysterol-binding protein family in metabolism, transport, and signaling

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Received 17 July 2007; received after revision 14 August 2007; accepted 12 September 2007
Online First 15 October 2007

Abstract. OSBP (oxysterol-binding protein) and ORPs (OSBP-related proteins) constitute an enigmatic eukaryotic protein family that is united by a signature domain that binds oxysterols, sterols, and possibly other hydrophobic ligands. The human genome contains 12 OSBP/ORP family members genes, while that of the budding yeast *Saccharomyces cerevisiae* encodes seven OSBP homologues (Osh). Of these, Osh4 (also referred to as Kes1) has been the most widely studied to date. Recently, three-dimensional crystal structures of Osh4 with and without sterols bound within the core of the protein were determined. The core consists of 19 anti-parallel β-sheets that form a near-complete β-barrel. Recent work has suggested that Osh proteins facilitate the non-vesicular transport of sterols *in vivo* and *in vitro*, while other evidence supports a role for Osh proteins in the regulation of vesicular transport and lipid metabolism. This article will review recent advances in the study of ORP/Osh proteins and will discuss future research issues regarding the ORP/Osh family.

Keywords. Oxysterol-binding protein, sterol, phospholipid, vesicular transport, nonvesicular transport.

Introduction

Oxysterols are 27-carbon oxygenated derivatives of cholesterol produced through both enzymatic and non-enzymatic mechanisms [1, 2]. Oxysterols are present in tissues at very low concentrations, but they are potent signaling molecules within the cell [3]. They induce a large number of their effects by acting as ligands for nuclear receptors of the liver X receptor (LXR) family [4, 5]. However, not all effects of oxysterols on cell biology can be attributed to gene regulation [3]. The search for additional oxysterol-responsive proteins led to the discovery of the founding member of the oxysterol-binding protein family, OSBP [6, 7]. OBSP was identified as a high-affinity cytosolic receptor for oxysterols; however, it did not appear to directly affect transcription, as treatment of cells with 25-hydroxycholesterol did not result in OSBP translocation to the nucleus but instead resulted in its association with Golgi membranes [8]. Recent work has implicated OSBP/OSBP-related proteins (ORPs) in the direct control of lipid synthesis and lipid transport in cells. OSBP/ORPs have been implicated as direct transporters of sterols [9, 10], while other studies are consistent with OSBP/ORPs acting as sterol sensors that in turn modulate cellular functions that include signal transduction, vesicular transport, and lipid metabolism.

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The OSBP/ORP family

The OSBP signature motif (EQVSHHPP) and the OSBP-related protein domain (ORD) are identifiable in proteins of very divergent eukaryotic groups including *Opisthokonta* (animals and true fungi), *Amoebozoa* (*i.e. Dictyostelium discoideum*) and *Plantae* (Plants) [11–13]. To date work on OSBP/ORP proteins has been limited mainly to animals and the budding yeast *Saccharomyces cerevisiae*. In the human genome, there are 12 genes that, through pre-mRNA splicing, code for at least 16 predicted ORPs [14]. OSBP and ORPs can be subdivided into six subfamilies based upon sequence homology. The N-terminal regions are variable and can contain the FFAT (two phenylalanones in an acidic tract) motif, PH (pleckstrin homology) domains, and ankyrin repeats, while the C-terminus is composed of the ORD domain [14]. In addition, ORPs 5 and 8 contain a putative C-terminal transmembrane domain [14].

The *S. cerevisiae* genome encodes seven OSBP homologues (Osh) [15]. Three of the yeast Osh proteins contain long N-terminal extensions that, similar to their mammalian counterparts, contain the FFAT motif, PH domains, and ankyrin repeats [15, 16]. The other four yeast Osh proteins consist of only the ORD domain. One of these genes, *OSH4,* is also commonly referred to as *KES1* [12, 17].

The roles of the various N-terminal extensions for each OSBP/ORP family member are not well defined. The FFAT motif mediates interactions between OSBP/ORP family members and VAP [VAMP (vesicle-associated membrane protein)-associated protein] in both mammals and yeast [16, 18–20]. VAP is an integral ER protein that mediates association of proteins containing a FFAT motif with this organelle. The PH domains of various OSBP/ORP family members have been found to bind phosphoinositides [21, 22], while ankyrin motifs mediate protein-protein interactions. The PH domains and ankyrin motifs likely also mediate localization of OSBP/ORP proteins, but their roles in this regard have yet to be studied *en masse* in great detail.

Yeast ORP/Osh Proteins

Structure of Kes1/Osh4. Recently, the lab of James Hurley produced a crystal structure of Kes1/Osh4 with and without sterols bound to the core of the protein [10]. The core of the structure is 19 anti-parallel β-sheets that nearly form a complete anti-parallel β-barrel (Fig. 1). This structure is able to accommodate both sterol and oxysterol binding [10]. Sterol binding was through hydrogen bonding that was primarily indirect with the peptide chain through bridging water molecules. The structure also contained a flexible lid region. In the sterol-bound structure, the lid protects the sterol molecule from the solvent. Crystals could not be obtained for the holo protein unless the N-terminal 29 amino acids corresponding to the lid were removed, suggesting the lid is flexible [10]. Structure alignment algorithms predict that the human and yeast ORD domains have the same general structure.

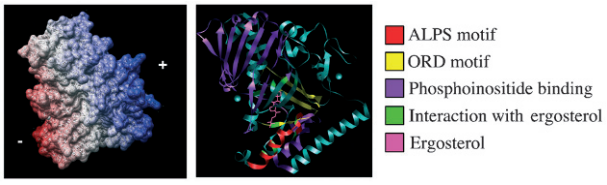

Figure 1. Structure of Kes1/Osh4. On the left is a relative surface map with red indicating a more negatively charge surface and blue a more positively charged surface. The surface charge map was generated using the Chimera molecular modeling package [71, 72]. On the right is a ribbon diagram in the same orientation as the surface map. Known motifs and domains are indicated [10, 33, 34, 38, 45]. The following abbreviations are used: ALPS, ArfGAP1 lipid packing sensor (also the lid domain that is thought to sequester sterols within the binding pocket); ORD, OSBP-related domain.

Kes1/Osh4 as a prototypical Osh protein: Sterol binding and transport? The transport of newly formed sterols from the ER to the plasma membrane is not inhibited by blocking bulk ER/Golgi-derived vesicular transport [23–26]. This suggests that the classical vesicular transport pathways to the plasma membrane are not the sole means to transport sterols; protein-mediated non-vesicular transport could accomplish the movement. Based on structure, in mammalian cells sterol carrier protein 2, Neimann-Pick type C2 protein, and the steroidogenic acute regulatory protein (StAR)-related lipid transfer (START) domain proteins could transport sterols in such a manner [27–30]. The crystal structures of the START domain and Kes1/Osh4 reveal a similar(ish) three-dimensional structure to accommodate sterol binding [10]. This suggests that in yeast the Osh proteins could be sterol transporters. Indeed, simultaneous inactivation of the function of all seven yeast *OSH* genes resulted in a ~3.5-fold increase in sterol concentration along with defects in endocytosis [15, 31, 32].

In vitro experiments using Kes1 and several mutant Kes1 proteins showed that Kes1 could bind to cholesterol and oxysterols with a disassociation constant of ~0.3 μM [10]. Kes1 was also able to extract cholesterol from liposomes and transfer it to acceptor membranes *in vitro*, although extraction ($t_{1/2}$ of 0.3–3 min) was much more efficient than transfer (many

minutes to several hours) [9]. *In vivo* experiments using the uptake of radiolabeled cholesterol from the medium and conversion to sterol ester in the ER were used to assess *in vivo* non-vesicular sterol transfer activity of Osh proteins [9]. While cells deficient in the activity of all seven yeast Osh proteins showed a large reduction in sterol transfer (~80%), it was not completely blocked [9]. The proteins Osh3 and Osh5 were essentially the sole contributors to the transfer of sterols. We found that cells lacking either *osh3* or *osh5* are hypersensitive to the actin monomer-sequestering drug latrunculin B, while deletion mutants of the other *OSH* genes demonstrated sensitivity to latrunculin B similar to wild-type cells (our unpublished observation). It is currently unclear if the decrease in plasma-to-ER transport of sterols mediated by Osh3 and Osh5 (either directly or indirectly) is due to their effects on actin dynamics or *vice versa*.

It is still not clear if Osh proteins are truly lipid transporters. One possibility for Osh protein regulation of sterol transport through indirect means is *via* their regulation of phosphoinositide levels. Phosphoinositide levels are increased in yeast cells with inactivated *KES1/OSH4* [33]. Decreasing the level of phosphoinositides was demonstrated to reduce plasma membrane-to-ER movement of sterols [9], and phosphoinositides are also direct regulators of the alterations in actin dynamics required for endocytosis. Indeed, yeast cells depleted of all seven Osh proteins exhibited depolarization of the actin cytoskeleton and were defective in endocytosis [31, 32].

Kes1/Osh4 as an atypical Osh protein. Much of the recent genetic and biochemical examination of the Osh family has been performed with Kes1/Osh4. However, compared to the other Osh proteins, Kes1/Osh4 is unique in several ways. Mutations in *kes1/osh4* were originally isolated due to their bypass of the essential function of *SEC14* [17, 34]. Sec14 is an essential phosphatidylcholine/phosphatidylinositol transfer protein whose inactivation results in an inability to carry out Golgi-derived vesicular transport [35, 36]. Inactivation of *KES1/OSH4,* but no other *OSH*, results in alleviation of the growth and vesicle transport defects that occur upon inactivation of Sec14 function [15]. In addition, overexpression of the other six yeast Osh proteins did not reverse *kes1/osh4*-mediated bypass of *sec14* defects [15]. Within the Osh family, Kes1/Osh4 is a specific regulator of Sec14 function.

As ORP/Osh proteins had been implicated in the regulation of cholesterol and sphingolipid metabolism, this suggested that inactivation of *KES1/OSH4* may alter sterol metabolism. Inhibition of sterol metabolism could lead to alleviation of the growth and vesicular transport defects that occur when *SEC14* is inactivated. To investigate this further, bulk sterol composition was altered in two ways in cells carrying a temperature-sensitive allele of *SEC14*, *sec14*^{ts} [17]. First, in *sec14*^{ts} cells the *ERG* genes within the sterol biosynthetic pathway were inactivated, and second, *sec14*^{ts} cells were treated with zaragozic acid (a squalene synthase inhibitor) or lovastatin (which inhibits HMG-CoA reductase). None of these alterations in sterol metabolism had any effect on secretion or growth of *sec14*^{ts} cells when grown at permissive or non-permissive temperatures for function of the *sec14*^{ts} allele as compared to a wild-type control [17]. This suggests that inactivation of *OSH4/KES1* does not bypass the requirement for *SEC14* through inhibition of membrane sterol synthesis.

A role for Osh proteins in the regulation of vesicular transport to sites of polarized cell growth has also been uncovered. Increased expression of Kes1/Osh4 prevented the growth defect in cells carrying temperature-sensitive alleles of the Rho GTPase family member *CDC42* [32]. Cdc42 localizes to sites of polarized growth in a cell cycle-dependent fashion, and Kes1/Osh4 was required for localization of Cdc42 to polarized growth sites [32, 37]. General secretory transport is not affected by ablation of Osh proteins; however, exocytosis of Bgl2, a β-1,3-glucanase that is transported to sites of polarized growth, was defective in yeast strains lacking the function of all seven Osh proteins. Localization of Rho1 and Sec4 (a Rab GTPase that regulates exocytosis) was also defective in cells without Osh function [32], while transport of Sec3, a component of the exocist complex itself, was not affected. Other Osh family members were also capable of preventing the growth defect of cells containing a temperature-sensitive allele of *CDC42*. These results indicate that Osh family members have overlapping functions in localization of a subset of proteins that are transported to sites of polarized growth.

Recently, a genome-wide screen in yeast for genes whose inactivation allows for growth in the absence of *SEC14* revealed that a major function of Kes1 is the regulation of vesicular transport at the Golgi through modulation of Golgi phosphatidylinositol 4-phosphate (PI-4P) levels and availability [33]. This is likely direct, as Kes1 and its human homologues (ORP1S) have been demonstrated to bind phosphoinositides *in vitro* under various conditions, and synthesis of Golgi PI-4P is required for Kes1 to localize to the Golgi in cells [34, 38, 39]. Golgi PI-4P is synthesized by the essential PI 4-kinase Pik1 [40–43]. Inactivation of either Pik1 or Sec14 function reduced cellular PI-4P levels to 50% normal, and inactivation of *KES1* restored growth and PI-4P levels to wild-type levels in

Table 1. Yeast Osh proteins.

Name	Domains/Motifs	Localization	Abundance (molecules/cell)
Osh1	Ank, FFAT, PH, ORD	Golgi & nucleus-vacuole junction	850
Osh2	Ank, FFAT, PH, ORD	Peripheral patches near the bud neck	1000
Osh3	GOLD, FFAT, PH, ORD	Cytoplasm	590
Osh4/Kes1	ALPS, ORD, PIP binding	Golgi & cytoplasm	32000
Osh5	ORD	Cytoplasm	1700
Osh6	ORD	Cytoplasm and plasma membrane	2500
Osh7	ORD	Cytoplasm and plasma membrane	2350

Ank, ankyrin repeat; FFAT, two phenylalanines in an acidic tract; PH, pleckstrin homology domain; ORD, oxysterol binding protein-related domain; GOLD, Golgi dynamics domain; ALPS, ArfGAP1 lipid packing sensor motif.

these cells [33]. In cells lacking Pik1or Sec14 function, a fluorescent protein that binds to PI-4P was no longer observed at the Golgi, and inactivation of *KES1* in these cells reestablished the localization of the PI-4P reporter for the Golgi. Whether Kes1 directly affects Pik1 activity has yet to be determined, but it has clearly been demonstrated that a major function of Kes1 is regulation of Golgi PI-4P function.

Membrane binding by Kes1/Osh4. Kes1/Osh4 is found both in the cytosol and associated with the Golgi [17, 33]. Using a combination of deletion and point mutants of Kes1/Osh4 and its human homologue, a phosphoinositide (primarily PI-4P and PI-4,5P_2)-binding site that is distinct from the sterol-binding region has been determined [34, 38, 39]. Inactivation of the Golgi-resident PI 4-kinase Pik1 results in relocalization of Kes1 from the Golgi to the cytoplasm [34], and point mutants of Kes1/Osh4 that decreased phosphoinositide binding prevented Kes1/Osh4 from inhibiting Sec14 function. Thus, PI-4P binding is required for Kes1/Osh4 function and Golgi localization.

A recent study from the lab of Peter Mayinger implies that Osh4 may be one of the most abundant PI-4P-binding proteins in yeast cell extracts [39]. Yeast cells contain ~ 32 000 molecules of Osh4 per cell, while the total of the other six Osh proteins combined is ~ 10 000 molecules (Table 1) [44]. As Osh4 represents three quarters of the Osh proteome, through its shear abundance and ability to bind the low-abundance lipid PI-4P, which is present at ~ 80 000 molecules/yeast cell, it may influence cell biology in ways that the other Osh proteins cannot.

Kes1/Osh4 is also unique within the Osh family in that it possesses an ArfGAP1 lipid-packing sensor (ALPS) motif comprised of its N-terminal 29 amino acids [45], the same region of Kes1/Osh4 thought to act as a lid that sequesters sterols within the protein. Experiments on full-length Kes1/Osh4 as well as the first 29 amino acids of Kes1 revealed that this region is a *bona fide* ALPS [45]. Kes1/Osh4 has been implicated in the regulation of Golgi vesicular transport, and the ALPS domain preferentially allows it to bind curved versus planar membranes. Sterol binding presumably has the potential to regulate the association of Kes1 with curved membranes, but this has yet to be examined. Indeed, the co-regulation of Kes1/Osh4 function by the three different lipid/membrane-binding regions has yet to be assessed in detail but points to a role for Kes1/Osh4 in the regulation of vesicular transport through sensing and responding to membrane lipid composition.

Yeast Osh6 and Osh7. Recent studies involving yeast Osh6 and Osh7 suggest that they may influence endosomal sorting or, conversely, are subject to regulation by Vps4, an endosomal AAA-ATPase [46]. Osh6 and Osh7 directly interact with Vps4. When incubated in the presence of ergosterol, the interaction of Osh6 or Osh7 with Vps4 is abolished [46]. Deletion of *VPS4* increased Osh6 and Osh7 interaction with membranes in cells, and this was accompanied by a decrease in sterol esterification [46]. Overexpression of *OSH7* in cells with an inactivated *VPS4* gene prevented the sterol esterification defect [46]. These results suggest that Osh6 and Osh7, in coordination with Vps4, may facilitate the transport of ergosterol out of the endosomal pathway and into an esterified sterol storage pool.

Human ORPs complementing Kes1 function in yeast. Inactivation of *KES1* allows for growth of cells lacking *SEC14*. The four human homologues with an amino acid sequence most similar to yeast Kes1 are ORP1S, ORP2, ORP9, and ORP10S. Expression of human ORP1S or ORP9S in yeast lacking functional Kes1 and Sec14 re-imposed the growth inhibition and block in Golgi-derived vesicular transport, while human ORP2 and ORP10S did not [38, 47, 48], suggesting that human ORP1S and ORP9 share at least one conserved function with Kes1/Osh4. To investigate

this further, we expressed human ORP1S, ORP2, ORP9S, and ORP10S in a yeast strain in which all seven of the *OSH* genes are disrupted, which is kept alive with a plasmid-borne temperature-sensitive *osh4–1* (*kes1*^{ts}) allele. ORP1S but not ORP9S, ORP2, or ORP10 was able to support growth of the Osh-deficient strain at the non-permissive temperature for the *osh4–1* allele (our unpublished observation). This suggests that ORP1S and Kes1/Osh4 have evolutionarily conserved functions.

Mammalian OSBP/ORPs

OSBP and lipid metabolism. OSBP contains an N-terminal PH domain and a C-terminal ORD shared by all OSBP family members [11, 49], and it translocates to the Golgi upon addition of its ligand (25-hydroxycholesterol) to cells. The PH domain binds phosphatidylinositol 4-phosphate (PI-4P) and the small G protein Arf1, and both are required for its localization to the Golgi [50]. Overexpression of OSBP in Chinese hamster ovary (CHO) cells enhanced the biosynthesis of cholesterol and sphingomyelin; however, RNAi knockdown of OSBP implied that OSBP is not directly responsible for the effects of 25-hydroxycholesterol on cholesterol synthesis [51, 52]. Recent studies have demonstrated Insig-2 as the intracellular target responsible for the inhibition of cholesterol synthesis by 25-hydroxycholesterol [53, 54]. OSBP may instead be acting as a sensor of sterols that coordinates sterol and sphingolipid metabolism. Evidence for regulation of sphingolipid metabolism by OSBP originated from the characterization of its protein-protein interaction with VAP (VAMP-associated protein) [19, 20]. OSBP appears to regulate sphingolipid synthesis by recruiting CERT (ceramide transport protein), and this increases the rate of CERT-mediated transport of ceramide from the ER to the Golgi [52]. The ability of OSBP to enhance sphingolipid synthesis requires its ability to target to both the ER (through its FFAT motif and interaction with VAP) and the Golgi (through its PH domain and interaction with Arf1) [52]. How sterols regulate OSBP control of sphingolipid synthesis is under investigation.

OSBP and ERK signaling. OSBP has also been identified as a sterol-responsive regulatory scaffold that directly interacts with two protein phosphatases, serine/threonine phosphatase PP2A and the tyrosine phosphatase PTPPBS. These phosphatases attenuate the activity of the extracellular signal-related kinases 1 and 2 (ERK1/2) [55]. The OSBP/PP2A/PTPPBS oligomer had phospho-serine/threonine and phospho-tyrosine phosphatase activities toward phospho-ERK. Lowering cellular cholesterol levels resulted in disassembly of the oligomer and a concomitant increase in the level of phospho-ERK. Sterol binding by OSBP may act as a conformational switch for assembly and disassembly of an oligomeric protein complex that directly regulates ERK signaling.

ORP1L in endosome trafficking. Two splice variants of ORP1 have been identified: a short form that consists only of the C-terminal ligand-binding domain (ORP1S), and a longer N-terminally extended form (ORP1L) containing three ankyrin repeats and a PH domain [56]. Expression profiles demonstrate that ORP1S predominates in heart and skeletal muscle, while ORP1L is more abundant in the brain and lung [56]. In mammalian cell culture, ORP1L localizes to the late endosomal compartments and appears to influence endosomal trafficking through Rab7 by regulating its GTP-GDP cycling [22]. GTP-bound Rab7 simultaneously bound Rab7-interacting lysosomal protein (RILP) and ORP1L [57]. Subsequently, RILP binds directly to the p150^{Glued} subunit of dynactin to allow it to bind dynein. Dynactin is a 1.2 MDa multiprotein complex that regulates the binding of dynein, also a 1.2 MDa protein complex, to microtubules. Dynein is the major motor for transport of vesicles along microtubules. Subsequently, the 2.4 MDa dynein-dynactin complex is transferred, *via* ORP1L within the RILP-Rab7-ORP1L complex, to βIII spectrin [57]. βIII spectrin is found on late endosomes and is thought to act as the membrane receptor for the dynein-dynactin complex. Thus, movement of late endosomes to the minus end of microtubules occurs only after Rab7, RILP, and ORP1L activities facilitate dynactin-dynein interaction with βIII spectrin [57]. The role of sterol binding by ORP1L in regulation of late endosome trafficking has yet to be examined.

ORP2 and alterations in lipid metabolism. Recent work from the lab of Vesa Olkkonen demonstrated that expression of ORP2 results in a decrease in free cholesterol within cells, and this correlated with enhanced cholesterol efflux [58]. This appears to be the result of increased transport of cholesterol out of the ER. Consistent with this observation, CHO cells constitutively overexpressing ORP2 showed increased levels of HMG (3-hydroxy-3methylglutaryl)-CoA reductase and the low-density lipoprotein (LDL) receptor, both of which are regulated by ER cholesterol content [58]. Cells overexpressing ORP2 also had a reduction in triglycerides and a shift in polyunsaturated fatty acids from neutral lipids to phospholipids [59]. Recently it was shown that ORP2

can bind to 25-hydroxycholesterol; whether ORP2 directly binds and regulates cholesterol levels in the ER is not known [60].

ORP4 interacts with intermediate filaments. The lab of Neale Ridgway has characterized both ORP4L and ORP4S in CHO cells. ORP4L is the closest homologue of OSBP and, like OSBP, binds to 25-hydroxycholesterol [61]. Addition of oxysterol to cells did not affect localization of either ORP4L or ORP4S. ORP4S colocalized with the intermediate filament vimentin, and its overexpression caused the filaments to collapse and aggregate [61]. ORP4L displayed a more diffuse staining pattern in cells but does colocalize with vimentin [62]. The function of vimentin has not been firmly established, but it appears to serve as a scaffolding network for proteins that regulate cell signaling, metabolism, and vesicular transport. The collapse of the vimentin network upon overexpression of ORP4S was similar to that observed due to treatment with the microtubule depolymerizing agent nocodazole, implying that ORP4S also plays a role in the regulation of microtubule function [61]. ORP4S-overexpressing cells loaded with LDL-derived cholesterol displayed a 40% reduction in cholesterol esterification, suggesting that ORP4S interaction with vimentin filaments may inhibit cholesterol transport [61]. Sterol binding by ORP4S does not influence the binding to vimentin *in vitro*. The direct role of ORP4 in vimentin organization has yet to be determined.

ORP9 and Akt. ORP9 is also expressed in long (ORP9L) and short (ORP9S) forms [18]. Only ORP9S is expressed in mast cells, where it is directly phosphorylated by protein kinase c-β (PKCβ) [63]. A co-immunoprecipitation complex of ORP9S and PKCβ also contained Akt (also known as protein kinase B) [63]. Akt is a protein kinase that regulates numerous signaling pathways that control cell cycle, survival, and glucose metabolism. Depletion of ORP9L from HEK293 cells by RNAi resulted in a 3-fold increase in Akt phosphorylation, indicating that ORP9L is a negative regulator of Akt phosphorylation [63]. The role of PKCβ phosphorylation on ORP9L/S function and the whether the regulation of Akt phosphorylation by ORP9L/S is direct need to be clarified.

ORPs and membrane proximal sites. Several membranes within the cell come within close distance to neighboring organelles. The majority of these involve the ER coming within close proximity with other organelles [64]. Recently, several articles have reviewed and proposed functions for membrane contact sites in assembly of signaling complexes and in lipid and ion transport [65, 66]. The term "contact sites" may be a somewhat misleading term; an alternative and perhaps more apt name would be "membrane-proximal sites". Recent work from the Ridgway lab suggests that OSBP and CERT transit between the ER and Golgi and are not simultaneously bound to both [52]. The action of this diffusion could lead to the concentration of VAP in membrane-proximal sites. This would lead to shorter diffusible barriers for CERT and may lead to the increased transport of ceramide to the *cis*-Golgi.

In yeast, Osh1 is localized to the Golgi as well as to membrane-proximal sites termed nuclear-vacuolar junctions [67, 68]. Nuclear-vacuolar localization requires the ankyrin repeat domain of Osh1, while Golgi localization is dependent on its PH domain [68]. Osh1 localization to nuclear-vacuolar junctions is through Nvj1. Nvj1 is a nuclear envelope-localized protein that associates with the vacuolar protein Vac8 to form nuclear-vacuolar junction patches. The role of Osh1 in lipid movement at the Golgi and between the vacuole and the nucleus has yet to be determined.

The localization of ORP/Osh protein to membrane-proximal sites, coupled with the ability of members of this protein family to bind sterols, has prompted suggestions that members of this protein family could act as direct non-vesicular sterol transporters between membranes. However, this issue has yet to be clearly resolved.

OSBP/ORP-trangenic mice. Cells with inactivated OSBP/ORPs and their yeast counterparts, or cells overexpressing these proteins, have altered lipid metabolism. The mechanisms behind these alterations are only now beginning to be resolved. Alterations in lipid metabolism have also been observed in OSBP/ORP-knockout and -transgenic mice. Adenovirus-mediated hepatic overexpression of OSBP, ORP1L, or ORP3 revealed that OSBP, but not ORP1L or ORP3, affected serum lipid profiles [69]. OSBP increased triacylglycerol levels in livers, the rate at which triacylglycerol was secreted from the liver, and the level of triacylglycerol in serum [69]. Suppression of OSBP in cultured hepatocytes reduced triacylglycerol synthesis. SREBP1-c (a major transcriptional activator of lipogenic gene expression) mRNA levels correlated with triacylglycerol levels and OSBP expression [69]. High levels of OSBP resulted in increased expression SREBP1-c, and OSBP knockdown prevented insulin-mediated induction of SREBP1-c mRNA and hepatic triacylglycerol synthesis [69]. The levels of mRNA for other genes that participate in the regulation of lipid metabolism, including fatty acid synthase (catalyzes *de novo* fatty

acid synthesis) and Insig-1, were also increased upon overexpression of OSBP [69]. Increased fatty acid synthase could supply the fatty acid required to increase triacylglycerol synthesis. Insig-1 regulates SREBP1-c activity. SREBPs are ER/Golgi membrane-bound transcription factors whose release from the membrane for translocation to the nucleus is regulated by Insig-1. Insig-1 binds to a protein complex that includes SREBP1 and traps the complex in the ER, preventing SREBP1 from translocating to the nucleus to regulate gene expression. Insig-1 also binds to HMG-CoA reductase, the rate-limiting step in cholesterol synthesis, resulting in its degradation. The role of increased Insig-1 transcript due to increased OSBP in the regulation of lipid metabolism has not been determined. Although OSBP clearly regulates hepatic triacylglycerol metabolism, we are still awaiting a detailed mechanism to determine if these alterations are direct or are downstream effects. ORP1L was also overexpressed in macrophages in mice. Bone marrow from the mice overexpressing ORP1L was transplanted into LDL receptor-homozygous null mice [70]. The LDL receptor binds to LDL particles in serum and is a major mechanism by which cells take up cholesterol and triacylglycrerols from their external environment. LDL receptor-null mice are prone to atherosclerotic lesions due to accumulation of LDL in serum. In LDL receptor-null mice, there was a 2-fold increase in lesion size in mice that received bone marrow from mice with increased expression of ORP1L in their macrophage [70]. Cholesterol-loaded macrophages from ORP1L-transgenic mice also displayed a 30% reduction in cholesterol efflux from cells [70]. These results demonstrate that ORP1L can act as a modulator of atherosclerotic lesion formation, but the biochemical mechanism awaits further research.

As there are at least 16 members of the OSBP/ORP family in mammals, transgenic knockout mice as well as RNAi studies in cells will be valuable tools in distinguishing cellular properties modulated by specific ORPs. Combined with in-depth biochemical assessment of OSBP/ORP protein, this should allow for determination of the mechanisms and functions of the members of this protein family.

Future issues

The studies of yeast Kes1/Osh4, including the determination of its structure by X-ray crystallography, have determined that Kes1/Osh4 function is regulated by sterols, phosphoinositides, and membrane curvature at three distinct sites on the protein. Kes1 clearly regulates the function of Golgi PI-4P, and its binding to membranes is responsive to membrane lipid content and curvature. How sterols regulate Kes1 function is still unclear. In addition, protein binding partners for Kes1/Osh4 have yet to be identified and would go a long way to determining the mechanism by which Kes1/Osh4 regulates vesicular transport.

The role of ORP/Osh proteins as regulators of sterol metabolism and/or movement in cells needs to be clarified. Are some members of this protein family direct carriers of sterols within cells? Or do they regulate processes within cells that impact sterol movement? Is the shared function of Osh proteins regulation of sterol transport, cytoskeletal organization, or sphingolipid synthesis? All of these have been implied but have yet to be tested directly *en masse*. Furthermore, do the ORP/Osh proteins respond differently depending upon whether cholesterol (or ergosterol), oxysterols, or other lipophilic molecules are bound? Do accessory molecules (*e.g.* Vps4) aid in the loading and unloading of ligands?

Clearly, mammalian OSBP/ORPs have very different effects on cell biology in the works published to date. While it appears that the ORP genes arose through gene duplication, they have evolved additional functions beyond their as-yet-unrecognized primordial role. Perhaps envisioning the ORD as a modular protein domain with potentially divergent properties in a given protein is a more appropriate view. In this way, much like a PH, C1, PX, and other known lipid binding domains, knowing the presence of the ORD domain in a protein gives researchers a starting point with respect to a mode of regulation for protein function by lipids. However, one should not be surprised when differences in functions involving these proteins are discovered, much like those for the many proteins that contain other types of lipid-binding domains.

Acknowledgements. Research in the lab of C. R. M. is supported by the Canadian Institutes of Health Research, the National Sciences and Engineering Research Council, the Canadian Foundation for Innovation, and the Canada Research Chairs Fund.

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