A Detour for Yeast Oxysterol Binding Proteins*

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Christopher T. Beh1*, Christopher R. McMaster2‡, Keith G. Kozminski3, and Anant K. Menon4§

From the 1Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada, the 2Departments of Pediatrics and Biochemistry & Molecular Biology, Atlantic Research Centre, Dalhousie University, Halifax, Nova Scotia B3H 4H7, Canada, the 3Departments of Biology and Cell Biology, University of Virginia, Charlottesville, Virginia 22904, and the 4Department of Biochemistry, Weill Cornell Medical College, New York, New York 10065

Oxysterol binding protein-related proteins, including the yeast proteins encoded by the OSH gene family (OSH1–OSH7), are implicated in the non-vesicular transfer of sterols between intracellular membranes and the plasma membrane. In light of recent studies, we revisited the proposal that Osh proteins are sterol transfer proteins and present new models consistent with known Osh protein functions. These models focus on the role of Osh proteins as sterol-dependent regulators of phosphoinositide and sphingolipid pathways. In contrast to their posited role as non-vesicular sterol transfer proteins, we propose that Osh proteins coordinate lipid signaling and membrane reorganization with the assembly of tethering complexes to promote molecular exchanges at membrane contact sites.

Sterols, including cholesterol in mammalian cells and ergosterol in fungi, constitute ~30–40% of plasma membrane (PM)5 lipids and play a critical role in the nanoscale organization of the PM bilayer (1). Sterol-enriched membrane domains serve as platforms to cluster and concentrate specific lipid-modified and integral membrane proteins that function in cell signaling, secretory transport, and cytoskeletal organization (1). Sterols are synthesized in the endoplasmic reticulum (ER) and rapidly transported to the PM by a non-vesicular mechanism that is unaffected by drugs or genetic mutations that block membrane-mediated protein secretion (2, 3). As sterols are largely water-insoluble, their non-vesicular transport between membranes is predicted to require sterol transfer proteins (STPs), which extract a sterol from a donor membrane and, after enclosing the bound sterol in a shielded pocket, transfer the sterol to an acceptor membrane. To exchange sterols between membranes, sterol-loaded STPs might diffuse through the cytoplasm or transfer sterols at membrane contact sites (MCSs), where two membranes are closely apposed (Fig. 1).

One consequence of a rapid non-vesicular transport mechanism is that the membranes involved must be close to equilibrium with respect to sterol levels. To account for the fact that sterols are more concentrated in the PM than elsewhere in the cell, it has been proposed that the lipid environment of the PM sequesters sterols (3–5). In the PM, sphingolipids as well as phospholipids with saturated acyl chains partner with sterols, lowering their chemical activity (or effective concentration) to a level similar to that in the ER (Fig. 1). Thus, even though the anterograde and retrograde flux of sterols between the ER and PM might be equivalent, the PM is enriched in sterols relative to the ER (4, 5).

The identity of yeast STPs is a mystery (6–9). As soluble sterol binding proteins that associate with organelle membranes, oxysterol binding protein (OSBP)-related proteins (ORPs) are potential candidates. Initial reports supported the idea that ORPs are directly involved in sterol transport. However, it is difficult to differentiate between sterol binding proteins that transfer sterols and those that regulate transport without being carriers themselves. Indeed, recent studies focusing on yeast ORPs (see below) suggest that the principal role of ORPs in vivo is to coordinate membrane lipid organization with the assembly of membrane-tethering complexes.

Osh Proteins: Non-vesicular STPs?

OSBP, the canonical mammalian ORP, was originally identified as a cytosolic protein that binds oxysterols (10, 11), which are oxygenated derivatives of cholesterol and are important regulators of cholesterol metabolism (12). OSBP is representative of the larger ORP superfamily that is conserved from yeast to man (13–16), and as discussed below, these proteins bind a variety of sterols. As might be expected of a STP, OSBP shuttles between cellular compartments in response to sterol binding (17–19).

The budding yeast genome encodes seven ORPs (OSH1–OSH7) (Table 1) that can be divided into “short” and “long” classes (Fig. 2). Long ORPs contain N-terminal extensions that can include a phosphoinositide (PIP)-binding pleckstrin homology (PH) domain, a Golgi dynamics domain, ankyrin repeats, and/or FFAT (FF (phenylalanine) in an acidic tract) motifs that bind vesicle-associated membrane protein-associated protein (VAP) homologs (20). All ORPs share homology through a region broadly defined as the ORP-related domain (ORD) motif (21), although the ORD motif is almost the entire length of short ORPs. Yeast Osh proteins can diffuse through the cytoplasm as can most other ORPs (except mammalian ORP5 and ORP8, which contain predicted transmembrane domains) (22), but a variety of different domains (e.g. PH...
domains in long Osh proteins) confer membrane targeting to all Osh proteins.

The structure of the most abundant yeast ORP homolog, Osh4/Kes1p (hereafter referred to as Osh4p), was determined in complexes with oxysterols, cholesterol, and ergosterol, suggesting that all ORPs bind a wide range of sterol ligands (23). Given the modest differences in Osh4p affinities for oxysterols versus non-oxysterated sterols and the relative abundance of ergosterol (cholesterol in mammalian cells) in cellular membranes, ergosterol and cholesterol appear to be the primary sterols bound by ORPs in vivo (23).

The modular architecture of Osh4p is consistent with the presumed structural requisites needed for sterol transfer between membranes (Fig. 2). Osh4p is a β-barrel protein in which the bound sterol is contained head down in a “beer mug” sealed by a small lid (23). Sterol capture may simply involve placing the Osh4p mug “mouth down” on top of the membrane surface, enabling the sterol to be ensconced in the binding cavity. Within the cavity, the sterol makes van der Waals contacts with Osh4p residues near the mug rim, and the sterol 3-ΟΗ headgroup interacts through hydrogen bonds with a number of water molecules inside the Osh4p mug (23). In fact, the Osh4p mug contains 15 water molecules, which provide a surprisingly “watery” environment for containing a hydrophobic lipid. A direct hydrogen bond between Osh4p Gln-96 and the sterol head also contributes to ligand binding (23). The sterol is ultimately enclosed within Osh4p by the flexible N-terminal lid, which might retain the captured sterol (Fig. 2).

The precise function of the lid in sterol binding is unclear. A “lidless” version of Osh4p has essentially the same affinity for cholesterol as wild-type Osh4p (Kd ∼ 0.3 μM) (23), indicating that the lid is not required for sterol retention. However, the lid might have other functions. The Osh4p lid sequence was identified as an ArfGAP1 lipid-packing sensor (ALPS)-like motif, which is an amphipathic helix that preferentially interacts with curved membranes (24). As it is unclear whether other Osh proteins contain bona fide ALPS helices, the broader relevance of the ALPS motif to the Osh protein family is uncertain. Alternatively, Osh4p might use its lid as a “bulldozer” to penetrate the bilayer and capture its sterol ligand. The A10/T4 helix of the phosphatidylinositol (PI)/phosphatidylcholine (PC) transfer protein Sec14p similarly acts like a bulldozer to penetrate the bilayer to the depth of the phospholipid acyl chains embedded in the outer leaflet to help scoop the lipid out of the membrane and into the Sec14p-binding cavity (25). Despite the ambiguities surrounding the role of the Osh4p lid, the structural attributes of Osh4p are generally consistent with the proposed role of ORPs as STPs.

In addition to a sterol, Osh4p can also bind PI4P (26). The crystal structure of Osh4p in a complex with PI4P shows that sterol and PI4P binding is mutually exclusive because the two binding sites overlap (Fig. 2). Indeed, when Osh4p is incubated with vesicles containing both sterols and PI4P, the two lipids compete for extraction by the protein (26). Moreover, the Osh4p-PI4P structure shows that specific but conserved residues bind PI4P, suggesting that all ORPs bind PI4P (26). The binary switch between lipid-bound Osh4p conformers presents new mechanistic possibilities for Osh proteins and ORPs.

Revisiting the Case for Osh Proteins as STPs

In budding yeast, none of the seven OSH genes is required for cell growth, but the deletion of all seven OSH genes results in cell lethality (13). Expressing any single OSH gene alone averts this lethality (13), indicating that each OSH gene is capable of providing the essential function(s) of the entire OSH gene family. When oshΔ osh4-1ts cells (oshΔ refers to the deletion of all OSH genes; osh4-1ts is a temperature-sensitive OSH4 mutation) are grown at elevated temperatures, the last remaining protein encoded by osh4-1ts is inactivated, and free sterols accumulate in internal membranes (27). In addition, the rate of retrograde transfer of exogenously added cholesterol from the PM to the ER is increased (26). However, it is disconcerting that, in these assays, the elimination of Osh4p alone has no effect on sterol transport, despite the fact that Osh4p is >10-fold more abundant than any of the other Osh proteins (28). Although these results suggest a role for Osh proteins in retrograde sterol transport in vivo, the sterol-trafficking defects in oshΔ osh4-1ts cells might be indirect.

Osh4p was shown to increase the rate of cholesterol exchange between vesicle populations in vitro (28). When cholesterol-containing donor vesicles were prepared with specific PIPs to stimulate transfer, each Osh4p molecule transferred ~20 cholesterol molecules/h to acceptor vesicles that were present in 10-fold excess (28). PI(4,5)P2 and phosphatidylycerine
that bind the surface of Osh4p (Fig. 2) were also transferred, albeit at a lower rate (28). Recent in vitro studies with improved temporal resolution showed that Osh4p exchanges sterols from donor vesicles to a 10-fold excess of PI4P-containing acceptor vesicles with an initial transport rate of $\frac{2000}{H11011}$ sterols/Osh4p/h (26). Because PI4P and sterol binding by Osh4p is mutually exclusive, it was proposed that Osh4p acquires PI4P at the PM and exchanges it for a sterol at the ER (26). This vectorial exchange would be driven by PI4P hydrolysis catalyzed by the ER-localized Sac1p PI4P phosphatase (30, 31). However, in vivo, the exchange of sterol between the ER and PM is an equilibrium rather than vectorial (Fig. 1) (3, 4, 29). The fact that an Osh4p mutation that impairs sterol binding does not inactivate the protein, as predicted for a STP, also challenges this model (32).

In vivo, the rate of sterol transfer between the ER and PM is $\frac{16,000}{H11022}$ ergosterol molecules/Osh protein/h (Fig. 1), an order of magnitude greater than the measured in vitro rate. In comparison, in vitro, the mammalian lipid transfer protein STARD4 exchanges dehydroergosterol (DHE) between vesicles at a rate of $\frac{420}{H11011}$ molecules/STARD4/h (9), which is comparable with Osh4p-mediated sterol transport. Because even nonspecific sterol-binding compounds like methyl β-cyclodextrin (MCD) redistribute sterols between liposomes, the relevance of the in vivo rate of sterol transfer between the ER and PM is an equilibrium rather than vectorial (Fig. 1) (3, 4, 29). The fact that an Osh4p mutation that impairs sterol binding does not inactivate the protein, as predicted for a STP, also challenges this model (32).
vitro liposome assay requires confirmation in vivo. In the case of STARD4, microinjection of MCD into STARD4-silenced cells restores sterol transfer to the ER, indicating that the in vitro assays are relevant to STARD4 activity in vivo (9). Unfortunately for the study of Osh proteins, such experiments are not technically feasible in yeast.

Reservations about rates of Osh4p-mediated sterol transfer in vitro and the weak in vivo dependence of retrograde sterol transport on Osh proteins prompted new studies that revisited the case for Osh proteins as STPs. Using a variety of new assays to monitor retrograde and anterograde transport, sterol transfer between the ER and PM was shown to be essentially unaltered in Osh-deficient cells (29). In a live-cell assay tracking the retrograde transport of DHE, a natural fluorescent sterol, sterol transport was visualized in budding yeast for the first time (29). In hypothetically grown cells, DHE enters the PM, and during an aerobic chase period, it moves to the ER and then to lipid droplets. In Osh-deficient cells, this retrograde transfer is slowed by ~3-fold. Based on these findings, it is clear that mechanisms other than those involving Osh proteins are required for sterol transport within cells.

Because the bulk of newly synthesized ergosterol is transported from the ER to the PM, another assay tested if Osh proteins affect sterol transfer in the anterograde direction (29). Following de novo synthesis in the ER, pulse-chase-labeled ergosterol was tracked by subcellular fractionation in wild-type and oshΔ osh4Δ cells. No matter whether Osh proteins were inactivated or not, at the end of the chase, the ergosterol profiles were nearly identical, with most labeled ergosterol concentrated in PM fractions in proportion to the pool of endogenous ergosterol. Other experiments showed that MCD extracts accessible sterols from the outer leaflet of the PM ~25-fold more efficiently in Osh-deficient cells relative to wild-type cells (29). Instead of a direct role in sterol transfer, these results suggest that Osh proteins affect the organization and sorting of sterols between bilayer leaflets and/or in membrane domains. Consistent with this reasoning, genetic studies indicate a functional interaction between OSH4 and DRS2, which encodes an ATP-dependent phospholipid flippase (34). This interaction might reflect a wider role of Osh proteins as regulators of enzymes and proteins that control the composition and organization of membrane bilayers.

It is a straightforward prediction that a STP would be nonfunctional if stripped of its capacity to bind and thereby transport sterols. As confirmed in vitro, mutations that abolish sterol interactions were designed based on the Osh4p crystal structure (23). Unlike mutations that affect the general association of Osh4p with membranes, the Y97F substitution specifically disrupts water-mediated hydrogen bonding required for Osh4p binding to sterols (23). When tested in vivo, however, elimination of sterol binding by the Osh4p Y97F substitution did not inactivate the protein. Rather, this allele is a gain-of-function mutation that causes lethality when Osh4p Y97F is expressed at levels comparable with wild-type Osh4p (32). A possible molecular explanation for the OSH4Y97F gain-of-function phenotype is provided by the fact that sites for sterol and PI4P binding within Osh4p are mutually exclusive (Fig. 2). If Osh4p function is normally induced by PI4P and repressed by sterols, then preventing sterol binding by Osh4p would end the competition between the lipid ligands for internal binding sites, and PI4P would be predicted to be constitutively bound. Consistent with this model, OSH4 gain-of-function lethality, whether caused by OSH4Y97F or OSH4 overexpression, is suppressed by mutations affecting PI4P metabolism (i.e. sac1Δ) (32). These genetic results suggest that sterols are inhibitory ligands of Osh4p activity, not transported cargo.

Osh Proteins at Organelle MCSs

The simple model that Osh proteins are STPs does not appear to be consistent with all the in vivo evidence. However, these proteins might play an indirect role in the intermembrane exchange of sterols and other lipids by regulating the assembly of contact sites between organelle membranes where sterol transfer might occur (Fig. 1). The best example of such a site is the nucleus-vacuole junction (NVJ), which is where Osh1p is localized (35, 36). The NVJ is a MCS involved in piecemeal microautophagy of the nucleus, in which portions of the yeast nucleus are directly transferred into the vacuolar lumen for degradation (37). Inactivation of all OSH genes inhibits piecemeal microautophagy of the nucleus (36), suggesting that NVJ assembly is a shared function among all Osh proteins. Of relevance to sterol transfer is the possibility that PM/ER MCSs might mediate molecular exchanges. Osh2p and Osh3p each contain a FFAT motif and a PIP-binding PH domain (Fig. 2). The FFAT motif is recognized by the ER membrane protein Scs2p (38). Binding to both Scs2p and PIPs might enable Osh2p and Osh3p to promote and/or stabilize contacts between the ER and PM. Consistent with this idea, the deletion of SCS2 disrupts the association of the cortical ER with the PM (39). Although Osh2p and Osh3p are found in the cytoplasm, a significant fraction of Osh3p can be detected on the cortical ER closest to the PM, and SCS2 overexpression drives even more of these proteins to the cortical ER (38–42). Osh6p and Osh7p are also implicated in PM/ER MCS formation (40, 41), but neither protein contains a FFAT motif required for Scs2p-mediated recruitment to the cortical ER, and other results suggest that these short Osh proteins are associated with endosomal membranes (43). Despite the localization of certain Osh proteins to MCSs, there is no direct evidence to support the idea that they promote lipid exchange at those sites.

Osh Proteins Promote Vesicle/PM Tethering

Osh proteins share an overlapping role in promoting membrane contact between exocytic vesicles and the PM (Fig. 3). During polarized exocytosis in yeast, vesicles are targeted to the PM, where localized membrane expansion occurs to support formation of the bud. At the PM, the docking of exocytic vesicles involves the association of vesicle- and PM-localized sub-units of the exocyst complex (44), which effectively form a MCS. The assembly of the exocyst complex is regulated in part by the Rab GTPase Sec4p and the Rho family GTPases Cdc42p, Rho1p, and Rho3p (45). After the exocyst complex attaches a vesicle to the PM, membrane fusion ensues. In the absence of all OSH gene function, undocked vesicles accumulate within buds, as observed either by electron microscopy (27) or when vesicles marked with GFP-Sec4p are tracked in vivo (32, 46). An in vivo
assay of polarized exocytosis, using β-1,3-glucanase (Bgl2p) as a marker of vesicular transport (47), affirmed that major defects in polarized exocytosis result after Osh protein inactivation (46). More specifically, genetic and physical interactions directly implicate Osh proteins in the regulation of exocyst complex assembly (32, 46). Many directly implicate Osh proteins in the regulation of exocyst (46). More specifically, genetic and physical interactions in polarized exocytosis result after Osh protein inactivation marker of vesicular transport (47), affirmed that major defects in MCS formation.

known if vesicle tethering to the PM represents a unique Osh protein inactivation marker of vesicular transport (47), affirmed that major defects in MCS formation. Osh proteins might sequester PI4P or activate a PI4P phosphatase for PI4P turnover and Ypt32p/Sec4p exchange.

Despite the absence of a canonical PH domain, which are not found in the other short Osh proteins (Fig. 1) (13, 41, 50, 52, 53). Inactivation of the Golgi-specific PI 4-kinase Pik1p blocks PI4P synthesis and decreases Osh4p association with the Golgi, suggesting that PI4P is required in vivo for Osh4p membrane association (50). The deletion of OSH4 also suppresses mutations in PI4P kinase PIK1 and SEC14 (which encodes a PI/PC transfer protein), both of which affect PI4P synthesis and vesicle biogenesis in the Golgi (50, 53). Like the elimination of Sac1p (the ER/Golgi-localized PI4P phosphatase), the deletion of OSH4 restores PI4P levels in cells with conditional PIK1 or SEC14 mutations (50, 53). Indeed all OSH genes share an overlapping function in activating Sac1p because, in Osh-deficient cells, there is an ~20-fold increase in PI4P levels, well beyond that observed when just OSH4 is deleted (41).

Osh4p and Sac1p play dual roles during vesicle biogenesis at the Golgi by decreasing PI4P but also by increasing PI levels (Fig. 3) (50, 53). Lower PI4P levels inhibit Sec14p-dependent production of diacylglycerol, which is required for vesicle budding from the Golgi (54). In addition, Osh4p and Sac1p promote PI4P dephosphorylation, and the resulting pool of PI is used for complex sphingolipid synthesis in the Golgi. Consistent with the notion that Osh4p activates Sac1p to produce this PI pool, complex sphingolipid levels are decreased in osh4Δ cells. By antagonizing Sec14p, Osh4p might delay vesicle formation long enough for PI produced from Sac1p PI4P dephosphorylation to be incorporated into complex sphingolipids. Because sterols and sphingolipids are enriched in polarized exocytic vesicles (56), this mechanism might coordinate lipid biosynthesis with the sorting of sterol/sphingolipid membrane domains into nascent vesicles. Indeed, in osh4Δ cells, some proteins that partition into sterol/sphingolipid domains are missorted and not properly delivered from the Golgi to the PM (57). If sterol binding causes Osh4p inhibition, which is alleviated by the activated Y97F mutation that abolishes Osh4p sterol binding, then sterol-enriched membrane domains might favor Osh4p inactivation. In a negative feedback model, the generation of sterol/sphingolipid domains induces Osh4p exchange of PI4P for a sterol, and sterol-bound Osh4p cannot stimulate Sac1p-dependent PI4P turnover. As a result, PI4P levels increase, and Sec14p-dependent vesicle budding proceeds.

Osh Proteins as Regulators of PIP and Sphingolipid Metabolism

Osh1p, Osh2p, and Osh3p contain bona fide PIP-binding PH domains contained within their extra N-terminal extensions, which are not found in the other short Osh proteins (Fig. 1) (13, 48, 49). Despite the absence of a canonical PH domain, Osh4p interacts with a variety of PIPs (50–52) and specifically binds PI4P as discussed above (26). In addition, a PIP-binding domain was mapped to a surface region originally described as an atypical PH domain (50), but from the Osh4p structure, it corresponds to a surface region that binds anionic lipids (Fig. 2). Point mutations in this PIP-binding region inhibit the association of Osh4p with membranes in vitro and in vivo (50, 52). Because this region is conserved, it suggests that all ORPs, long or short, have a general capacity to associate with PIPs.

Although Osh4p is the focus of most studies analyzing ORP regulation of PIPs, Osh4p has a unique role in PIP metabolism distinct from that of other Osh proteins. Osh4p is observed in the cytoplasm, on endosomes, in post-Golgi vesicles, and on the Golgi, where it is involved in regulating PI4P metabolism (32, 41, 50, 52, 53). Inactivation of the Golgi-specific PI 4-kinase Pik1p blocks PI4P synthesis and decreases Osh4p association with the Golgi, suggesting that PI4P is required in vivo for Osh4p membrane association (50). The deletion of OSH4 also suppresses mutations in PI4P kinase PIK1 and SEC14 (which encodes a PI/PC transfer protein), both of which affect PI4P synthesis and vesicle biogenesis in the Golgi (50, 53). Like the elimination of Sac1p (the ER/Golgi-localized PI4P phosphatase), the deletion of OSH4 restores PI4P levels in cells with conditional PIK1 or SEC14 mutations (50, 53). Indeed all OSH genes share an overlapping function in activating Sac1p because, in Osh-deficient cells, there is an ~20-fold increase in PI4P levels, well beyond that observed when just OSH4 is deleted (41).

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MINIREVIEW: Sterol/PI4P-dependent Regulation by Osh Proteins
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Sac1p and PI4P Are Effectors of Osh Proteins for Membrane Tethering

New functional assays and genetic analyses also support a model for Osh proteins as a novel class of sterol-dependent regulators of PI(P) signaling and metabolism. In this model, the Sac1p PI4P phosphatase appears to be particularly important as a presumptive effector for several Osh proteins. For example, the deletion of SAC1 suppresses both the lethality of the Osh4p Y97F gain-of-function mutation and most growth defects of cells in which Osh4p is overexpressed (32). This result suggests that Osh4p is an upstream regulator of Sac1p signaling. Indeed, much of the functional diversity of Osh proteins can be explained if their primary activity is to sequester and present PI4P to cognate signaling proteins and enzymes, such as Sac1p.

The overlapping shared role of all Osh proteins in exocyst complex-mediated vesicle docking is likely to involve, in part, PI4P regulation (Fig. 3). En route to the PM, PI4P levels decrease in exocytic vesicles, thereby triggering the release of the Ypt32p GTPase from Sec2p. Once released, Sec2p acts as a guanine nucleotide exchange factor (GEF), directly activating Sec4p to initiate the first steps in exocyst complex formation (58). By promoting Sac1p-dependent PI4P hydrolysis or by directly sequestering PI4P themselves, Osh proteins might promote this Ypt32p/Sec4p GTPase swap. Indeed, OSH4 (KES1) was originally identified as a suppressor of kre11-1, a mutation affecting the Kre11/Trs65p subunit of the TRAPP (transport particle) II complex, which acts as a GEF for Ypt32p activation (59, 60). Thus, Osh4p and potentially other Osh proteins are functionally linked to the TRAPP II complex and Ypt32p.

The role of Osh proteins at PM/ER MCSs is also proposed to involve Sac1p regulation (Fig. 3). Localization of Osh3p to PM/ER MCSs depends on PI4P levels, and many Osh proteins, including Osh3p, induce Sac1p PI4P phosphatase activity (41). Osh3p and Osh7p physically interact with Sac1p, albeit weakly, as complexes are detectable only after incubating isolated membrane fractions with chemical cross-linkers and by fusing a lengthy 13-Myc epitope onto Sac1p to boost antibody binding (41). However, in vivo, the interaction might involve a larger complex that includes Scs2p, a scaffolding protein that is required for full Sac1p phosphatase activity (41). In Drosophila melanogaster, Sac1 interacts with VAP-33-1 (a fly member of the VAP/Sec2 protein family), which in turn interacts with an ORP homolog (61). However, the inferred role for Sac1p in PM/ER MCS formation has not been established. For that matter, it is still to be determined whether Osh3p, with or without other Osh proteins, is required for PM/ER MCS formation.

Although important, Sac1p cannot be the only effector of Osh protein regulation because the cellular effects of SAC1 and OSH mutations are not the same (32). These findings indicate that other downstream effectors of Osh proteins must exist. The physical interaction of Osh4p with the exocyst complex suggests that one of these effectors might be an exocyst complex subunit (32). Other potential Osh protein effectors include other Sac1-domain phosphatases, such as Inp51/Sjl1p, Inp52/Sjl2p, and Inp53/Sjl3p. Consistent with this idea, OSH7 overexpression suppresses the growth defects of a ymr1<sup>ts</sup> inp52/sjl2Δ inp53/sjl3Δ triple phosphatase mutant (62).

Do Osh Proteins Provide a Valid Model for ORP Functions in Other Organisms?

Osh4p is the best understood yeast Osh protein and arguably the best studied ORP in general. However, as it actually represents a fungus-specific clade of the ORP superfamily (20), it is valid to question whether yeast Osh proteins (Osh4p in particular) truly represent the activities of ORPs in other organisms. Not only do the 12 human ORP genes share sequence homology with yeast OSH genes, but several can functionally replace them. When expressed in yeast, human ORP15 rescues the inviability of Osh-deficient cells (63). In addition, expression of human ORP15 or ORP95 in yeast complements osb4Δ phenotypes with respect to the suppression of SEC14 mutations (51, 63, 64). These results suggest that ORPs share common functions even between species. Apart from any role as STPs, certainly yeast and metazoan ORPs have been implicated in many different signaling pathways, as reviewed previously (63, 65).

It remains to be seen if the proposed role of Osh4p and other Osh proteins in coordinating lipid signaling and biosynthesis with secretory trafficking applies to mammalian ORPs. Like the long Osh proteins, many ORPs interact with PIPs through PH domains. Also like Osh4p, the canonical mammalian OSBP coordinates sterol and sphingolipid synthesis between compartments in a PI4P-dependent manner (66, 67). The cellular compartmentalization of lipid biosynthesis is sometimes different in yeast compared with mammalian cells, which is the case for complex sphingolipids. Nevertheless, OSBP couples sterol binding with the regulation of PI 4-kinase IIa and the PI4P-dependent recruitment of CERT (ceramide transport protein) to the Golgi (67). Unlike yeast cells that lack CERT, CERT-mediated transfer of ceramide to the Golgi is critical for the metabolism of complex sphingolipids in mammalian cells. Despite the differences between yeast and mammalian cells, the general paradigm of ORPs as sterol- and PI4P-dependent regulators of lipid metabolism and signaling appears to be applicable.

Understanding the functional roles of ORPs has taken on a new imperative with the recent discovery that human ORPs are specific targets of ORPphilins, a diverse group of nanomolar inhibitors that prevent cancer cell growth (68). In some cases, these compounds vaguely resemble a sterol fused to a PIP, implying that they confer inhibition by simultaneously occupying both sterol- and PI4P-binding sites. How these drugs affect ORP activities is still unknown, but studies of yeast Osh proteins suggest that Sac1p and PI4P regulation might be the ultimate targets.

REFERENCES

1. Lingwood, D., and Simons, K. (2010) Lipid rafts as a membrane-organizing principle. Science 327, 46–50
2. Urbani, L., and Simoni, R. D. (1990) Cholesterol and vesicular stomatitis virus G protein take separate routes from the endoplasmic reticulum to the plasma membrane. J. Biol. Chem. 265, 1919–1923
3. Baumann, N. A., Sullivan, D. P., Ohvo-Rekilä, H., Simonot, C., Pottekat, A., Klaassen, Z., Beh, C. T., and Menon, A. K. (2005) Transport of newly synthesized sterol to the sterol-enriched plasma membrane occurs via non-vesicular equilibration. Biochemistry 44, 5816–5826
MINIREVIEW: Sterol/P4P-dependent Regulation by Osh Proteins

4. Maxfield, F. R., and Menon, A. K. (2006) Intracellular sterol transport and distribution. *Curr. Opin. Cell Biol.* 18, 379–385

5. Mesmin, B., and Levine, T. P. (2003) Oxysterol binding proteins: in

6. Reiner, S., Micолод, D., Zellnig, G., and Schneider, R. (2006) A genome-wide screen reveals a role of mitochondria in anaerobic uptake of sterols in yeast. *Mol. Biol. Cell* 17, 90–103

7. Fei, W., Alfaro, G., Muthusamy, B. P., Klaassen, Z., Graham, T. R., Yang, H., and Beh, C. T. (2008) Genome-wide analysis of sterol-lipid storage and trafficking in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 7, 401–414

8. Sullivan, D. P., Georgiev, A., and Menon, A. K. (2009) Triticum suicide selection identifies proteins involved in the uptake and intracellular transport of sterols in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 8, 161–169

9. Mesmin, B., Pipala, N. H., Lund, F. W., Sokolov, A., Eliezer, D., and Maxfield, F. R. (2011) STARD4 abundance regulates sterol transport and sensing. *Mol. Biol. Cell* 22, 4004–4015

10. Kandutsch, A. A., Chen, H. W., and Shown, E. P. (1977) Binding of 25-hydroxycholesterol and cholesterol to different cytoplasmic proteins. *Proc. Natl. Acad. Sci. U.S.A.* 74, 2500–2503

11. Dawson, P. A., Van der Westhuyzen, D. R., Goldstein, J. L., and Brown, M. S. (1989) Purification of oxysterol binding protein from hamster liver cytosol. *J. Biol. Chem.* 264, 9046–9052

12. Schroepfer, G. J., Jr. (2000) Oxysterols: modulators of cholesterol metabolism and other processes. *Physiol. Rev.* 80, 361–554

13. Beh, C. T., Cool, L., Phillips, J., and Rine, J. (2001) Overlapping functions of the yeast oxysterol binding protein homologos. *Genetics* 157, 1117–1140

14. Jaworski, C. J., Moreira, E. L. A., Lee, R., and Rodriguez, I. R. (2001) A family of 12 human genes containing oxysterol-binding domains. *Genomics* 78, 185–196

15. Lehto, M., Laitinen, S., Chintetti, G., Johannsen, M., Ehnholm, C., Staels, B., Ikonen, E., and Olkkonen, V. M. (2001) The OSBP-related protein family in humans. *J. Lipid Res.* 42, 1203–1213

16. Aniss, A. M., Apostolopoulos, J., Dworkin, S., Burton, L. E., and Sparrow, R. L. (2002) An oxysterol binding protein family identified in the mouse. *DNA Cell Biol.* 21, 571–580

17. Ridgway, N. D., Dawson, P. A., Ho, Y. K., Brown, M. S., and Goldstein, J. L. (1992) Translocation of oxysterol binding protein to Golgi apparatus triggered by ligand binding. *J. Cell Biol.* 116, 307–319

18. Ridgway, N. D., Lagace, T. A., Cook, H. W., and Byers, D. M. (1998) Differential effects of sphingomyelin hydrolysis and cholesterol transport on oxysterol binding protein phosphorylation and Golgi localization. *J. Biol. Chem.* 273, 31621–31628

19. Wyles, J. P., McMaster, C. R., and Ridgway, N. D. (2002) Vesicle-associated membrane protein-associated protein-A (VAP-A) interacts with the oxysterol binding protein to modify export from the endoplasmic reticulum. *J. Biol. Chem.* 277, 29908–29918

20. Raychaudhuri, S., and Prinz, W. A. (2010) The diverse functions of oxysterol binding proteins affect Cdc42p- and Rho1p-mediated cell polarization. *Curr. Opin. Cell Biol.* 22, 1521–1536

21. Harsay, E., and Bretscher, A. (1995) Parallel secretory pathways to the cell surface in yeast. *J. Cell Biol.* 129, 79–94

22. Sha, B., Phillips, S. E., Bankaitis, V. A., and Luo, M. (1998) Crystal structure of the *Saccharomyces cerevisiae* phosphatidylinositol transfer protein. *Nature* 391, 506–510

23. de Saint-Jean, M., Delfosse, V., Douguet, D., Chicanne, G., Payrastre, B., Bourguet, W., Antonny, B., and Drin, G. (2011) Osh4p exchanges sterols for phosphatidylinositol 4-phosphate between lipid bilayers. *J. Cell Biol.* 195, 965–978

24. Beh, C. T., and Rine, J. (2004) A role for yeast oxysterol binding protein homologs in endocytosis and in the maintenance of intracellular sterol distribution. *J. Cell Sci.* 117, 2983–2996

25. Raychaudhuri, S., Im, Y. J., Hurley, J. H., and Prinz, W. A. (2006) Non-vesicular sterol movement from plasma membrane to ER requires oxysterol binding protein-related proteins and phosphoinositides. *J. Cell Biol.* 173, 107–119

26. Bourguet, W., Antonny, B., and Drin, G. (2011) Osh proteins regulate membrane sterol organization but are not required for sterol movement between the ER and PM. *Traffic* 12, 1341–1355

27. Whitters, E. A., Cleves, A. E., McGee, T. P., Skinner, H. B., and Bankaitis, V. A. (1993) Sac1p is an integral membrane protein that influences the cellular requirement for phospholipid transfer protein function and inositol in yeast. *J. Cell Biol.* 122, 79–94

28. Georgiev, A. G., Sullivan, D. P., Kersting, M. C., Dittman, J. S., Beh, C. T., and Menon, A. K. (2011) Osh proteins regulate membrane sterol organization but are not required for sterol movement between the ER and PM. *Traffic* 12, 1341–1355

29. Toulmay, A., and Prinz, W. A. (2011) Lipid transfer and signaling at or-ganelle contact sites: the tip of the iceberg. *Curr. Biol.* 21, 144, 717–728

30. Schulz, T. A., Choi, M. G., Raychaudhuri, S., Mears, J. A., Ghirlando, R., Hindraco, J. G., and Goldfarb, D. S. (2003) Osmotic lysis and other processes. *Physiol. Rev.* 83, 307–319

31. Park, H. O., and Bi, E. (2007) Central roles of small GTPases in the development of cell polarity in yeast and beyond. *Mol. Biol. Cell* 18, 889–903

32. Stefan, C. J., Manford, A. G., Baird, D., Yamada-Hanff, J., Mao, Y., Emr, S. D. (2011) Osh proteins regulate phosphoinositide metabolism at ER-plasma membrane contact sites. *Cell* 144, 389–401

33. Toulmay, A., and Prinz, W. A. (2011) Lipid transfer and signaling at organelle contact sites: the tip of the iceberg. *Curr. Opin. Cell Biol.* 23, 458–463

34. Wang, P., Zhang, Y., Li, H., Chieu, H. K., Munn, A. L., and Yang, H. (2005) AAA ATPases regulate membrane association of yeast sterol binding proteins and sterol metabolism. *EMBO J.* 24, 2989–2999

35. Boyd, C., Hughes, T., Pypaert, M., and Novick, P. (2004) Vesicles carry most exocyst subunits to exocytic sites marked by the remaining two subunits, Sec3p and Exo70p. *J. Cell Biol.* 167, 889–901

36. Park, H. O., and Bi, E. (2007) Central roles of small GTPases in the development of cell polarity in yeast and beyond. *Microbiol. Mol. Biol. Rev.* 71, 48–96

37. Kozminski, K. G., Alfaroo, G., Dighe, S., and Beh, C. T. (2006) Homologs of oxysterol binding proteins affect Cdc42p- and Rho1p-mediated cell polarization in *Saccharomyces cerevisiae*. *Traffic* 7, 1292–1242

38. Harsay, E., and Bretscher, A. (1995) Parallel secretory pathways to the cell surface in yeast. *J. Cell Biol.* 131, 297–310

39. Levine, T. P., and Munro, S. (1998) The pleckstrin homology domain of oxysterol binding protein recognizes a determinant specific to Golgi membranes. *Curr. Biol.* 8, 729–739
MINIREVIEW: Sterol/PI4P-dependent Regulation by Osh Proteins

49. Roy, A., and Levine, T. P. (2004) Multiple pools of phosphatidylinositol 4-phosphate detected using the pleckstrin homology domain of Osh2p. J. Biol. Chem. 279, 44683–44689

50. Li, X., Rivas, M. P., Fang, M., Marchena, J., Mehrbrau, B., Chaudhary, A., Feng, L., Prestwich, G. D., and Bankaitis, V. A. (2002) Analysis of oxysterol binding protein homolog Kes1p function in regulation of Sec14p-dependent protein transport from the yeast Golgi complex. J. Cell Biol. 157, 63–77

51. Fairn, G. D., and McMaster, C. R. (2005) Identification and assessment of the role of a nominal phospholipid-binding region of ORP1S (oxysterol binding protein-related protein 1 short) in the regulation of vesicular transport. Biochem. J. 387, 889–896

52. LeBlanc, M. A., and McMaster, C. R. (2010) Lipid binding requirements for oxysterol binding protein Kes1 inhibition of autophagy and endosome-trans-Golgi trafficking pathways. J. Biol. Chem. 285, 33875–33884

53. Fairn, G. D., Curwin, A. J., Stefan, C. J., and McMaster, C. R. (2007) The oxysterol binding protein Kes1 regulates Golgi apparatus phosphatidylinositol 4-phosphate function. Proc. Natl. Acad. Sci. U.S.A. 104, 15352–15357

54. Kearns, B. G., McGee, T. P., Mayinger, P., Gedvilaita, A., Phillips, S. E., Kagiwada, S., and Bankaitis, V. A. (1997) Essential role for diacylglycerol in protein transport from the yeast Golgi complex. Nature 387, 101–105

55. Sullivan, D. P., Ohvo-Rekilä, H., Baumann, N. A., Beh, C. T., and Menon, A. K. (2006) Sterol trafficking between the endoplasmic reticulum and plasma membrane in yeast. Biochem. Soc. Trans. 34, 356–358

56. Klemm, R. W., Eising, C. S., Surma, M. A., Kaiser, H. J., Gerl, M. J., Sampayo, J. L., de Robillard, Q., Ferguson, C., Proszynski, T. J., Shevchenko, A., and Simons, K. (2009) Segregation of sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. J. Cell Biol. 185, 601–612

57. Proszynski, T. J., Klemm, R. W., Gravert, M., Hsu, P. P., Gloor, Y., Wagner, J., Koza, K., Grabner, H., Walzer, K., Bagnat, M., Simons, K., and Walch-Solimena, C. (2005) A genome-wide visual screen reveals a role for sphingolipids and ergosterol in cell surface delivery in yeast. Proc. Natl. Acad. Sci. U.S.A. 102, 17981–17986

58. Mizuno-Yamasaki, E., Medkova, M., Coleman, J., and Novick, P. (2010) Phosphatidylinositol 4-phosphate controls both membrane recruitment and a regulatory switch of the Rab GEF Sec2p. Dev. Cell 18, 828–840

59. Jiang, B., Brown, J. L., Sheraton, J., Fortin, N., and Bussey, H. (1994) A new family of yeast genes implicated in ergosterol synthesis is related to the human oxysterol binding protein. Yeast 10, 341–353

60. Jones, S., Newman, C., Liu, F., and Segev, N. (2000) The TRAPP complex is a nucleotide exchanger for Ypt1 and Ypt31/32. Mol. Biol. Cell 11, 4403–4411

61. Giot, L., Bader, J. S., Brouwer, C., Chaudhuri, A., Kuang, B., Li, Y., Hao, Y. L., Ooi, C. E., Godwin, B., Vitos, E., Vijayadamanodar, G., Pochart, P., Machinieni, H., Welsh, M., Kong, Y., Zerhusen, B., Malcolm, R., Varrone, Z., Collis, A., Minto, M., Burgess, S., McDaniel, L., Stimpson, E., Spriggs, F., Williams, J., Neurath, K., Ioiime, N., Agee, M., Voss, E., Furtak, K., Renzulli, R., Aanensen, N., Carrolla, S., Bickelhaupt, E., Lazovatsky, Y., DaSilva, A., Zhong, J., Stanyon, C. A., Finley, R. L., Jr., White, K. P., Braverman, M., Jarvie, T., Gold, S., Leach, M., Knight, J., Shimkets, R. A., McKenna, M. P., Chant, J., and Rothberg, J. M. (2003) A protein interaction map of Drosophila melanogaster. Science 302, 1727–1736

62. Parrish, W. R., Stefan, C. J., and Emr, S. D. (2005) PtdIns(3)P accumulation in triple lipid phosphate deletion mutants triggers lethal hyperactivation of the Rho1p/Pkc1p cell integrity MAP kinase pathway. J. Cell Sci. 118, 5589–5601

63. Fairn, G. D., and McMaster, C. R. (2008) Emerging roles of the oxysterol binding protein family in metabolism, transport, and signaling. Cell. Mol. Life Sci. 65, 228–236

64. Fairn, G. D., and McMaster, C. R. (2005) The roles of the human lipid binding proteins ORP9S and ORP10S in vesicular transport. Biochem. Cell Biol. 83, 631–636

65. Yan, D., and Olkkonen, V.M. (2008) Characteristics of oxysterol binding proteins. Int. Rev. Cytol. 265, 253–285

66. Perry, R. I., and Ridgway, N. D. (2006) Oxysterol binding protein and vesicle-associated membrane protein-associated protein are required for sterol-dependent activation of the ceramide transport protein. Mol. Biol. Cell 17, 2604–2616

67. Banerji, S., Ngo, M., Lane, C. F., Robinson, C. A., Minogue, S., and Ridgway, N. D. (2010) Oxysterol binding protein-dependent activation of sphingomyelin synthesis in the Golgi apparatus requires phosphatidylinositol 4-kinase Ia. Mol. Biol. Cell 21, 4141–4150

68. Burgett, A. W., Poulsen, T. B., Wangkanont, K., Anderson, D. R., Ikikuchi, C., Shimada, K., Okubo, S., Fortner, K. C., Mimaki, Y., Kuroda, M., Murphy, J. P., Schwalb, D. J., Petrella, E. C., Cornella-Taracido, I., Schirle, M., Tallarico, J. A., and Shair, M. D. (2011) Natural products reveal cancer cell dependence on oxysterol binding proteins. Nat. Chem. Biol. 7, 639–647

69. Saad, H. Y., and Higuchi, W. I. (1965) Water solubility of cholesterol. Proc. Natl. Acad. Sci. U.S.A. 51, 102–105

70. Yeagle, P. L. (1985) Lanosterol and cholesterol have different effects on mammalian cells. J. Cell Biol. 102, 17981–17986

71. Radhakrishnan, A., Goldstein, J. L., McDonald, J. G., and Brown, M. S. (1987) A genome-wide visual screen reveals a role for sphingolipids and sterols during formation of secretory vesicles at the trans-Golgi network. J. Cell Biol. 185, 601–612

72. Maxfield, F. R., and van Meer, G. (2010) Cholesterol, the central lipid of mammalian cells. Curr. Opin. Cell Biol. 22, 422–429