

# The OSBP-related proteins (ORPs): global sterol sensors for co-ordination of cellular lipid metabolism, membrane trafficking and signalling processes?

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## Abstract

Protein families related to OSBP (oxysterol-binding protein) are present in eukaryotes from yeast to human. The functions of the ORPs (OSBP-related proteins) have remained largely enigmatic. Even though they have been implicated in the function of ERJs (endoplasmic reticulum junctions), it is evident that any single model for their mechanism of action is insufficient. The existing evidence points in many different directions, such as integration of sterol and sphingomyelin metabolism, regulation of neutral lipid metabolism, control of signalling cascades, regulation of secretory vesicle generation, and function in the microtubule-based motility of endo/lysosomes. Some of these functions could involve ERJ and non-vesicular transport of lipids, but this is unlikely to be the unifying feature. We believe, rather, that the common denominator for ORP function is acting as sterol sensors that relay information to a spectrum of cellular processes.

## The ORP [OSBP (oxysterol-binding protein)-related protein] family

Families of proteins displaying homology to the C-terminal sterol-binding domain of OSBP are present in practically all eukaryotic organisms for which sequence information is available. The C-terminal domain is denoted ORD (OSBP-related ligand-binding domain) (Figure 1). In humans, the gene family consists of 12 members, and extensive splice variation increases the number of encoded protein products substantially. The ORPs minimally comprise an ORD, but most of them carry an N-terminal portion that typically contains a PH domain (pleckstrin homology domain). The fact that *Saccharomyces cerevisiae* has seven ORP genes (*OSH1–OSH7*) suggests a fundamental role of these genes and their products in the physiology of eukaryotic cells. In accordance with this, each human tissue or cell type expresses a large number of ORPs. Even though most ORP mRNAs are practically ubiquitous, there are marked quantitative tissue- and cell type-specific differences in their expression [1].

## Intracellular targeting of the ORPs

Most ORPs are cytosolic proteins that associate peripherally with membrane organelles, and some show affinity for cyto-

skeletal elements. Of the human ORPs, eight (OSBP, ORP1–4, ORP6, ORP7 and ORP9) possess a short sequence motif denoted FFAT (two phenylalanine residues in an acidic tract) with the consensus sequence EFFDAXE, in the region between the PH domain and the ORD [2] (Figure 1). This motif interacts with VAPs [VAMP (vesicle-associated membrane protein)-associated proteins], transmembrane proteins of the ER (endoplasmic reticulum). ORP5 and ORP8 have instead a C-terminal membrane anchor that has, in the case of ORP8, been shown to specify ER targeting (D. Yan, M. Lehto and V.M. Olkkonen, unpublished work). The PH domain in the N-terminal region is in several cases known to bind membrane phosphoinositides [3–5] (Figure 1). For OSBP, the interaction of the PH domain with phosphatidylinositol 4-phosphate is known to be crucial for targeting of this protein to the Golgi complex and essential for its function [3,6]. The PH domains of several other ORPs target the plasma membrane, but the monomeric PH domains alone are often insufficient for effective targeting, and other flanking determinants are involved [4,7]. In the absence of bound ligand, the C-terminal ORD appears to have a negative regulatory impact on the targeting function of the N-terminal PH domain region. In the case of OSBP, phosphorylation of specific serine residues in the central region of the amino acid sequence plays a role in the Golgi association of the protein [8]. ORP1L localizes to LEs (late endosomes)/lysosomes and is the only human protein that carries an additional N-terminal extension with three ankyrin repeats. This region interacts with the LE GTPase Rab7 and thus plays a distinct role in the membrane targeting of ORP1L [5].

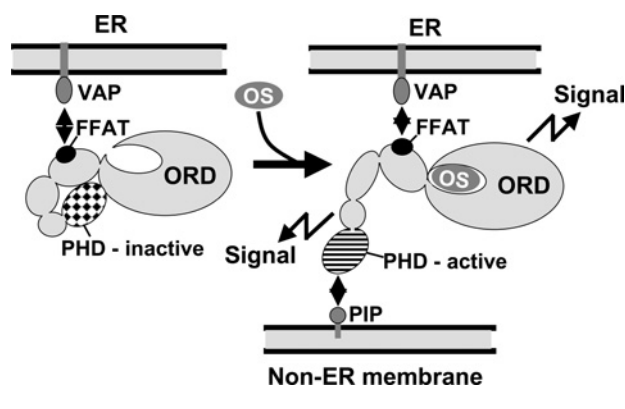
**Key words:** endoplasmic reticulum junction, lipid metabolism, membrane trafficking, Osh4p, oxysterol-binding protein (OSBP), oxysterol-binding-protein-related protein (ORP).

**Abbreviations used:** CERT, ceramide transport protein; ER, endoplasmic reticulum; ERJ, ER junction; LE, late endosome; 25OH, 25-hydroxycholesterol; OSBP, oxysterol-binding protein; ORD, OSBP-related ligand-binding domain; ORP, OSBP-related protein; PH domain, pleckstrin homology domain; VAP, VAMP (vesicle-associated membrane protein)-associated protein.

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**Figure 1 | A schematic model for OSBP/ORP function**

OSBP and most ORPs interact with ER VAPs via an FFAT motif. Binding of oxysterol (OS) to the ORD results in a conformational change and activation of a PH domain (PHD), which interacts with phosphoinositides (PIP) in non-ER membranes. The ligand binding and additional protein-protein interactions induce downstream transduction of signals that impact on cellular lipid metabolism, vesicle transport and signalling cascades. It is also possible that the ORP transfers the bound sterol from one membrane or protein complex to another.



Importantly, *S. cerevisiae* Osh1p localizes in part to the nucleus–vacuole junction, an ERJ (ER junction) characteristic of this organism, and Osh2p as well as Osh3p seems to target ER–plasma membrane junctions [2,9]. Together with the observed dual localization of human ORPs at the ER and specific non-ER compartments, these findings prompted us to propose a model for ORP function in the generation or regulation of ERJs [10,11].

### Function of OSBP and ORPs in lipid metabolism

OSBP is the most extensively studied member of the ORP protein family. Overexpression of the protein in the presence of its ligand, 25OH (25-hydroxycholesterol) was shown to significantly enhance the synthesis of sphingomyelin [12], but also sterol homeostasis was affected by excess OSBP [6]. A mutant of OSBP was found to limit a fluorescent ceramide analogue to sites in the ER, suggesting that the function of OSBP involves the transport of ceramide from ER to the Golgi sites where sphingomyelin synthase is active [13]. The shift of OSBP to a Golgi location upon 25OH treatment of cells coincides with Golgi translocation and activation of the recently discovered CERT (ceramide transport protein) [14]. The prevailing hypothesis that arises from these observations is that OSBP acts as a sterol sensor whose function is to integrate, possibly via regulation of CERT function, the cellular sterol status with sphingomyelin metabolism [15]. A study employing siRNA (small interfering RNA)-mediated knock-down of OSBP expression demonstrated that OSBP plays no role in the maintenance of sterol homeostasis [16], and the effects under the overexpression conditions are most likely to be due to sequestration of endogenous cellular

oxysterols by an excess of the protein. Our recent *in vivo* studies with OSBP show that adenoviral overexpression of this protein in mouse liver induces marked changes in serum lipoproteins, supporting an important role in the homeostatic control of lipid metabolism (D. Yan, M. Lehto, L. Rasilainen, C. Ehnholm, S. Ylä-Herttua, M. Jauhiainen and V.M. Olkkonen, unpublished work).

In addition to OSBP, overexpression of ORP2 in cultured cell models was reported to have an impact on cellular sterol, neutral lipid and phospholipid metabolism [17,18], and that of ORP4S was shown to inhibit esterification of low-density-lipoprotein-derived cholesterol [19]. However, knockdown studies are required to obtain reliable information on the physiological role of these mammalian proteins. In support of ORP function in lipid or sterol metabolism, disruption of the yeast *OSH* genes leads to defects in the intracellular distribution of ergosterol [20]. Whether the effects that OSBP/ORPs have on lipid metabolism involve the function of ERJs remains to be elucidated.

### Functional insight provided by high-resolution structure of *S. cerevisiae* Osh4p

The *S. cerevisiae* ORP Osh4p is suggested to act as a negative regulator of Golgi secretory function [21]. The high-resolution structure of the Osh4p complexed with five different sterols (cholesterol, ergosterol, 7-hydroxycholesterol, 20-hydroxycholesterol and 25OH) was recently solved [22]. The protein has a sterol-binding pocket formed by a  $\beta$ -sheet consisting of 19  $\beta$ -strands in an antiparallel arrangement. The sheet bends to an almost complete roll that is, in the presence of bound ligand, closed by a lid comprising sequences at the N-terminal portion of the protein. Ligand binding stabilizes the closed conformation of the lid, which adopts markedly different conformations depending on whether ligand is present or not. In the present study, a dual function as both sterol transporter and mediator of sterol signals was suggested for Osh4p. How this function connects to secretory vesicle biogenesis is as yet unclear, but it may involve interplay of Osh4p with the small GTPase Arf1p, a central regulator of vesicle formation at the Golgi [21]. Similarly to the ORDs of several human ORPs, Osh4p was demonstrated to bind phosphoinositides. It now appears that this phosphoinositide interaction represents binding of acidic phospholipids to a charged surface determinant, and we find it likely that most, if not all, ORPs possess a sterol-binding pocket similar to that of Osh4p (M. Suchanek, G. Wohlfahrt, M. Lehto, R. Hynynen, C. Thiele and V.M. Olkkonen, unpublished work).

### Function of ORPs in cell signalling

In addition to lipid metabolism and membrane trafficking, functions relating to cell signalling have been assigned to ORPs. Sugawara et al. [23] convincingly demonstrated a role of a *Caenorhabditis elegans* ORP in a transforming growth factor  $\beta$  signalling pathway, and OSBP homologues

in *Drosophila* and *Dictyostelium* have been implicated in cell cycle control [24] and regulation of the slug-fruiting body switch [25] respectively. These findings could reflect direct involvement of ORPs in signal transduction pathways or cell cycle regulation, but the observed effects may also arise indirectly via alterations in lipid metabolism. However, Wang et al. [26] recently presented evidence for a direct role of OSBP as a sterol sensor that regulates the dephosphorylation and hence the activity of extracellular-signal-regulated kinases.

## A new function for an ORP: ORP1L is an effector of Rab7

We have recently assigned a new type of function for an ORP in intracellular membrane trafficking. ORP1L was shown to interact directly with the LE small GTPase Rab7 and to induce recruitment of the dynactin complex to the surface of the LE. Excess ORP1L was shown to stabilize GTP-bound Rab7 on the LE membranes and to cause clustering of the compartments in the juxtannuclear region, dependent on intact microtubules and on dynein-dynactin minus-end-directed motor complexes [5]. ORP1L binds sterols (M. Suchanek, G. Wohlfahrt, M. Lehto, R. Hynynen, C. Thiele and V.M. Olkkonen, unpublished work), prompting the hypothesis that it acts as a sensor that integrates LE/lysosome sterol status with organelle motility and protein or lipid trafficking in the late endocytic pathway. However, experiments employing ORP1L overexpression have so far failed to show an effect on the egress of cholesterol from late endocytic compartments (M. Johansson, R. Vikstedt, M. Jauhiainen and V.M. Olkkonen, unpublished work). Although ORP1L does carry an FFAT motif and interacts *in vitro* with VAP, we have not detected significant association of the protein with ER membranes.

## Conclusion

With increasing information on the function of ORPs, it has become evident that any single model for their mechanisms of action is insufficient. The functional evidence points in many different directions, such as integration of sterol and sphingomyelin metabolism, regulation of neutral lipid metabolism, control of signalling cascades, regulation of secretory vesicle generation at the Golgi complex, and function in the microtubule-based motility of endo/lysosomes. Some of these functions could involve ERJs and non-vesicular transport of lipids, but this is unlikely to be the unifying feature. We believe, rather, that the common denominator for ORP function is to act as sterol sensors that relay information

to a spectrum of cellular processes that are integrated with the sterol status.

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