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REVIEW

A family of sterol sensors/transporters at membrane contact sites: Regulation of ORP-VAP complexes by sterol ligands

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Characteristics of the OSBP-related protein family

The functions of lipids as signaling compounds and their interorganelle transport are topics that have recently moved to the center stage of cell biological and biomedical research. In this context the concept of membrane contact sites (MCS), sites of close apposition of organelle membranes, is an emerging major theme. An increasing number of studies have revealed crucial roles of such contacts as sites with prominent functions in interorganelle lipid transport and metabolism as well as signaling nodes, and molecular machineries operating at MCSs are being identified (reviewed by [1-4]). One of the protein families reported to localize at membrane contacts are the Oxysterol-binding protein/OSBP-related proteins (ORPs), sterol/phospholipid binding proteins implicated in a variety of cellular functions: lipid metabolism and transport, vesicle transport and signaling cascades [5,6].

The unifying feature of the ORPs is a unique β-barrel-like OSBP-related ligand-binding domain (ORD; [7, 8]), which mediates binding of sterols and/or phospholipids [9-12]. In addition, most of the ORPs pertain a FFAT (two phenylalanines in an acidic tract) motif, which mediates interaction with VAMP-associated proteins (VAPs), type II integral membrane proteins of the endoplasmic reticulum (ER) [13-16]. Alternatively, certain ORPs carry a carboxy-terminal trans-membrane segment, which targets the ER [17, 18]. In addition to ER-targeting determinants, most ORPs have an amino-terminal pleckstrin homology (PH) domain, which interacts with phosphoinositide species at specific organelle membranes [19]. This dual targeting mode of the ORPs has indicated a functional role of these proteins at MCSs [20-26]. The PH domain-containing ORPs are designated 'long' (L), while those lacking a PH domain are categorized as 'short' (S).

A break-through in understanding the structure and function of ORPs was achieved when the structure of a 'short' subtype yeast ORP, Osh4p, in complex with ergosterol, cholesterol, and 7-, 20- and 25-hydroxycholesterol, was solved [8]. The analysis revealed a β-barrel-like fold that accommodates a bound sterol with the 3β-hydroxyl group facing the bottom of the ligand-binding pocket and a lid structure that closes the pocket and thereby stabilizes the ligand-bound conformation of Osh4p. The human ORPs studied thus far show varying affinities for different oxysterols with K_ds in the nM-μM range and but also bind cholesterol with a somewhat lower affinity [20, 27, 28]. For example OSBP, the archetype member of the ORP family, binds 25-hydroxycholesterol (25OHC) with K_d of 10 nM [9, 10, 29] in comparison to K_d of 170 nM for cholesterol [11, 30]. Similar to OSBP, OSBP2/ORP4 and ORP1 display a high affinity for 25OHC, whereas a close homologue

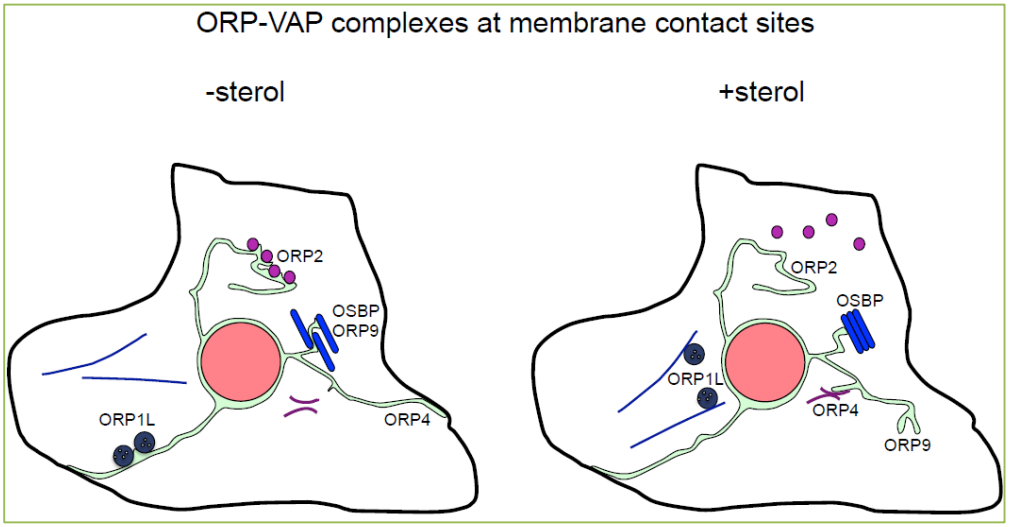

Figure 1. Ligand binding of ORP proteins (1) alters the subcellular targeting of ORP–VAP complexes (ORP2, ORP4, ORP9), **(2)** modifies organelle morphology (OSBP, ORP1, ORP2, ORP9) and **(3)** regulates organelle movement or distribution (ORP1, ORP2).

of ORP1, ORP2, shows only a low affinity (K_d=3.9 μM) for this oxysterol but a high affinity for 22(R) OHC [27, 31]. Interestingly, ORP9 was found not to bind 25OHC, cholesterol being thus far the only sterol it has been shown to interact with [32]. Furthermore, not all ORPs may have the capacity to bind sterols: Yeast Osh3p [33], Osh6p and Osh7p [34] were suggested to be selective for glycerophospholipid ligands.

Subcellular localization of ORP-VAP complexes is regulated by sterol ligands

The distinct intracellular distributions of the ORP family members suggest specific functions of these proteins at distinct subcellular locations. Due to the capacity of several ORPs to simultaneously bind two distinct organelle membranes – ER and non-ER ones – they are reported to localize at MCSs ([35, 36]; Figure1). OSBP-VAP complexes anchored to the ER via VAP have been shown to colocalize with the Golgi apparatus at sites that most likely represent ER-Golgi MCSs, this localization being enhanced by 25OHC liganding of OSBP or cellular sterol depletion. Such treatments also induce a clustering of Golgi membranes in a condensed juxtanuclear arrangement ([35-37]; Figure 1). Earlier studies suggested that a conformational change in OSBP triggered by 25OHC binding enables PI4P-mediated membrane targeting of the protein via the PH domain and thereby enhances the localization of OSBP at Golgi [37, 38]. The study of Mesmin *et al.* [12] provided evidence that OSBP in fact acts as a bidirectional transporter of cholesterol and PI4P at the ER-Golgi interface. The ORD of OSBP transports cholesterol forward from the ER to the Golgi membranes and PI4P in the opposite direction; The PI4P is hydrolyzed in the ER, which is suggested to provide a means or energizing the forward transport of cholesterol. The PI4P in Golgi membranes also has another function: OSBP-VAP complexes target the Golgi via binding of its PH domain to PI4P. The authors also showed that binding of 25OHC to OSBP inhibits its lipid transporter function, suggesting that the localization of OSBP at clustered juxtanuclear Golgi membranes in 25OHC-treated cells does not reflect an activation of OSBP's lipid transport function but rather that the high-affinity oxysterol ligand occupies the ligand pocket, locks the protein at the Golgi, and precludes its dynamic lipid transfer function [12]. In addition to a lipid transporter function such as that described above, ORP-VAP complexes have been reported to organize lipid modifying enzymes at MCSs [25, 26].

Besides OSBP, ORP9L (long variant of ORP9) is another ORP family member associated with ER and Golgi membranes. Endogenous cellular ORP9L was localized to Golgi membranes, and ORP9 was shown to mediate PI4P-dependent transfer of cholesterol *in vitro* [32]. On the other hand, overexpressed ORP9L and ORP9L-VAP complexes have been localized to aberrant enlarged ER structures [36, 39]. In contrast, a sterol-binding deficient mutant, ORP9L (ΔDLTK), in complex with VAP, distributed at normal-appearing ER and Golgi compartments (Figure 1). These quite distinct localization patterns suggest a crucial role of sterol liganding in the function of ORP9L. This protein could act in concert with OSBP in lipid transport at ER-Golgi MCSs, and insertion of cholesterol within the ORD of ORP9L most likely induces a conformational change that alters the subcellular targeting of ORP9L-VAP complexes and their putative interactions with other protein

and/or lipid partners.

The closest homologue of OSBP is OSBP2/ORP4; Like OSBP and ORP9, this protein carries a FFAT motif for ER targeting but associates prominently with vimentin intermediate filaments and to a lesser extent with the plasma membrane ([28, 35, 36, 40]; Figure 1). The association of ORP4-VAP complexes with the plasma membrane is enhanced under sterol depletion conditions (Figure 1). The plasma membrane sites harboring these complexes may represent sites engaged in active signal transduction, as ORP4 was reported to play an essential role the viability/proliferation of several cell types [36, 41]. The role of ORP4-VAP complexes associated with vimentin filaments is poorly understood, but previous studies show a reorganization of vimentin filaments to bundle-like structures in cells overexpressing ORP4 [28, 40]. One can envision that ORP4-VAP complexes at the vimentin network represent contacts of the ER with vimentin, with an unknown function. Moreover, they could mediate the reported involvement of vimentin in Golgi organization, endo-lysosomal protein sorting and/or cholesterol/shingolipid metabolism [42-46].

ORP1L-VAP complexes bring ER membrane into contact with late endosomes (LE) and lysosomes via the interaction of ORP1 with the LE GTPase Rab7. Together with Rab7 and its second effector protein, RILP (Rab7-interacting lysosomal protein), which connects directly to dynein/dynactin motor complexes, ORP1L regulates the mobility and subcellular distribution of LE ([20, 24, 36, 47]; Figure 1). Cellular sterol depletion or overexpression of a sterol binding deficient mutant ORP1L(ΔELSK) increases ER-LE contacts which results in smaller scattered LEs with reduced motility [20, 24, 36], while overexpression of the wild-type ORP1L induces clustering and fusion of LE driven to the juxtanuclear region of cells by microtubule-dependent transport (Figure 1). Why the ER association and motility of LE should be regulated by the cellular sterol status has remained poorly understood. However, a plausible hypothesis is that a more intimate communication of the major lipid synthetic subcellular organelle, the ER, with endosomes is required under sterol depletion conditions.

ORP2 is the closest homologue of ORP1; unlike all other human ORPs, this protein is only present as a 'short' variant that lacks a PH domain. ORP2-VAP complexes localize at ER domains that interact with lipid droplets ([27, 36]; Figure 1). Binding of the high-affinity oxysterol ligand 22(R)OHC releases ORP2 from the lipid droplet surface, whereas sterol binding deficient ORP2(ΔELSK)-VAP complexes cause increased clustering of lipid droplets in the perinuclear region of cultured hepatocytes [27, 35, 36]. This sterol-dependent localization of ORP2-VAP complexes most likely impacts neutral lipid metabolism as RNA interference experiments suggested that ORP2-VAP complexes promote the synthesis and inhibit the hydrolysis of cellular triglycerides [36]. Of note, the subcellular localization of ORP2-VAP complexes is upon 22(R)OHC treatment shifted from bulky ER elements decorated with lipid droplets to a more diffuse pattern with membrane rings encircling lipid droplets and plasma membrane aspects, suggesting that reduction of the lipid droplet affinity of ORP2-VAP complexes allows their more dynamic redistribution [35].

Conclusions

ORP-VAP complexes constitute part of the newly discovered molecular machinery operating in the lipid transport and signaling events at membrane contact sites. In addition to acting as lipid transporters, ORP-VAP complexes organize protein complexes with lipid modifying enzymatic activity at MCSs. Sterol binding by ORPs not only represents an interaction with a lipid substrate to be transported, but also acts as a regulatory switch between different modes of localization and function of ORP-VAP complexes.

Conflicting interests

The authors have declared that no competing interests exist.

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