examination of the conformation and dynamics of ubiquitin chains. The current trend of linkage type driving specific conformations and differential recognition implies an incredibly rich signaling landscape, especially when one considers mixed chains that contain more than one linkage type. Do these mixed chains send mixed signals (Nakasone et al., 2013), or could specific adaptors recognize unique conformations of mixed chains? What signal is sent by a substrate tagged with multiple homotypic or heterotypic chains? Are there adapters that simultaneously recognize multiple chain types? Future work to decode the incredible information content stored in ubiquitin chains and translate the relationship between chain type and downstream response should shed light on an emerging and important aspect of cellular regulation.

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A Protein Pair with PIPs Inside

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http://dx.doi.org/10.1016/j.str.2013.06.010

Oxysterol binding protein (OSBP) and many of its homologs transfer sterol in vitro, but in vivo, they are not major sterol transporters. In this issue of Structure, Tong and colleagues find that the yeast OSBP homolog, Osh3, binds PI(4)P but not sterol, supporting the view that PI(4)P regulation, not sterol transport, is the key activity for OSBP homologs.

Oxysterol binding protein (OSBP) was discovered 25 years ago because its carboxy-terminal OSBP-related protein (ORP) domain (ORD) binds oxysterols. Families of OSBP homologs (Osh1–7 in yeast) containing highly related ORDs are found in all eukaryotes, but their cellular function remains enigmatic. Crystallographic studies of yeast Osh4 revealed that it binds a single sterol in an internal cavity closed by a lid (Im et al., 2005), and in vitro assays showed that OSBP, Osh4, and many other OSBP homologs move sterols between populations of vesicles. This suggested that OSBPs might be the long-sought intracellular sterol transport proteins needed to facilitate non-vesicular transport of sterols between membranes.

Significantly, this hypothesis failed; the bidirectional flow of sterol between the ER and PM was unaffected in a strain of yeast lacking all seven Osh proteins (Georgiev et al., 2011), indicating that OSBPs are not responsible for bulk intracellular sterol transport. These results left a large question mark over the field: what is the true function of this protein family? Interaction with another lipid had been known for a long time; PI(4,5)P2 binds to polybasic patches on the outside surface of ORDs as well as to PH domains encoded as accessory domains in long OSBP homologs, most obviously to achieve targeting to membranes such as the PM or trans-Golgi network (TGN). The OSBP-phosphoinositide interaction generated further interest when PIPs in an acceptor membrane were found to alter the ability of Osh4 to take up sterol from a donor (Schulz et al., 2009). This was explained in detail with a further crystal structure of Osh4 that, unexpectedly, contained PI(4)P inside the binding cavity, where it competed for sterol because the binding sites partially overlapped (de Saint-Jean et al., 2011). This dual specificity could mean that Osh4 traffics sterol and PI(4)P in opposite directions, driven possibly by a phosphatidylinositol phosphorylation cycle powered by PI 4-kinases on PM/TGN and the Sac1 PIP 4-phosphatase on the ER, which is linked to OSBP homologs both directly and indirectly (Forrest et al., 2013, Stefan et al., 2011). As noted above, this trafficking itinerary would not move the bulk of sterol to PM/TGN. The focus on phosphoinositides is further justified by the observation that an Osh4 variant (Y97F), which is unable to bind sterol, nevertheless exhibits a gain of function and enhanced effects on PI(4)P turnover (Alfaro et al., 2011).
All structures determined so far have been of Osh4, but Tong et al. (2013; in this issue of Structure) carried out a crystallographic study of Osh3. Their most interesting findings relate to the Osh3 ORD, which they studied in isolation as the entire protein was not amenable to analysis. Crystals of Osh3-ORD/PI(4)P are similar to those of Osh4/PI(4)P, except that the acyl chains of PI(4)P are arranged differently. For both Osh3 and Osh4, the most highly conserved primary structural element, a polybasic motif, interacts not with sterol but with the PI(4)P headgroup, which supports the importance of the interaction with PI(4)P. However, unlike Osh4, crystals of Osh3-ORD with sterol were impossible to obtain. Closer examination showed that Osh3-ORD does not interact with sterol at all, because the cavity of Osh3 has different shape constraints from Osh4 that exclude the rigid planar four ring structure of sterol. Also Tong et al. (2013) repeated old findings that Osh3 can rescue the defect associated with a lack of all seven Osh proteins (Beh et al., 2001) and showed, by testing a selection of point mutants, that function in this assay correlates with binding to PI(4)P. Together, these findings imply that the conserved function that is shared by all yeast Osh proteins is binding to PI(4)P and that sterol binding is not a universal aspect of Osh function. Given the high degree of sequence homology between yeast and other ORs, these findings may be applicable to ORs in other species.

This finding leaves many unanswered questions. First, why was the lack of sterol binding by Osh3 missed previously? In a survey of Osh1–7, Osh3 was reported to transport cholesterol to about 15% of the extent of Osh4 (Schulz et al., 2009), and if that activity turns out to be equivalent to background, then other ORs (Osh1, Osh6, and Osh7) may also turn out not to transport sterol. Second, the essential function of Osh proteins could be more than just mediating delivery of PI(4)P to Sac1, because jasP1 yeast cells are not as sick as cells lacking all seven Osh proteins. One possibility is that, when occupied by PI(4)P, the ORD’s external conformation changes. Thus, ORs could act as PI(4)P receptors, with groups of specific effectors. A similar phenomenon occurs for OSBP bound to sterol (Wang et al., 2005), and the same thing has been suggested for Osh4, which signals upstream of TORC1 (Mousley et al., 2012). While the lid region of Osh4 and Osh3 (Figure 1B) suggests a mutually exclusive binding of α-tocopherol binding protein, a lipid binding/transfer protein from a different family, suggested a mutually exclusive binding of α-tocopherol and PI(4,5)P2 (Kono et al., 2013), analogous to the concept being proposed here. The possibility that different ORs all bind PI(4)P with different counter ligands should be tested not only by direct biochemical approaches, but also by determining if Osh4 (or another sterol binding OSBP) can rescue phenotypes associated with Osh3 in isolation.

ACKNOWLEDGMENTS

Financial support from the Medical Research Council UK (MR/J006580/1), the Lowe Syndrome Trust (both to T.P.L.) and the National Institutes of Health (GM/10141 to A.K.M.) is gratefully acknowledged.

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Figure 1. Diagrams of Known and Possible Lipid Complexes Formed by Osh4 and Osh3

Complexes have been identified between Osh4 (magenta) and PI(4)P or sterol (A) and between Osh3 (green) and PI(4)P (B). A second lipid (indicated by the question mark) may also form complexes with Osh3, but narrowings in the cavity do not allow sterol. Key amino acids indicated are positives that coordinate the PI(4)P headgroup (“+”) and hydrophilics at the bottom of the cavity (triangles); black coloring indicates residues conserved between different ORPs.