

# Transport of phosphatidylserine by Osh6 in budding yeast

Alenka Čopič<sup>1\*</sup>, Juan Martín D'Ambrosio<sup>1</sup>, Véronique Albanèse<sup>1</sup>, Nicolas-Frédéric Lipp<sup>2</sup>, and Guillaume Drin<sup>2</sup>

<sup>1</sup>*Institut Jacques Monod, CNRS, Université Paris Diderot, Sorbonne Paris Cité, 75013 Paris, France.*

<sup>2</sup>*Institut de Pharmacologie Moléculaire et Cellulaire, UCA and CNRS, 660 route des lucioles, 06560 Valbonne, France.*

\* **Corresponding author:** alenka.copic@ijm.fr

The ORP/Osh proteins represent a large family of proteins found throughout the animal, plant and fungal kingdoms, important for cellular lipid homeostasis. In general, these proteins function at membrane contact sites, where they exchange lipids between cellular compartments, thereby maintaining cellular lipid gradients. We study two members of this family, Osh6 and Osh7, which in *Saccharomyces cerevisiae* transport phosphatidylserine (PS) from the endoplasmic reticulum (ER) to the plasma membrane (PM), where PS is highly enriched. Using *in vitro* and cellular approaches, we have demonstrated that Osh6/7 transport PS against its concentration gradient by exchanging it with phosphatidylinositol-4-phosphate, a lipid that is synthesized at the PM and hydrolyzed at the ER by the phosphatase Sac1. Unlike many other members of the OPR/Osh family, Osh6/7 are single-domain proteins. The lipid-binding (ORD) domain of Osh6 has therefore not only to accommodate two different lipid species, but also be able to selectively and rapidly bind to two very different types of membrane surfaces. We show that the N-terminal part of the Osh6 molecule, which forms a lid covering the lipid binding pocket of the ORD domain, is involved in regulating the binding and release of Osh6 from lipid membranes upon ligand capture. Furthermore, we show that specific protein-protein interactions are required for targeting of Osh6 to membrane contact sites. Our results give insight into the dynamics and regulation of lipid exchange between cellular compartments.