

**P013** Afg2p regulates the interaction between Scs2p and Osh1p and intracellular sterol metabolism

Penghua Wang, Yong Zhang, Cong Jin Low and Hongyuan Yang

*Department of Biochemistry, National University of Singapore, Singapore 119260*

We have previously shown that the yeast AAA ATPase, Vps4p, regulates the membrane association of oxysterol binding proteins Osh6/7p and sterol metabolism. Here we further examine the relationship between other AAA proteins and OSBP proteins. We demonstrated that Osh1p interacted with Afg2p, an AAA ATPase highly homologous to Cdc48p, both physically and functionally. Osh1p and Afg2p were identified in the same protein complex by yeast two hybrid and *in vitro* pull-down analyses. In addition, inactivation of Afg2p caused more Osh1p to associate with the ER membrane in an Scs2p dependent manner. Furthermore, increases in free sterols and sterol esters upon inactivation of Afg2p can be suppressed by the overexpression of OSH1. We also examined the role(s) of Scs2p and Nvj1p in Osh1p function. Overexpression of OSH1 alone in wild type cells results in a significant increase of free sterols and sterol esters, and nystatin sensitivity. However, overexpression of OSH1 in *scs2?* or *nvj1?* fails to elicit such changes, suggesting the effect of OSH1 on sterol metabolism requires SCS2 and NVJ1. In addition, the effects of OSH1 overexpression can also be neutralized by NVJ1 overexpression. These data collectively suggest that Afg2p, Osh1p and Scs2p cooperate to regulate cellular sterol metabolism, possibly transport.