How do membrane-embedded enzymes recognize and process their (lipid) substrates?

Matthijs A. KOL, Anton I.P.M. de KROON, and Joost C. HOLTHUIS

Bijvoet Center / Institute of Biomembranes , Utrecht University, Netherlands

We are studying the molecular details of substrate recognition of two sets of lipid metabolizing membrane proteins, both of which have been linked to cancer via their substrates or products. Indeed, the distribution of lipids throughout the membranes of the cell is important for protein and cellular function, since some lipid classes are important signalling molecules in apoptosis and proliferation while others modulate protein activity.

Yeast Opi3p and mammalian PEMT perform SAM-dependent methylation of the headgroup of PE to synthesize PC and SAHcy. PEMT accepts PE, as well as mono- and dimethylated PE as a substrate, whereas Opi3p has strongly reduced activity on PE.

In mammalian cells, levels of DAG (pro-survival) and ceramide (pro-apoptotic) are influenced by the activities of sphingomyelin synthase family members SMS1, SMS2 and SMSr, which catalyze head-group transfer reactions. SMS1 is a genuine sphingomyelin (SM)-synthase and only accepts PC as a head group donor. SMS2 accepts both PE and PC, whereas SMSr accepts PE and has been proposed to serve as a sensor of ceramide.

The heterologous expression of enzymatically active, tagged versions of the wt proteins in yeast and *Pichia* will be reported, alongside with initial functional data on hybrid proteins and point mutants.