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MEMBRANE TRAFFICKING

Lipid sorting and clustering

Intracellular trafficking ensures the proper delivery of proteins and lipids to different parts of the cell. However, the process by which lipids are sorted and enriched at the plasma membrane remains unclear. Klemm *et al.* now provide new insights into this process by showing that, in budding yeast, the two plasma membrane components ergosterol (equivalent to cholesterol in mammalian cells) and sphingolipid are sorted at the *trans*-Golgi network (TGN) and transported in specific secretory vesicles to the cell surface.

> “…sorting of plasma membrane lipids occurs at the TGN.”

The authors engineered the expression of a green fluorescent protein (GFP)-tagged form of a transmembrane raft protein, FusMid, for the immunopurification of TGN-derived secretory vesicles from live yeast cells. Likewise, an engineered TGN/endosomal protein, Gap1–GFP, was used for purification of the donor compartment. Electron microscopy and western blot analyses revealed that these two subcellular compartments are morphologically distinct and have different protein compositions, and comparative quantitative lipidomics further uncovered their differential membrane constituents. The authors found that ergosterol and the sphingolipid mannosyl-di-IPC ($M(IP)_2C$) are enriched in FusMid vesicles compared with the TGN/endosomes, which suggests that lipid sorting occurs at the TGN. The enrichment of these two lipid raft-associated lipid species further indicates that a raft-clustering mechanism might be involved in the formation of secretory vesicles.

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Next, the authors investigated the functional consequences of raft-associated lipid enrichment in the trafficking between the TGN and secretory vesicles. Using fluorescent C-Laurdan spectrophotometric analysis, the authors observed that the FusMid vesicle membrane is more ordered than that of the TGN/endosomes, which suggests that modulation of the membrane architecture occurs during the trafficking process and further corroborates the involvement of raft clustering in TGN sorting. Taken together, the authors propose that enrichment of lipid raft-associated ergosterol and sphingolipid might promote raft coalescence and subsequent structural changes in the vesicle bilayer, leading to possible selective segregation of proteins and lipids during FusMid vesicle formation at the TGN.

Through technological advances in purifying TGN-derived secretory vesicles and quantitative measurements of lipid composition, Klemm *et al.* have shown the specific enrichment of the plasma membrane lipids ergosterol and sphingolipid by TGN/vesicle trafficking of a lipid raft protein to the cell surface. This is the first evidence that sorting of plasma membrane lipids occurs at the TGN. Furthermore, the enrichment of raft-associated lipids implicates the involvement of raft clustering in TGN sorting and vesicle formation. These findings have set the foundation for the further understanding of this sophisticated membrane trafficking system.

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ORIGINAL RESEARCH PAPER Klemm, R. W. *et al.* Segregation of sphingolipids and sterols during formation of secretory vesicles at the *trans*-Golgi network. *J. Cell Biol.* 11 May 2009 (doi:10.1083/jcb.200901145)

FURTHER READING van Meer, G. *et al.* Membrane lipids: where they are and how they behave. *Nature Rev. Mol. Cell Biol.* **9**, 112–124 (2008) | Ikonen, E. Cellular cholesterol trafficking and compartmentalization. *Nature Rev. Mol. Cell Biol.* **9**, 125–138 (2008)