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MEMBRANE TRAFFICKING

Ceramide buds in

Cargo proteins that are destined for lysosomal degradation are first incorporated into intraluminal vesicles (ILVs), which form by the invagination of endosomes; this process turns endosomes into multivesicular endosomes (MVEs). Inward budding of endosomes can generate a second population of ILVs that are subsequently released as so-called exosomes to the extracellular environment. But how cargo is sorted to different ILVs with different destinations has previously been unknown. Trajkovic *et al.* now report that exosome biogenesis requires the sphingolipid ceramide, whereas the ESCRT (endosomal sorting complex required for transport) machinery is only needed for ILVs that are sorted to lysosomes.

The authors used a mouse oligodendrocyte cell line that contains large numbers of MVEs to study the formation and release of exosomes containing proteolipid protein (PLP). Ultracentrifugation analysis showed that large amounts of PLP are present in the exosome-containing pellet, which also contains other known exosomal proteins. Indeed, immunoelectron microscopy confirmed that PLP resides on exosomes.

So how is exosomal cargo sorted from non-exosomal cargo in endosomes? PLP associates with raft-type microdomains, whereas epidermal growth factor receptor (EGFR; which is sorted into ILVs that are trafficked to lysosomes) colocalizes with the ubiquitin-binding protein Hrs, which sorts proteins for degradation. In addition, sorting of EGFR into lysosome-targeted ILVs requires the ESCRT machinery. By contrast, depletion of ESCRT components or expression of a dominant-negative form of an ESCRT component does not affect the intraendosomal transport of PLP.

Mass spectrometry analysis of the lipid composition of exosomes revealed that they are enriched in ceramide — a lipid that is produced from sphingomyelin by sphingomyelinases. Inhibition or depletion of sphingomyelinase enzymes reduces the secretion of PLP-containing exosomes. To explore the functional significance of ceramide, the authors generated giant unilamellar vesicles with a liquid-ordered 'raft-like' phase and a liquid-disordered phase. After adding sphingomyelinase, small vesicles start to bud from the liquid-ordered phase and accumulate into the lumen of the large lipid vesicles. The authors propose that ceramide, possibly thanks to its cone-shaped structure, induces inward budding of endosomes to produce ILVs that are subsequently secreted as exosomes.

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ORIGINAL RESEARCH PAPER Trajkovic, K. *et al.* Ceramide triggers budding of exosome vesicles into multivesicular endosomes. *Science* **319**, 1244–1247 (2008)
FURTHER READING Williams, R. L. & Urbé, S. The emerging shape of the ESCRT machinery. *Nature Rev. Mol. Cell Biol.* **8**, 355–368 (2007)