Nature Reviews Molecular Cell Biology | AOP, published online 9 February 2011; doi:10.1038/nrm3063

ENDOCYTOSIS

One ubiquitin does the trick

ESCRTs (endosomal sorting complexes required for transport) mediate cargo sorting to multivesicular bodies (MVBs), and this is primarily mediated by their ubiquitin-binding domains (UBDs). UBDs also mediate ESCRT ubiquitylation through a process known as coupled ubiquitylation, but whether this has a physiologically relevant effect on ESCRT function was unclear. Now, Stringer and Piper show that ESCRT-0 ubiquitylation is not essential for cargo sorting into MVBs.

The authors used several approaches to investigate whether ubiquitylation of ESCRT-0 affects cargo sorting. First, they generated yeast strains with a mutated version of the ESCRT-0 component vacuolar protein sorting-associated 27 (Vps27) that lacked all Lys residues (and could therefore not undergo Lys-linked ubiquitylation), and observed that sorting was normal. A yeast strain that does not require the other ESCRT-0 component (Hse1) for sorting and that carried the mutated Vps27 had the same phenotype. Furthermore, after identifying Rsp5 (reverses SPT-phenotype 5) as the main ubiquitin ligase modifying ESCRT-0, they found that sorting of cargo was completely blocked in Rsp5-mutant cells. However, this could be reversed by fusing one ubiquitin to the carboxyl terminus of the cargo. This suggests that, in the absence of ESCRT-0 ubiquitylation, addition of one ubiquitin molecule to the cargo is sufficient to mediate sorting.

> “… addition of one ubiquitin molecule to the cargo is sufficient to mediate sorting.”

Although it seems that ESCRT-0 ubiquitylation is not required for its role in sorting, the authors reasoned that their approach might not completely eliminate ESCRT-0 ubiquitylation, as, for example, other ubiquitin ligases could be having an effect. To test this further, they generated cargo proteins fused to the catalytic domain of deubiquitylating enzymes (DUBs). These proteins did not undergo MVB sorting and instead localized to the cell surface. Importantly, although fusion of the DUB catalytic domain to the ESCRT-0 subunit Hse1, and even ESCRT-I, blocked cargo sorting, this could be rescued by fusing one non-deubiquitylatable ubiquitin molecule to the cargo protein.

BRAND X

So, it seems that the lack of ESCRT-0 ubiquitylation can be compensated for by the addition of a single ubiquitin molecule to the cargo. It remains to be determined whether coupled ubiquitylation of other ESCRTs is important for their function.

Rachel David

ORIGINAL RESEARCH PAPER Stringer, D. K. & Piper, R. C. A single ubiquitin is sufficient for cargo protein entry into MVBs in the absence of ESCRT ubiquitination. *J. Cell Biol.* **192**, 229–242 (2011)
FURTHER READING Hurley, J. H. & Hanson, P. I. Membrane budding and scission by the ESCRT machinery: it's all in the neck. *Nature Rev. Mol. Cell Biol.* **11**, 556–566 (2010)