ENDOCYTOSIS

Joint efforts of ESCRTs

> “…the interacting residues in VPS25 and VPS20 are strictly conserved from yeast to humans…”

Sorting of ubiquitylated cargo proteins, such as transmembrane receptors, into multivesicular bodies (endosomes filled with intraluminal vesicles (ILVs)) is catalysed by the ESCRT (endosomal sorting complex required for transport) machinery. ESCRT-0, ESCRT-I and ESCRT-II bind cargo proteins, ESCRT-III remodels the membrane, and the Vps4–Vta1 complex recycles ESCRT-III following vesicle scission. The physical and functional link between ESCRT-II and ESCRT-III is pivotal but poorly understood. James Hurley and colleagues have now determined the crystal structure of a complex comprising the minimal interacting components of ESCRT-II and ESCRT-III, and demonstrated the functional relevance of these interactions for the activation by ESCRT-II of ESCRT-III-mediated vesicle budding and scission of ILVs *in vitro*.

The authors mapped the interacting regions of ESCRT-II and ESCRT-III to the second winged-helix domains of the human ESCRT-II subunit VPS25 and to the first helix of the ESCRT-III subunit VPS20, and determined the structure of the complex at 2.0 Å resolution. Mutations in the crystallographic interaction residues abolished binding in solution, thereby confirming the VPS25–VPS20 interaction. Sequence analysis showed that the interacting residues in VPS25 and VPS20 are strictly conserved from yeast to humans, which suggests that different species share a common mode of ESCRT-II–ESCRT-III interaction.

Using an *in vivo* assay for cargo sorting, Hurley and colleagues showed that cargo proteins are incorrectly sorted in yeast strains that express binding-defective VPS20 or VPS25 mutants. This suggests that the ESCRT-II–ESCRT-III interaction is essential for sorting cargo proteins. By contrast, ESCRT-II binding is not required for recruiting ESCRT-III to the endosomal membrane in yeast. This observation was confirmed by *in vitro* studies using liposomes, which showed that ESCRT-II and VPS20 bind independently to liposomes with nanomolar affinity.

The Hurley laboratory had previously developed an assay for the *in vitro* reconstitution of ESCRT-III-mediated ILV budding and scission into giant unilamellar vesicles (which mimic multivesicular bodies) using purified yeast ESCRT-III components. In the current study, they show that adding ESCRT-II to the *in vitro* assay accelerates ESCRT-III-dependent ILV production, whereas adding a binding-defective Vps25 mutant has no effect.

Finally, Hurley and co-workers modelled the binding of ESCRT-II to two VPS20 molecules, resulting in a composite ESCRT-II–$VPS20_2$ ‘supercomplex’ with a convex curvature that is complementary to that of an ILV. The authors propose that the ESCRT-II–$VPS20_2$ supercomplex might promote negative membrane curvature by functioning as a scaffold, in the same way that crescent-shaped BAR domains are thought to stabilize positive membrane curvature.

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Rubber ball

ORIGINAL RESEARCH PAPER Im, Y. J. *et al.* Structure and function of the ESCRT II-III interface in multivesicular body biogenesis. *Dev. Cell* 17 Aug 2009 (doi:10.1016/j.devcel.2009.07.008)
FURTHER READING Williams, R. L. & Urbé, S. The emerging shape of the ESCRT machinery. *Nature Rev. Mol. Cell Biol.* **8**, 355–368 (2007)