Nature Reviews Molecular Cell Biology | AOP, published online 9 April 2010; doi:10.1038/nrm2888

ENDOCYTOSIS

Division of labour between ESCRTs

The ESCRT complexes are known to mediate multivesicular body (MVB) biogenesis, but elucidating the mechanism by which they achieve this has been difficult. Using reconstitution approaches with large synthetic vesicles known as GUVs (giant unilamellar vesicles), Wollert and Hurley reveal the role of ESCRT-0–ESCRT-III in cargo sorting and MVB biogenesis.

Ubiquitylation of endocytosed cargo acts as the main signal to direct cargo to the MVB pathway. ESCRT-0 has five ubiquitin-binding domains (UBDs), so it could have a role in clustering ubiquitylated cargo. Indeed, yeast ESCRT-0 colocalized with ubiquitin in synthetic liposomes, but not when a ubiquitin mutant was used that abrogates its interaction with the ESCRT-0 UBD.

Following cargo clustering, the membrane forms invaginations by an unknown mechanism. Reconstitution of GUVs with both ESCRT-I and ESCRT-II at subphysiological concentrations was sufficient to induce membrane bud formation and to confine the cargo within the buds. Because the two complexes localized at the bud necks and did not enter the GUV lumen, the authors speculate that they mediate bud formation by inducing and stabilizing bud necks. The interaction of ESCRT-I with ESCRT-0 was partly responsible for localizing ubiquitylated cargo adjacent to the buds, as GUV experiments with mutated ESCRT-0 that cannot interact with ESCRT-I showed reduced colocalization of ESCRT-0–ubiquitin with membrane buds.

The final step in MVB formation is scission of membrane buds. The ESCRT-III subunits vacuolar protein sorting 20 (Vps20), Snf7 and Vps24 are known to induce membrane bud formation at superphysiological concentrations (when added in this order) and to scaffold and cleave the membrane bud necks. Therefore, the authors assessed whether they could also induce scission in membrane buds formed by ESCRT-I and ESCRT-II. Vps20 colocalized with ESCRT-II on the outside of the bud necks and could not induce scission alone. When Snf7 was added, it also colocalized with Vps20 and seemed to be poised for scission. The reaction could be completed by the addition of Vps24.

So, this study shows a clear division of labour between ESCRTs: ESCRT-0 clusters ubiquitylated cargo, ESCRT-I and ESCRT-II induce membrane bud formation and ESCRT-III mediates membrane scission. Furthermore, it highlights an unusual mechanism of budding and scission in which the proteins do not line the inside of the bud, as occurs during, for example, clathrin-dependent endocytosis.

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DIGITAL VISION

ORIGINAL RESEARCH PAPER Wollert, T. & Hurley, J. H. Molecular mechanism of multivesicular body biogenesis by ESCRT complexes. *Nature* 21 Mar 2010 (doi: 10.1038/nature08849)

FURTHER READING Williams, R. L. & Urbé, S. The emerging shape of the ESCRT machinery. *Nature Rev. Mol. Cell Biol.* **8**, 355–368 (2007)