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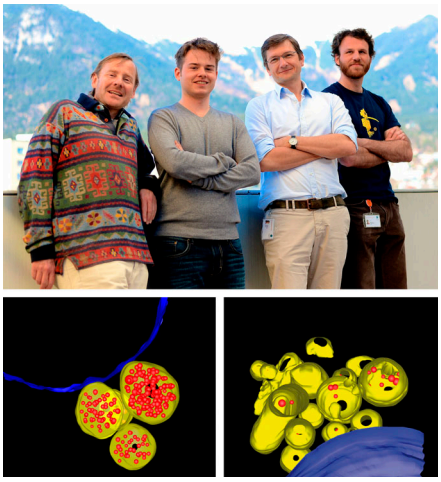
First remodel, then recycle

Study reveals how the AAA-ATPase Vps4 helps the ESCRT-III complex form multivesicular bodies.

Focal Point



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(Top row, left to right) Michael Hess, Manuel Alonso Y Adell, David Teis, Georg Vogel, and colleagues (not pictured) reveal how the AAA-ATPase Vps4 helps the ESCRT-III complex form intraluminal vesicles (ILVs) in the interior of multivesicular bodies (MVBs). Vps4 is known to disassemble the ESCRT-III complex once ILVs have budded off into the MVB lumen. But Adell et al. show that the ATPase also acts at an earlier step, helping to constrict the neck of nascent ILVs, most likely by remodeling the structure of ESCRT-III filaments. Compared with a control yeast cell (bottom left), fewer ILVs (red) form when Vps4's association with ESCRT-III is reduced by mutations in the Vps2 and Snf7 subunits (bottom right), and budding vesicles show wider connections to the limiting membrane (yellow) of MVBs.

Multivesicular bodies (MVBs) are specialized endosomes that promote the degradation of membrane proteins by delivering them to lysosomes. This process is regulated by a series of endosomal sorting complexes required for transport (ESCRTs) (1). The ESCRT-0, -I, and -II complexes sequester ubiquitinated membrane proteins and recruit the ESCRT-III complex to drive the invagination and release of cargo-laden vesicles into the interior of the MVB. The AAA-ATPase Vps4 then binds and disassembles the ESCRT-III complex so that its subunits can be recycled for further rounds of intraluminal vesicle (ILV) formation (2). Adell et al. reveal that Vps4 also participates in an earlier step of the pathway, helping ESCRT-III proteins to constrict the neck of nascent ILVs (3).

The ESCRT-III complex consists of four subunits, Vps20, Snf7, Vps24, and Vps2, which, when recruited by a component of the ESCRT-II complex, assemble into ring- or spiral-shaped filaments on the surface of MVBs (4, 5). In vitro, ESCRT-III assembly is sufficient to induce the formation of vesicles in the lumen of artificial liposomes (6). But, says David Teis from the Biocenter, Innsbruck Medical University in Austria, these in vitro vesicles are larger and more irregular than the ILVs formed in MVBs, suggesting that additional proteins play a role in vivo. "We wondered whether the only function of Vps4 is to recycle ESCRT-III or whether, in vivo, it somehow helps to shape ILVs."

> "Vps4 has to do something more than just disassemble the complex."

Every ESCRT-III subunit carries a so-called MIM domain, capable of binding to the MIT domain of Vps4. To investigate how Vps4 interacts with the ESCRT-III complex in vivo, Teis and colleagues, led by Manuel Alonso Y Adell, systematically replaced the endogenous ESCRT-III proteins of budding yeast with versions carrying mutated MIM domains. "The MIM domains of two subunits—Snf7 and Vps2—were critical for recruiting Vps4 and stabilizing its association with the ESCRT-III complex," Teis explains.

The Vps4–ESCRT-III interaction was especially reduced in yeast expressing mutant versions of both Snf7 and Vps2. Accordingly, ESCRT-III disassembly was dramatically slowed in these cells. "Vps4 still binds a little bit, but the disassembly process is very inefficient," Teis says. The sorting and degradation of membrane proteins was strongly perturbed in double mutant yeast, a similar phenotype to that of *Vps4* knockout cells, which can't recycle the ESCRT-III complex and therefore fail to transport membrane proteins correctly. But the sorting defects of yeast expressing mutant Snf7 and Vps2 weren't due to the trapping of the ESCRT machinery on endosomes.

"The key experiment was when we started swapping the different MIM domains around," says Teis. For example, the researchers replaced the MIM domain of Snf7 with the MIM domain of Vps2 and expressed this chimera in *Vps2* mutant yeast. "This chimeric protein fully supported Vps4 recruitment and ESCRT-III disassembly," Teis explains, "but it didn't support ILV formation, suggesting that Vps4 has to do something more than just disassemble the complex." This additional role is inhibited when Vps4's association with ESCRT-III is altered by a rearrangement of the subunits' MIM domains.

To investigate how Vps4 might contribute to ILV formation, Adell et al. enlisted the help of Georg Vogel and Michael Hess, who examined the MVBs of mutant yeast by electron tomography. Yeast carrying mutations in the MIM domains of both Snf7 and Vps2, with their reduced rates of ESCRT-III recycling, formed very few ILVs. Those that did form were larger than normal, and, as they grew inwards, the bud necks connecting them to the outer membrane of the MVB were much wider than those seen in wild-type cells. ILV budding was completely blocked by inhibiting ESCRT-III assembly, indicating that the complex induces or stabilizes the necks of nascent vesicles. But, because these necks are wider when ESCRT-III's association with Vps4 is perturbed, Adell et al. think that the AAA-ATPase helps constrict the bud neck and complete vesicle scission, probably by remodeling ESCRT-III filaments before it disassembles them. "This requires the coordinated binding of Vps4 to Vps2 and Snf7," says Teis. "Now we want to understand how it does this."

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