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Cryo-EM Structure of Dodecameric Vps4p and Its 2:1 Complex with Vta1p

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The type I AAA (ATPase associated with a variety of cellular activities) ATPase Vps4 and its co-factor Vta1p/LIP5 function in membrane remodeling events that accompany cytokinesis, multivesicular body biogenesis, and retrovirus budding, apparently by driving disassembly and recycling of membrane-associated ESCRT (endosomal sorting complex required for transport)-III complexes. Here, we present electron cryomicroscopy reconstructions of dodecameric yeast Vps4p complexes with and without their microtubule interacting and transport (MIT) N-terminal domains and Vta1p co-factors. The ATPase domains of Vps4p form a bowl-like structure composed of stacked hexameric rings. The two rings adopt dramatically different conformations, with the "upper" ring forming an open assembly that defines the sides of the bowl and the lower ring forming a closed assembly that forms the bottom of the bowl. The N-terminal MIT domains of the upper ring localize on the symmetry axis above the cavity of the bowl, and the binding of six extended Vta1p monomers causes additional density to appear both above and below the bowl. The structures suggest models in which Vps4p MIT and Vta1p domains engage ESCRT-III substrates above the bowl and help transfer them into the bowl to be pumped through the center of the dodecameric assembly.

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Keywords: Vps4; Vta1; AAA ATPase; HIV budding; Cryo-EM

Introduction

A common set of cellular machinery, the 'class E' vacuolar protein sorting (Vps) proteins, functions in vesiculation processes that accompany the final abscission stage of cytokinesis,[1,2] retrovirus budding,[3–12] and protein sorting at the late endosomal multivesicular body.[13–16] Most class E proteins function as subunits of three hetero-oligomeric endosomal sorting complexes required for transport (ESCRTs) that are recruited to different membranes to function in vesicle formation. Although their precise functions are not yet fully understood, ESCRT-I and ESCRT-II form stable, discrete complexes that help concentrate ubiquitylated protein cargoes and recruit ESCRT-III subunits, whereas ESCRT-III and ESCRT-III-like proteins co-assemble on membranes and appear to function directly in protein sorting, membrane remodeling, and/or fission events. Finally, the ESCRT machinery is apparently recycled by Vps4, an ATPase that binds ESCRT-III subunits and is required for their release from the membrane.[17,18] As the only class E protein with enzymatic activity, Vps4 imparts directionality to the budding process and may also play a direct role in protein sorting and membrane fission. Vps4 ATPase activity is enhanced by association with a co-factor, Vta1p (called LIP5 in higher eukaryotes).

Vps4 proteins belong to the large family of AAA (ATPase associated with a variety of cellular activities) ATPases that are spread widely throughout all kingdoms of life (reviewed in Refs. 19–22). These enzymes typically assemble into oligomeric rings that use the energy generated by ATP hydrolysis to drive conformational changes that cause macro-

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Abbreviations used: AAA, ATPase associated with a variety of cellular activities; cryo-EM, electron cryomicroscopy; ESCRT, endosomal sorting complex required for transport; MIT, microtubule interacting and transport; Vps, vacuolar protein sorting; FSC, Fourier shell correlation.