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Regulation of Vps4 ATPase activity by ESCRT-III

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Abstract

Multivesicular body (MVB) formation occurs when the limiting membrane of an endosome invaginates into the intralumenal space and buds into the lumen, bringing with it a subset of transmembrane-cargoes. Evagination of the endosomal membrane from the cytosol is topologically similar to the budding of retroviral particles and cytokinesis, wherein membranes bud away from the cytoplasm, and the machinery responsible for MVB sorting has been implicated in these phenomena. The AAA-ATPase Vps4 performs a critical function in the MVB sorting pathway. Vps4 appears to dissociate the endosomal sorting complexes required for transport (ESCRTs) from endosomal membranes during the course of MVB sorting, but it is unclear how Vps4 ATPase activity is synchronized with ESCRT release. We have investigated the mechanisms by which ESCRT components stimulate the ATPase activity of Vps4. These studies support a model wherein spatial and temporal regulation of Vps4 activity is impacted via distinct mechanisms during MVB sorting.

Introduction

The endosomal network coordinates protein sorting between the Golgi, plasma membrane, and lysosome, thereby impacting protein composition within these subcellular compartments. Multivesicular Bodies (MVBs) are endosomal intermediates that arise when the limiting membrane of the endosome invaginates and buds into the endosomal lumen. Fusion of the MVB with the lysosome results in the delivery of the intralumenal vesicles to the hydrolytic lumen of the lysosome for degradation. Entry into this degradative pathway is highly regulated. Ubiquitin modification of endosomal proteins is the major signal for cargo inclusion into the MVB pathway. MVB sorting requires the function of the ESCRTs and cargo selection is thought to occur through ubiquitin binding domains contained therein. ESCRTs can be broken into three complexes (-I, -II, and -III) conserved throughout eukaryotes. Additional factors critical for the pathway include a set of adaptor proteins that are more divergent than the ESCRTs themselves (Hrs/Vps27, Gga's, Tom/Tollip), deubiquitinating enzyme complexes (Doa4-Bro1 in yeast), and an AAA-ATPase (Vps4/SKD1) and its modulators Ist1 and Vta1/SBP1/Lip5 (recently reviewed in [1, 2]. Vps4 recruitment to the site of MVB formation occurs via interactions with the ESCRT-III family members [3, 4]. The precise significance of these associations and the consequences of mechanical energy generated through Vps4 ATP hydrolysis remain unclear, however one effect appears to be the removal of ESCRTs from the endosomal membrane; additional speculation suggests that Vps4 ATP hydrolysis generates force for membrane deformation during intralumenal vesicle budding.

ESCRT-III assembly

ESCRT-III is unique among the ESCRTs in that the complex transiently assembles on the endosomal membrane; by contrast, ESCRT-I and -II exist as complexes in the cytoplasm that are transiently recruited to the site of MVB sorting [3, 5, 6]. Yeast possess six ESCRT-III subunits, four of which are essential for MVB function and are referred to as "core"

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subunits (Vps20, Snf7, Vps2 and Vps24) and 2 regulatory subunits (Did2 and Vps60) [3, 7]. Mammals express 11 members of the ESCRT-III family, of which all but one (CHMP7) are homologs of the yeast proteins [8]. Overexpression studies in mammalian cells have revealed that CHMP4 (homolog of yeast Snf7) can polymerize into filaments on the membrane and that these filaments are associated with membrane deformations consistent with the topology of invagination in MVB formation [9]. This observation has suggested that assembly of ESCRT-III itself may facilitate the membrane deformation permitting intralumenal vesicle formation, although disassembly by Vps4 also is required to complete the process.

Alignment of the ESCRT-III subunits reveals they have highly similar charge composition and secondary structure, and structural studies of CHMP3 (homolog of yeast Vps24) have defined the five helix core arrangement present throughout the family [10]. However, the carboxyl-termini are more divergent and have not been crystallized with the core. The conserved amino-terminus has been implicated in membrane association and ESCRT-III oligomerization, while the carboxyl-termini (containing α6) seem to be more flexible and may be capable of adopting distinct closed and open conformations in the monomeric and oligomeric states [11]. This model has been supported by recent small-angle X-ray scattering analyses of CHMP-3 that suggest ionic-dependent repositioning of the carboxyl-terminus [12]. The carboxyl-termini, along with α4 and α5 of the core, have also been implicated in mediating interactions with regulators of MVB sorting.

ESCRT-III effector interactions

ESCRT-III is responsible for coordinating a number of activities required at a late stage of MVB sorting, including the recruitment of the AAA-ATPase Vps4 and its regulators (Ist1, Vta1) as well as deubiquitinating enzymes such as Doa4, AMSH and UBPY [3, 7, 13-17]. The contributions of distinct ESCRT-III subunits to this process and the mechanisms enabling this specificity are becoming apparent. Snf7 and the human CHMP-4 proteins (Snf7 homologs) bind in a specific manner to the Bro1 domain proteins Bro1 and Alix, respectively, with the Bro1-Snf7 interaction facilitating recruitment of the ubiquitin isopeptidase Doa4 [15, 18, 19]. CHMP1 (Did2), CHMP2 (Vps2) and CHMP3 (Vps24) can interact with two mammalian deubiquitinating enzymes, AMSH and UBPY, to facilitate their recruitment directly [17, 20, 21]. These interactions are mediated by three helix MIT domains present within AMSH and UBPY. Vps4 harbors a MIT domain as well and binds to these same three ESCRT-III subunits [22, 23]. Determination of the Vps4 MIT domain in complex with the carboxyl-termini of CHMP1 and Vps2 revealed that type 1 MIT-interaction motifs (MIM1) in α6 of Did2/CHMP1, Vps2/CHMP2 and Vps24/CHMP3 are recognized by the surface formed by the second and third helices of the MIT domain [24, 25]. This MIM1 is similar to the Snf7 α6 sequence recognized by the Bro1 domain, except distinct spacing of hydrophobic residues discriminate between the two epitopes [18]. Vps4 also binds Vps20/CHMP6 via the MIT domain; however, this interaction occurs via a surface formed by the first and third MIT helices and recognizes a distinct MIM (MIM2) in the loop connecting CHMP6 α4 and α5 [26]. The ESCRT-III α4-α5 region has also been implicated in interactions between Did2 and the Vps4 inhibitor Ist1 and between Vps60/CHMP5 and the Vps4 activator Vta1/Lip5 [7, 14, 16]. The Vta1/Lip5 amino-terminus mediating this association also interacts with the Did2/CHMP1 α6, and elucidation of the structure of the Vta1 amino-terminus revealed an arrangement of dual MIT motifs [7, 26, 27]. These observations highlight two elements (Bro1 domains and MIT motifs) recognizing these ESCRT-III subunits and two regions of ESCRT-III proteins critical for interactions with effectors (the α6 amphipathic helix and the α4-α5 region). The conformational changes in ESCRT-III subunits between monomeric and oligomeric states likely impact associations with effectors through repositioning α4-α5 and the carboxyl-terminal helix. The assembly of

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ESCRT-III oligomers is anticipated to present a multitude of distinct epitopes important for coordinating cargo deubiquitination and ESCRT disassembly.

Function of the Vps4 oligomer

The mechanisms by which the assembly and disassembly of ESCRT-III permit membrane invagination remain unclear, but nucleotide hydrolysis by Vps4 plays a critical role in this process. Vps4 is a type 1 AAA ATPase with a single AAA domain per monomer. Type 2 AAA ATPases (such as p97 and NSF) contain two AAA domains per subunit and exist as stable hexamers with one ring of AAA domains generating force through ATP hydrolysis while the second ring stabilizes the hexameric form [28]. By contrast, type 1 AAA ATPases, including Vps4, have been suggested to follow a cycle of ATP-stimulated oligomerization, oligomerization-stimulated ATP hydrolysis and subsequent dissociation. In the case of Vps4, this cycle has also been suggested to be coordinated with membrane recruitment. In this model, association with ESCRT-III facilitates Vps4 oligomerization while subsequent ATP hydrolysis leads to ESCRT release along with Vps4 disassembly and membrane dissociation [4, 29]. However, a number of recent studies suggest that this model is inaccurate. Vta1 has been demonstrated to stimulate Vps4 ATPase activity in part through promoting Vps4 oligomerization [30]; this suggests that the Vta1-Vps4 oligomer is stable throughout the ATPase cycle *in vivo*. Cryo-EM analysis of the Vps4 dodecamer has also revealed that the Vps4 oligomer exhibits 6-fold symmetry, suggesting that the upper and lower rings function differentially [31]. This asymmetry between rings is reminiscent of the type 2 AAA ATPases and also suggests that the Vps4 oligomer may remain assembled throughout the ATP hydrolysis cycle even in the absence of Vta1. While initial models proposed that Vps4 subunits hydrolyze ATP concomitantly, elegant studies with the bacterial AAA ATPase ClpX have demonstrated that the concerted hydrolysis does not occur and that ATP hydrolysis by even a single subunit can support substrate unfolding by the $ClpX_6$ ring [32, 33]. These observations suggest that the previous model of Vps4 function requires reassessment.

ESCRT-III activation of the Vps4 oligomer

The Vta1-Vps4 complex plays a critical role in coordinating disassembly of ESCRT-III, and MIT domains within both Vta1 and Vps4 contribute to this activity [4, 7]. In an attempt to understand the significance of the interactions between ESCRT-III and Vta1-Vps4, we have focused on the ability of ESCRT-III subunits to modulate Vps4 ATPase activity. These studies have revealed at least two distinct mechanisms by which ESCRT-III family members can stimulate Vps4 [7]. Direct interaction of Vps2 or Did2 with the MIT domain of Vps4 results in stimulation of Vps4 ATPase activity. Additionally, Did2 and Vps60 can stimulate Vps4 ATPase activity indirectly via the MIT domains of Vta1. Vta1 itself can also stimulate Vps4 ATPase activity, indicating that Vps4 ATPase activity is regulated on a number of levels. These observations suggest a coordination of ESCRT-III assembly with its disassembly as stimulated by Vta1-Vps4. How this process facilitates membrane deformation to complete MVB sorting remains to be determined, but *in vitro* reconstitution of this process is being pursued to examine this question.

The formation of the MVB represents a topological conundrum as membrane must exvaginate from the cytoplasm to form the intralumenal vesicle. The mechanisms by which the cytoplasmic ESCRT machinery accomplishes this task without being consumed by the process are as yet unclear. Formation of the ESCRT-III complex has been implicated in promoting the membrane deformation, and ATP hydrolysis by Vps4 is required to complete vesicle budding and recycle the ESCRT-III subunits. The coordination of ESCRT-III assembly with disassembly appears critical for this process, and the mechanisms by which

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the Vta1-Vps4 complex is activated to promote ESCRT-III disassembly are becoming clear. Further dissection of how Vta1-Vps4 dissociates ESCRT-III to permit completion of intralumenal vesicle budding should resolve a critical question of MVB sorting.

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Bibliography

1. Piper RC, Katzmann DJ. Biogenesis and Function of Multivesicular Bodies. Annu Rev Cell Dev Biol. 2007
2. Williams RL, Urbe S. The emerging shape of the ESCRT machinery. Nat Rev Mol Cell Biol. 2007; 8(5):355–68. [PubMed: 17450176]
3. Babst M, Katzmann DJ, Estepa-Sabal EJ, Meerloo T, Emr SD. Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting. Dev Cell. 2002; 3(2):271–82. [PubMed: 12194857]
4. Babst M, Wendland B, Estepa EJ, Emr SD. The Vps4p AAA ATPase regulates membrane association of a Vps protein complex required for normal endosome function. Embo J. 1998; 17(11):2982–93. [PubMed: 9606181]
5. Babst M, Katzmann DJ, Snyder WB, Wendland B, Emr SD. Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. Dev Cell. 2002; 3(2):283–9. [PubMed: 12194858]
6. Katzmann DJ, Babst M, Emr SD. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. Cell. 2001; 106(2):145–55. [PubMed: 11511343]
7. Azmi IF, Davies BA, Xiao J, Babst M, Xu Z, Katzmann DJ. ESCRT-III family members stimulate Vps4 ATPase activity directly or via Vta1. Dev Cell. 2008; 14(1):50–61. [PubMed: 18194652]
8. Horii M, Shibata H, Kobayashi R, Katoh K, Yorikawa C, Yasuda J, Maki M. CHMP7, a novel ESCRT-III-related protein, associates with CHMP4b and functions in the endosomal sorting pathway. Biochem J. 2006; 400(1):23–32. [PubMed: 16856878]
9. Hanson PI, Roth R, Lin Y, Heuser JE. Plasma membrane deformation by circular arrays of ESCRT-III protein filaments. J Cell Biol. 2008; 180(2):389–402. [PubMed: 18209100]
10. Muziol T, Pineda-Molina E, Ravelli RB, Zamborlini A, Usami Y, Gottlinger H, Weissenhorn W. Structural basis for budding by the ESCRT-III factor CHMP3. Dev Cell. 2006; 10(6):821–30. [PubMed: 16740483]
11. Shim S, Kimpler LA, Hanson PI. Structure/function analysis of four core ESCRT-III proteins reveals common regulatory role for extreme C-terminal domain. Traffic. 2007; 8(8):1068–79. [PubMed: 17547705]
12. Lata S, Roessle M, Solomons J, Jamin M, Gottlinger HG, Svergun DI, Weissenhorn W. Structural basis for autoinhibition of ESCRT-III CHMP3. J Mol Biol. 2008; 378(4):816–25.
13. Amerik AY, Nowak J, Swaminathan S, Hochstrasser M. The Doa4 deubiquitinating enzyme is functionally linked to the vacuolar protein-sorting and endocytic pathways. Mol Biol Cell. 2000; 11(10):3365–80. [PubMed: 11029042]
14. Dimaano C, Jones CB, Hanono A, Curtiss M, Babst M. Ist1 regulates vps4 localization and assembly. Mol Biol Cell. 2008; 19(2):465–74. [PubMed: 18032582]
15. Luhtala N, Odorizzi G. Bro1 coordinates deubiquitination in the multivesicular body pathway by recruiting Doa4 to endosomes. J Cell Biol. 2004; 166(5):717–29. [PubMed: 15326198]

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16. Shim S, Merrill SA, Hanson PI. Novel Interactions of ESCRT-III with LIP5 and VPS4 and their Implications for ESCRT-III Disassembly. Mol Biol Cell. 2008; 19(6):2661–72. [PubMed: 18385515]
17. Row PE, Liu H, Hayes S, Welchman R, Charalabous P, Hofmann K, Clague MJ, Sanderson CM, Urbe S. The MIT domain of UBPY constitutes a CHMP binding and endosomal localization signal required for efficient epidermal growth factor receptor degradation. J Biol Chem. 2007; 282(42): 30929–37. [PubMed: 17711858]
18. McCullough J, Fisher RD, Whitby FG, Sundquist WI, Hill CP. ALIX-CHMP4 interactions in the human ESCRT pathway. Proc Natl Acad Sci U S A. 2008; 105(22):7687–91. [PubMed: 18511562]
19. Kim J, Sitaraman S, Hierro A, Beach BM, Odorizzi G, Hurley JH. Structural basis for endosomal targeting by the Bro1 domain. Dev Cell. 2005; 8(6):937–47. [PubMed: 15935782]
20. Agromayor M, Martin-Serrano J. Interaction of AMSH with ESCRT-III and deubiquitination of endosomal cargo. J Biol Chem. 2006; 281(32):23083–91. [PubMed: 16760479]
21. Tsang HT, Connell JW, Brown SE, Thompson A, Reid E, Sanderson CM. A systematic analysis of human CHMP protein interactions: additional MIT domain-containing proteins bind to multiple components of the human ESCRT III complex. Genomics. 2006; 88(3):333–46. [PubMed: 16730941]
22. Scott A, Gaspar J, Stuchell-Brereton MD, Alam SL, Skalicky JJ, Sundquist WI. Structure and ESCRT-III protein interactions of the MIT domain of human VPS4A. Proc Natl Acad Sci U S A. 2005; 102(39):13813–8. [PubMed: 16174732]
23. Xiao J, Xia H, Yoshino-Koh K, Zhou J, Xu Z. Structural characterization of the ATPase reaction cycle of endosomal AAA protein Vps4. J Mol Biol. 2007; 374(3):655–70. [PubMed: 17949747]
24. Obita T, Saksena S, Ghazi-Tabatabai S, Gill DJ, Perisic O, Emr SD, Williams RL. Structural basis for selective recognition of ESCRT-III by the AAA ATPase Vps4. Nature. 2007; 449(7163):735–9. [PubMed: 17928861]
25. Stuchell-Brereton MD, Skalicky JJ, Kieffer C, Karren MA, Ghaffarian S, Sundquist WI. ESCRT-III recognition by VPS4 ATPases. Nature. 2007; 449(7163):740–4. [PubMed: 17928862]
26. Kieffer C, Skalicky JJ, Morita E, De Domenico I, Ward DM, Kaplan J, Sundquist WI. Two distinct modes of ESCRT-III recognition are required for VPS4 functions in lysosomal protein targeting and HIV-1 budding. Dev Cell. 2008; 15(1):62–73. [PubMed: 18606141]
27. Xiao J, Xia H, Zhou J, Azmi IF, Davies BA, Katzmann DJ, Xu Z. Structural basis of Vta1 function in the multivesicular body sorting pathway. Dev Cell. 2008; 14(1):37–49. [PubMed: 18194651]
28. Hanson PI, Whiteheart SW. AAA+ proteins: have engine, will work. Nat Rev Mol Cell Biol. 2005; 6(7):519–29. [PubMed: 16072036]
29. Babst M, Sato TK, Banta LM, Emr SD. Endosomal transport function in yeast requires a novel AAA-type ATPase, Vps4p. Embo J. 1997; 16(8):1820–31. [PubMed: 9155008]
30. Azmi I, Davies B, Dimaano C, Payne J, Eckert D, Babst M, Katzmann DJ. Recycling of ESCRTs by the AAA-ATPase Vps4 is regulated by a conserved VSL region in Vta1. J Cell Biol. 2006; 172(5):705–17. [PubMed: 16505166]
31. Yu Z, Gonciarz MD, Sundquist WI, Hill CP, Jensen GJ. Cryo-EM structure of dodecameric Vps4p and its 2:1 complex with Vta1p. J Mol Biol. 2008; 377(2):364–77. [PubMed: 18280501]
32. Hersch GL, Burton RE, Bolon DN, Baker TA, Sauer RT. Asymmetric interactions of ATP with the AAA+ ClpX6 unfoldase: allosteric control of a protein machine. Cell. 2005; 121(7):1017–27. [PubMed: 15989952]
33. Martin A, Baker TA, Sauer RT. Rebuilt AAA + motors reveal operating principles for ATP-fuelled machines. Nature. 2005; 437(7062):1115–20. [PubMed: 16237435]