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ESCRT complexes and the biogenesis of multivesicular bodies

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Summary

Multivesicular bodies (MVBs) are critical intermediates in the trafficking of ubiquitinated receptors and other cargo destined for lysosomes. The formation of MVBs by invagination of the endosomal limiting membrane is catalyzed by the ESCRT complexes, a process that has recently been visualized in three-dimensional detail by electron tomography. Structural and biochemical analysis of the upstream components, Vps27-Hse1, ESCRT-I, and ESCRT-II, shows how these complexes assemble and cluster cargo. Rapid progress has been made in understanding the assembly and disassembly of the ESCRT-III complex and the interactions of its subunits with MIT domain and other proteins. A key role for deubiquitination in the regulation of the system has been demonstrated. One central question remains largely unanswered, which is how the ESCRTs actually promote the invagination of the endosomal membrane.

The lysosome is the eukaryotic cell's main engine for the breakdown of membrane proteins and internalized materials. Cell surface receptors destined for the lysosome arrive through a pathway in which portions of the limiting membrane of endosomes containing these receptors invaginate into the lumen of the endosome [1-3]. At the stage when the endosome becomes filled with intraluminal vesicles (ILVs), it is referred to as a multivesicular body (MVB). Research into MVB biogenesis has exploded following the discovery in yeast of three protein complexes, Endosomal Sorting Complex Required for Transport (ESCRT) I, II, and III [4-6]. The ESCRT pathway is conserved throughout eukaryotes, where it plays essentially the same role in MVB biogenesis as in yeast. In animals, the ESCRT pathway is hijacked by viruses, which use the pathway to bud from the plasma membrane in a reaction that is topologically equivalent to the budding of ILVs [7-9]. Moreover, the ESCRT system is required for a third topologically equivalent process, the membrane scission event during cytokinesis [10,11].

The details of the ESCRT pathway have been described in several excellent reviews [12-17]. Here I summarize the main points (Fig. 1). Cargo is brought into the pathway by the Vps27-Hse1 complex (Table 1). Vps27 is targeted to endosomes via its FYVE domain, which binds to the endosomal lipid phosphatidylinositol 3-phosphate. Vps27 contains P(S/T)XP motifs that recruit the soluble heterotetrameric ESCRT-I complex to the endosomal membrane via the UEV domain of its Vps23 subunit. ESCRT-I recruits another soluble heterotetrameric complex, ESCRT-II. All three of the above mentioned complexes contain ubiquitin binding domains that interact with ubiquitinated cargo proteins. The ESCRT-III proteins are soluble monomers until recruited to the endosomal membrane, where they form an insoluble array. Recruitment is thought to be initiated by ESCRT-II binding to Vps20. Cargo is deubiquitinated following assembly of the ESCRT-III lattice. Finally, ILV formation and ESCRT-III disassembly by the ATPase Vps4 take place, either concurrently or sequentially.

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The structure of MVBs and the class E compartment

Electron tomography showed that in wild-type yeast, MVBs are roughly spherical, ~200 nm across, and filled with spherical ~24 nm ILVs [18] (Fig. 2A-C). In yeast and human [19,20] cells, defects in the ESCRT machinery not only interfere with normal MVB formation, they also manifest a distinctive abnormal subcellular structure, the class E compartment. The class E compartment consists of stacked flat cisternae-like membranes (Fig. 2D-F), which are not connected to each other [18]. It is not known what determines the morphology of the class E compartment. In *did2Δ* cells, which have an intermediate phenotype, the MVBs are elongated and irregular in shape, and the ILVs are enlarged (Fig. 2G-I) [18].

Cargo delivery into the ESCRT pathway

Monoubiquitination [21,22] and Lys-63 linked polyubiquitination [22-24] direct cargo into the endolysosomal pathway. Vps27-Hse1, a key upstream component of the pathway, binds ubiquitin via UIM motifs. While yeast Vps27 contains two UIMs, its human orthologs contains one UIM with a double-sided ubiquitin binding capability [25]. The yeast and human orthologs thus bind cooperatively to multiple ubiquitin moieties by different mechanisms. The Hse1 subunit of the Vps27-Hse1 complex seems to be involved mainly in the recruitment of ubiquitin ligases and deubiquitinating enzymes (DUBs) [30]. The complex heterodimerizes via two intertwined GAT domains [26], linking Vps27 architecturally to the GAT domain-containing GGA and TOM1L trafficking protein families. The GGAs [27] and the TOM1, TOM1L1, and TOM1L2 proteins [28] are like Vps27 in that they bind to ubiquitin and ESCRT-I. VPS27 appears to concentrate ubiquitinated cargo into microdomains, to which VPS27 recruits clathrin. Clathrin forms a flat coat over these microdomains, rather than the typical curved clathrin coated vesicles [29]. ESCRT-I is thought to either be recruited by VPS27 at the edges of the clathrin coat, or to enter the coat by displacing clathrin in an exchange reaction.

How the ESCRT machinery assembles on endosomes

In the conventional model for ESCRT assembly, ESCRT recruitment is initiated by Vps27, and ESCRT-I, II, and III then sequentially recruit one another from the cytosol. A recent ultrastructural study found that ESCRTs are localized to a wide range of endosomal and other membranes within cells, leading to the appearance under optical microscopy that they are cytosolic [31]. ESCRT-I is a heterotetramer of Vps23, Vps28, Vps37, and Mvb12 in both yeast [32-35] and humans [36,37], and the subunits are present in one copy each [35-38]. The binding sites for ubiquitinated cargo and ESCRT-II are at opposite ends of the 25 nm long ESCRT-I complex [35] (Fig. 3), which argues against the concept that ubiquitinated cargo is handed off from one ESCRT complex to another in a “conveyor belt” mechanism. Current thinking favors the idea that ESCRT-I and II co-assemble and cluster multiple ubiquitinated transmembrane proteins for packaging into ILVs [17,35].

In yeast, the C-terminal domain (CTD) of the Vps28 subunit of ESCRT-I recruits ESCRT-II [39,40] (Fig. 3). ESCRT-II consists of one molecule each of Vps22 and Vps36, and two molecules of Vps25. The Vps28-CTD domain binds to the NZF1 zinc finger in the N-terminus of the Vps36 subunit of ESCRT-II [38]. Human ESCRT-II lacks the NZF1 domain, leaving the mechanism for the putative human ESCRT-I/II interaction uncertain. The N-termini of Vps36 orthologs contain a pleckstrin homology (PH) domain variant called a “GLUE” domain. The human VPS36 GLUE domain binds to phosphoinositides [41] and ubiquitin [41-43]. The yeast Vps36 GLUE domain binds with moderate affinity and specificity to phosphoinositide-bearing membranes [40], but does not directly bind to ubiquitin. Two zinc fingers, the above-mentioned NZF1, and the ubiquitin-binding NZF2, are inserted within the sequence of the yeast

GLUE domain. Thus yeast ESCRT-II binds the same ligands as the human complex through a more complicated set of domains.

Structural and interaction data on Vps27-Hse1, ESCRT-I, and ESCRT-II are sufficient to form a reasonably complete picture of the assembly of the upstream half of the ESCRT machinery (Fig. 3). The individual interactions of the ESCRT complexes with lipids are of moderate to high (~ 100 nM to low micromolar) affinity, and the interactions with individual ubiquitinated cargo are exceptionally weak (0.1 to 2 mM). However, the assembled network is cross-linked by interactions between the ESCRT complexes, which makes it a multivalent platform that can bind tightly to the membrane and cluster multiple ubiquitinated cargo molecules.

Regulation of ESCRT-III assembly

There are six ESCRT-III or ESCRT-III-like proteins in yeast, Vps2, Vps20, Vps24, Snf7, Did2, and Vps60. Vps20 and Snf7 associate with each other, and probably act an early stage in ESCRT-III assembly. Vps20 binds directly to ESCRT-II, and reportedly to ESCRT-I as well [44]. There is no direct evidence that ESCRT-III comprises the membrane scission “machine” that makes and detaches ILVs. However, the ability of Snf7 and possibly other ESCRT-III subunits to form arrays has made ESCRT-III a prime candidate to fill such a role. The main evidence for array formation comes from *SNF7* overexpression studies [45,46]. Direct imaging of the SNF7 array shows that it consists of filaments arranged in a spiral pattern (P. Hanson, pers. comm.). The SNF7 array appears capable of deforming membranes into tubules that project away from the cytoplasm. This observation might be consistent with a direct role for Snf7 in the mechanics of ILV formation. Did2 and Vps2 appear to act at a later stage, where they are responsible for recruiting Vps4 [18,47,48].

ESCRT-III subunits are distinguished by a primarily basic N-terminus and an acidic C-terminus. The crystallization of a VPS24 construct provides most of what we know about ESCRT-III structure [49]. The crystallized VPS24 contains a deletion in its most C-terminal predicted helix, which leads to dimerization both in solution and in the crystal. A flat basic face formed by the two N-terminal helices presents a binding site for acidic phospholipids, while the C-terminal part of the crystallized structure is acidic. The most C-terminal helix, not present in the crystal, has been a topic of investigation in its own right. The C-terminal helix appears to autoinhibit ESCRT-III monomers with respect to array formation [46,50]. Deletion of this helix from VPS24 and SNF7 promotes the formation of insoluble membrane-bound aggregates in vivo that interfere with MVB biogenesis and sorting [46,50]. Furthermore, the C-terminal helices of ESCRT-III proteins play multiple roles in interacting with other proteins. The C-termini of Vps2 and Did2 contain a MIT (microtubule interacting and transport) domain-interacting motif (MIM), defined by six conserved residues that make up a contiguous surface on one face of the helix (Fig. 4) [47,48]. This suggests that binding of other proteins to the C-terminal helices of ESCRT-III monomers could initiate array formation. This might, for example, occur between ESCRT-III proteins Vps20 or Snf7 with ESCRT-II or Bro1, respectively. Reciprocally, ESCRT-III subunits within the array expose their C-terminal helices, making them available to recruit MIT-domain containing proteins such as Vps4.

Deubiquitination: the final signal for cargo entry into ILVs?

In yeast, the key DUB associated with the ESCRT pathway is Doa4. Doa4 is targeted to endosomes via an N-terminal predicted helical region [51]. Doa4 localization depends on the assembly of ESCRT-III, while its enzymatic activation is promoted by Bro1 [52]. Doa4 is not critical for MVB biogenesis, but in its absence, ILVs are fewer and smaller [52]. This is consistent with a lighter cargo load as ubiquitinated cargoes such as Cps1 and Gap1 are retained at the limiting membrane [51-53].

The major human DUBs implicated in the MVB pathway are AMSH and UBPY. Knockdown or catalytic inactivation of these enzymes markedly slows the degradation of EGF receptor [54-58]. AMSH and UBPY are recruited to endosomes by interactions between their N-terminal MIT domains and late-acting ESCRT-III subunits [54-57,59]. Remarkably, UBPY is catalytically activated by the HSE1 subunit of the early-acting VPS27-HSE1 complex [57]. UBPY thus appears to function at both early and late stages of MVB biogenesis. The findings in human cells are in good agreement with the yeast data, and support the concept that cargo deubiquitination is a critical late-stage signal for cargo entry into ILVs. This concept represents a significant change in thinking: the complete ubiquitination/deubiquitination cycle is considered essential, not just the initial ubiquitination of the cargo.

Regulation of ESCRT-III disassembly

The main thermodynamic driving force for MVB biogenesis is thought to be the consumption of ATP by Vps4. A complex of Vps4 with another protein, Vta1, [60-63] appears to disassemble the ESCRT-III lattice. In isolation, Vta1 is a rod-like homodimer, and it binds to the C-terminal β domain of Vps4 through a short conserved VSL region at its C-terminus [63]. Vta1 accelerates the ATPase activity of Vps4, and promotes the assembly of Vps4 into its functional form, a double hexameric ring. Vta1 directly binds to the ESCRT-III subunit Did2 [62]. Since Vps4 also directly binds to a subset of ESCRT-III subunits that includes Did2 [47,48], the multiplicity of binding sites in the Vps4:Vta1 complex suggests a mechanism for high cooperativity in binding to the ESCRT-III lattice. Current evidence favors a model in which Vps4 is an ATP-powered engine that pumps ESCRT-III monomers through the central pore of the double ring and into the cytosol [64]. A key question is whether Vps4 is involved directly in the mechanics of ILV scission, or indirectly by cleaning up the ESCRT-III lattice afterwards to enable additional rounds of scission.

Concluding remarks

The past two years have seen an explosion of structural and mechanistic insights into the targeting, assembly, and disassembly of the ESCRT machinery. The role of ubiquitination/deubiquitination circuits as regulatory elements is emerging in considerable detail, and there are hints that other modifications, such as phosphorylation, will also be important [37]. Advances in molecular structural analysis by x-ray crystallography and NMR have helped propel this field for the past few years, and EM tomography is now beginning to make equally important contributions at the level of subcellular structure. *In vitro* reconstitution of the ESCRT reaction should in principle be possible, given the identification and purification of many, if not all, of the factors involved. The field now appears ready to tackle the single most pressing issue head on, the mechanism of ILV formation and scission.

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Figure 1.

A model for the organization of the ESCRT system. In this scheme, Vps27-Hse1, ESCRT-I, and ESCRT-II are targeted to membranes by lipids (green), where they cluster cargo (red). ESCRT-I and II interact at a 1:1 stoichiometry. The stoichiometry of ESCRT-I binding to Vps27-Hse1 is undefined and for simplicity is shown as 1:1. ESCRT-II recruits ESCRT-III via its Vps20 subunit. Vps20 might nucleate the polymerization of Snf7, which forms filamentous arrays as demonstrated by Hanson, Heuser, and colleagues (personal communication). Other ESCRT-III subunits probably polymerize in a similar manner, and it is not clear whether homo- or heteromeric polymers predominate. Incorporation of Vps2 and Did2 into ESCRT-III initiates the depolymerization of ESCRT-III by Vps4:Vta1. ILVs are thought to be formed at some point during the polymerization/depolymerization cycle. This model is similar to the recently proposed “concentric circle” model [17].

Figure 2.

Multivesicular bodies and the class E compartment. Genotypes are identified at left. Panels A, D, and G show tomographic sections, and the remaining panels show three-dimensional reconstructions. Panels A, B, C, show normal yeast multivesicular bodies; D, E, F show the class E compartment; and G, H, I show multivesicular bodies under conditions of defective cargo deubiquitination. Reproduced from the **Journal of Cell Biology**, 2006, 175: 715–720 [18]. Copyright 2006, Rockefeller University Press.

Figure 3.

A structural model for the assembly and cargo clustering by A) Vps27-Hse1, B) ESCRT-I, and C) ESCRT-II. The structures shown are described in more detail in references [16,26,35].

Figure 4. MIT domains and MIM motifs coordinate the assembly and disassembly of ESCRT-III. The MIM of DID2B (red) bound to the MIT domain of VPS4A (orange) [48] illustrates the recognition of the C-terminal MIM regions of ESCRT-III subunits by Vps4. The structure of the C-terminally truncated dimer of VPS24 [49] is shown to illustrate the putative membrane binding “tiles” of the ESCRT-III array. The structure is colored according to electrostatic potential, with blue electropositive, and red electronegative.

Table 1

Nomenclature for ESCRT complex subunits

Complex	Yeast or generic protein	Synonyms (yeast)	Metazoan protein	Synonyms (metazoan)
Vps27-Hse1	Vps27 Hse1		VPS27 HSE1	HRS STAM
ESCRT-I	Vps23 Vps28 Vps37		VPS23 VPS28 VPS37A,B,C,D	TSG101
ESCRT-II	Mvb12 Vps22 Vps25		MVB12A,B VPS22 VPS25	EAP30 EAP20
ESCRT-III	Vps36 Vps2 Vps20 Vps24 Snf7 Vps60 Did2	Vps32	VPS36 VPS2A,B VPS20 VPS24 SNF7A,B,C VPS60 DID2	EAP45 CHMP2A,B CHMP6 CHMP3 CHMP4A, B, C CHMP5 CHMP1A, B
Vps4	Vps4 Vta1		VPA4A,B VTA1	SKD1 LIP5
Other	Bro1	Vps31	BRO1	ALIX, AIP1