Piecing together the ESCRTs

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Abstract
High-resolution structural analysis has characterized nearly all of the individual domains of ESCRT (endosomal sorting complex required for transport) subunits, all of the core structures of the soluble complexes and many of the interactions involving domains. Recent emphasis in structural studies has shifted towards efforts to integrate these structures into a larger-scale model. Molecular simulations, hydrodynamic analysis, small-angle X-ray scattering and cryo-EM (electron microscopy) techniques have all been brought to bear on the ESCRT system over the last year.

Introduction
Early structural studies of the ESCRT (endosomal sorting complex required for transport) system began almost a decade ago and have picked up momentum over the last 5 years. Thanks to the efforts of many laboratories, structures are known for nearly all of the constituent protein domains, the complexes of many of these domains with their partners and the truncated core assemblies of the soluble ESCRTs. One recent modelling effort highlighted that detailed information of the stoichiometries of ESCRTs, their structures and their footprints relative to the membrane are critical for understanding how they function in multivesicular body biogenesis [1]. The structural frontier in the ESCRT field has moved from solving structures of fragments to understanding how the structural fragments assemble together. The goal of structural analysis is to tell us how individual ESCRT components are integrated into a machine that clusters cargo and closes the necks of membrane buds.

Vps (vacuolar protein sorting) 27–Hse1
(has symptoms of class E mutants ¹)
Vps27–Hse1 is an early endosomal clathrin adaptor that binds to ubiquitinated membrane proteins and acts directly upstream of the ESCRTs. Vps27 and Hse1 constitutively form a 1:1 heterodimer via an antiparallel coiled coil and two intertwined GAT [GGA (Golgi-associated γ–adaptin ear homology domain ADP-ribosylation factor-interacting protein) and TOM (target of Myb)] domains [2] (Figure 1A). Vps27 contains two tandem UIMs (ubiquitin-interacting motifs), whereas Hse1 and its human orthologues STAM (signal-transducing adaptor molecule) have one. UIMs consist of a single α-helix [3] that binds ubiquitin via its Ile-44 patch [4] with low affinity [3]. Hrs (hepatocyte growth factor receptor substrate), the human orthologue of Vps27, contains one DUIM (double-sided UIM) [5]. The low-affinity interactions with ubiquitinated proteins are not the driving force for membrane targeting of Vps27. Rather, the FYVE domain of Vps27, which constitutively binds two Zn²⁺ ions, recognizes the endosomal lipid Pad1n3P in the membrane [6,7]. The C-terminal clathrin box motif of Vps27 or Hrs recruits clathrin association, which in turn concentrates ubiquitinated cargos into the restricted clathrin coats on endosomes [6]. The C-terminal unstructured regions also contain P(S/T)XP motifs that interact with ESCRT-I [6,7]. The emerging picture of Vps27–Hse1 and Hrs–STAM is that they are remarkably interaction-dense complexes that co-ordinate many interactions with membranes, cargo and coat proteins, and co-ordinate multiple ubiquitination and deubiquitination reactions.

There have been two main efforts to integrate this structural information. In the first, an EM (electron microscopy) reconstruction at 16 Å (1 Å = 0.1 nm) was obtained for a hexameric state of Hrs in the absence of its partner STAM [8], which allowed a model for the mutual orientations of domains to be constructed. We have found that, when co-expressed, full-length Hrs and STAM form a 1:1 heterodimer similar to that of Vps27–Hse1, and probably more reflective of the physiological Hrs–STAM complex (X. Ren and J.H. Hurley, unpublished work). For the Vps27–Hse1 complex, a coarse-grained Monte Carlo simulation of the 1:1 heterodimer bound to a membrane and ubiquitinated cargo suggests that the complex is open and dynamic, facilitating its interactions with cargo of a wide range of sizes and conformations [2]. This study has the limitation that no biophysical data on the overall conformation were available to constrain the simulation. The main short-term goal for structural studies of Vps27–Hse1 and Hrs–STAM is to develop an experimentally based holistic model for the entire complexes.

Key words: ALG-2 (apoptosis-linked gene 2)-interacting protein X (Alix), endosomal sorting complex required for transport (ESCRT), membrane, small-angle X-ray scattering (SAXS), ubiquitin.
Abbreviations used: AAA, ATPase associated with various cellular activities; Alix, ALG-2 complex required for transport (ESCRT), membrane, small-angle X-ray scattering and cryo-EM (electron microscopy) techniques have all been brought to bear on the ESCRT system over the last year.

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**ESCRT-I**

ESCRT-I is a 1:1:1:1 heterotetramer composed of Vps23, Vps28, Vps37 and Mvb12 (multivesicular body sorting factor of 12 kDa) in both yeast [9–13] and humans [14] (Figure 1B). ESCRT-I binds directly to mono-ubiquitinated protein cargo by its UEV (ubiquitin-conjugating enzyme E2 variant) domain, which interacts, like most other ubiquitin-binding domains, with the Ile-44 patch on ubiquitin [15,16]. The ESCRT-I UEV domain also interacts with HIV-1 Gag p6 [17] and a number of cellular proteins, including the
ESCRT-III subunits Vps20, Snf7, Vps2 and Vps24 become activated upon unknown mechanisms. The inhibitory negatively charged C-terminus (red helix, model) is released from the positively charged N-terminus (crystal structure of Vps24 as blueprint of all ESCRT-III subunits, PDB code 2GD5). The N-terminus binds to the membrane, whereas the C-terminus serves to recruit other ESCRT-III subunits (Did2, Vps60), which in turn recruit the AAA Vps4 via binding to the adaptor protein Vta1. Vps4 recycles the ESCRT-III subunits after intraluminal vesicles have been formed and may contribute to scission of nascent buds in an ATP-dependent manner. After release, ESCRT-III subunits became inactivated adopting the autoinhibited conformation.

above-mentioned Hrs and Vps27, through their P(S/T)XP sequences. The binding site for P(S/T)XP motifs does not overlap with the ubiquitin-binding site [18], so both ligands can bind simultaneously. A GPP (Gly-Pro-Pro)-based motif in the linker connecting the UEV and core regions of the Vps23 subunit of ESCRT-I binds to the midbody proteins CEP55 (centrosomal protein of 55 kDa) and targets ESCRT-I for membrane abscission in cytokinesis [19,20]. In yeast, the CTD (C-terminal domain) of Vps28 is responsible for binding to ESCRT-II [21] via NZF (Npl4 zinc finger) 1 in the N-terminus of the Vps36 of ESCRT-II [13]. The four-helix bundle domain has also been implicated in binding to ESCRT-III [22], although the interaction has not been characterized in detail. The current goal of recent structural analysis in our laboratory has been to integrate the collection of information described above.

The emerging shape of ESCRT-I is a nearly 25 nm long elongated structure, which consists of a headpiece attached to a 13 nm stalk [12] (Figure 2). A low-resolution model in solution was developed using separately solved crystal structures of the UEV [15] and Vps28-CTD [22], constraints from hydrodynamic measurements taken on full-length and deletion constructs of ESCRT-I [12]. Unlike EM or SAXS (small-angle X-ray scattering), hydrodynamic data provide no shape information. However, in spite of its limitations, hydrodynamic modelling has provided significant insights into cases of highly elongated structures where high-resolution structures of the components are known and are related by just a few degrees of freedom, such as ESCRT-I. The docked model of ESCRT-I with Vps27, ubiquitin and ESCRT-II places the stalk parallel to the plane of membrane, where the stalk is responsible for spatially organizing interactions on the membrane, yet does not contact the membrane directly. The rod-like structure disfavours a close contact between the ubiquitin- and ESCRT-II-binding sites, and suggests a cargo-clustering mechanism instead of a direct hand-off or ‘conveyor belt’ mechanism. This illustrates the power of integrated structural analysis to constrain likely models for the biological function of the ESCRTs.

ESCRT-II
ESCRT-II acts as a molecular hub for ESCRT assembly by connecting upstream cargo-binding components with the downstream putative membrane-remodelling machinery. ESCRT-II consists of one copy each of Vps22 and Vps36 and two copies of Vps25 [23–25]. The core of the ESCRT-II complex is a trilobal heterotetramer with a Y-shaped structure [23–25] (Figure 1C). Two Vps25 subunits form two lobes, and the tightly packed Vps22 and Vps36 form the third lobe. In all species, the N-terminus of Vps36 contains a phosphoinositide-binding GLUE [GRAM-like ubiquitin binding in EAP45 (ELL-associated protein 45)] domain [26,27]. ESCRT-II is targeted to endosomal membranes by the lipid-binding activities of both the Vps36 GLUE domain [26,27] and the H0 helix of Vps22 [25]. Yeast Vps36 has two Npl4-type zinc finger domains (NZF1 and NZF2) inserted in the GLUE domain. NZF1 binds to ESCRT-I as described above, whereas NZF2 of yeast Vps36 binds ubiquitin, and the interaction is essential for efficient cargo sorting in yeast [28]. The GLUE domain found in human Vps36 and other mammalian species lacks the NZF domain. Instead, ubiquitin...
binds directly to the GLUE domain [26,29,30]. Vps36 contains a conserved ESCRT-I-binding site in the linker between the GLUE domain and the core [25]. Both Vps22 and Vps36 subunits contribute to a dynamic helical domain that connects the GLUE domain to the rest of the ESCRT-II core [25]. A closed conformation of the intact ESCRT-II in solution was proposed on the basis of hydrodynamic studies [25], with the attendant limitations. The ESCRT-I- and membrane-binding sites are close to each other in the region of ESCRT-II where the GLUE domain, Vps22-H0 helix and core come together. On binding to the Vps28-CTD fragment of ESCRT-I, ESCRT-II precipitates, suggesting a conformational change occurs that exposes hydrophobic surfaces. The conformational dynamics of the quaternary ESCRT-I–ESCRT-II–membrane–ubiquitin interaction are a tantalizing issue that remains largely unexplored.

**ESCRT-III**

The ESCRT-III complex consists of six homologous proteins in yeast and 11 in humans. In contrast with the upstream soluble complexes, the subunits of ESCRT-III are not pre-assembled, but are located in the cytosol [31] as monomers in an autoinhibited conformation [32,33] (Figure 2). The crystal structure of a truncated version of human VPS24, where most of the C-terminus was deleted, has been solved [34]. The only other high-resolution structural information on ESCRT-III consists of short C-terminal fragments bound to MIT (microtubule-interacting and transport) domains of Vps4 [35,36] and to the Bro1 domain of ALIX [37]. SAXS profiles of two conformations of the full-length VPS24 protein have been obtained [38], consistent with the popular autoinhibited model in which the C-terminal helix closes with respect to the N-terminal core domain. Cryo-EM analysis of mammalian cells overexpressing human SNF7 revealed concentric filaments surrounding spherical membrane deformations [39]. This observation makes SNF7 a prime candidate for the membrane-deformation properties attributed to the ESCRTs, although there is currently no reason to think other ESCRT-III subunits might not be equally capable of producing such deformations. At a functional level, the connection, if any, between the observed membrane deformation and the putative bud-scission activity of the ESCRTs remains unclear. At a structural level, higher-resolution views of the ESCRT-III filaments allowing molecular analysis are eagerly awaited.

**Vps4–Vta1 (Vps20-associated 1)**

Vps4 is an AAA (ATPase associated with various cellular activities) whose deletion in yeast leads to profound trafficking defects, including formation of a class E compartment and accumulation of all ESCRT-III proteins on membranes. It functions in partnership with Vta1 to disassemble the ESCRT-III lattice. Vps4 is composed of an N-terminal MIT domain that binds to ESCRT-III C-termini as described above, a conserved AAA domain, a β domain and a C-terminal helix. The atomic structures of the MIT domain [35,36,40] and catalytic (ATPase plus β domain) region have been solved separately [41,42]. Vta1 contains two outlier MIT domains that bind to ESCRT-III [43,44]. The MIT domains are connected to the rod-like dimeric VSL (Vta1/SBP1/LIP5) domain [43] by an unstructured region. Vta1 stabilizes the Vps4 oligomer and promotes its association with ESCRT-III. Vps4 assembles into a large complex by stacking two hexameric or heptameric rings on top of each other, leaving a pore in the middle of the ring. Vps4 probably translocates its ESCRT III substrates in an ATP-dependent manner through the central pore of the complex to release them from the membrane [41]. Exciting progress has been made in integrating the MIT and catalytic domain information in cryo-EM analysis of the assembled Vps4 complex [45,46] and Vps4–Vta1 complex [46]. Important questions remain about the 12-fold oligomerization state of the assembly, asymmetry between the two rings and, above all, the precise mechanism of the ESCRT-III disassembly reaction.

**Bro1/Alix [ALG-2 (apoptosis-linked gene 2)-interacting protein X]**

Alix (Bro1 in yeast) is a multifunctional adaptor protein that is intimately involved in the ESCRT pathway. Alix is composed of an N-terminal Bro1 domain, a central V domain and a C-terminal PRD (proline-rich domain) (Figure 3). The Bro1 domain has a smoothly curved convex face, which might bind to negatively curved membranes [47]. The concave surface on the opposite side of the Bro1 domain binds to the ESCRT-III subunit Snf7 [CHMP4 (charged multivesicular body protein 4) in humans] [37,47]. The V domain is named for its shape [48] and is the locus for binding the LYPXL late motif of HIV-1 Gag [48–50]. The two domains are connected by a very short linker, which has made it possible to obtain an atomic structure of the nearly complete Alix protein, lacking only the PRD [48]. The structure and interactions of the PRD, both intra- and inter-molecular, are the last structural frontier in what is now a very well characterized system.

**Concluding remarks**

Impressive progress has been made in characterizing the core structural elements of essentially all of the ESCRT machinery on a piecemeal basis by X-ray crystallography and solution...
NMR. There is now a great deal of interest and energy going into understanding higher-order structure using a range of experimental and theoretical techniques in order to integrate the high-resolution, but fragmentary, information currently available. The ultimate goal would be to have a ‘hybrid’ structure of the ESCRT system in the appropriate conformation at each step in its reaction cycle. This still sounds like science fiction and is undoubtedly years away from being realized. Rapid progress in just the last year in EM studies of Vps4 and ESCRT-III assemblies, SAXS analysis of ESCRT-III monomers, and hydrodynamic analysis and Monte Carlo simulations of Vps27–Hse1, ESCRT-I and ESCRT-II offer hope for some exciting years to come.

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References


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