

# The ESCRT Complexes: Structure and Mechanism of a Membrane-Trafficking Network\*

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## Key Words

ubiquitin, sorting, endocytosis, lysosomes

## Abstract

The ESCRT complexes and associated proteins comprise a major pathway for the lysosomal degradation of transmembrane proteins and are critical for receptor downregulation, budding of the HIV virus, and other normal and pathological cell processes. The ESCRT system is conserved from yeast to humans. The ESCRT complexes form a network that recruits monoubiquitinated proteins and drives their internalization into luminal vesicles within a type of endosome known as a multivesicular body. The structures and interactions of many of the components have been determined over the past three years, revealing mechanisms for membrane and cargo recruitment and for complex assembly.

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**Proteasome:** large multiprotein complex that proteolyzes polyubiquitinated proteins, typically soluble proteins or membrane proteins that are substrates of ER-associated degradation

**Ubiquitin (Ub):** a highly conserved 76-amino-acid protein that can be covalently attached to itself or other proteins through an isopeptide bond with its terminal carboxylate

## INTRODUCTION

Targeted degradation is a fundamental mechanism of protein regulation and quality control (39, 44, 80). Soluble proteins are marked for degradation by the proteasome by their modification with polymeric chains of ubiquitin (Ub), a highly conserved 76-amino-acid protein. These polyubiquitin chains are added by a series of enzymes known as E1, E2, and E3. Polyubiquitin chains target proteins so modified to the regulatory subunit of the proteasome and thence to degradation by the catalytic subunit. This entire process is tightly regulated and highly specific, as errors can lead to inappropriate degradation or failure to degrade appropriate substrates resulting in severe consequences for the cell.

A strikingly different yet equally elaborate process governs the degradation of many,

if not most, transmembrane proteins. These proteins are covalently modified by a single Ub moiety, as opposed to a polyubiquitin chain (36, 40, 54, 87, 98). Monoubiquitinated membrane proteins are recognized by a series of receptors that contain specific monoubiquitin-binding domains. These receptors target monoubiquitinated membrane proteins through a series of trafficking steps that ultimately deliver them to their destruction in the lysosome. Unlike the proteasome, the lysosome is a membrane-delimited organelle. Lysosomal proteases and lipases efficiently degrade small internal vesicles loaded with membrane proteins. This delivery system has other functions in addition to protein degradation. Many lysosomal and vacuolar hydrolases arrive via this pathway, and the pathway also is used for antigen presentation. Much attention has focused on the pathway because it is coopted by HIV and other enveloped retroviruses in order to bud from cells (34, 66, 91, 101, 111).

## The MVB Sorting Pathway

The Ub-dependent downregulation of activated signaling receptors at the lysosome requires sorting of the receptors at the endosome into a unique class of vesicles that invaginate into the interior of the endosome. The endosomal compartment containing these vesicles is called the multivesicular body (MVB). Pioneering electron microscopy studies showed that ferritin-conjugated epidermal growth factor (EGF) bound to the EGF receptor is sorted into the luminal vesicles of MVBs en route to the lysosome (35, 37). Fusion of the MVB with the lysosomal membrane results in delivery of the luminal MVB vesicles and their contents into the lysosome, where the vesicles and the transmembrane receptor are degraded. This unique mechanism enables cells to degrade entire transmembrane proteins as well as lipids in the MVB vesicles. Membrane proteins that are excluded from the inner MVB vesicles remain within the limiting membrane of the

MVB. Following fusion with the lysosome, these proteins are transferred to the limiting membrane of the lysosome. Recent studies have demonstrated that Ub serves as a signal for efficient sorting into the MVB transport pathway (6, 53, 88, 108).

Studies in mammalian cells have revealed critical roles for MVBs in such seemingly distinct processes as growth factor receptor downregulation, antigen presentation, and retroviral budding. However, the simple yeast *Saccharomyces cerevisiae* has served as an important model system for the discovery of the molecular machinery essential for MVB sorting (53). An unexpectedly large number of protein complexes have been identified that directly bind to Ub-modified cargo and also appear to direct the complex process of receptor sorting and MVB vesicle formation (see **Table 1**). The conservation of these components in other organisms including humans highlights the importance of this transport route in all eukaryotic cells.

## Class E Vps Proteins

Genetic studies in yeast have identified more than 60 gene products involved in vacuolar protein sorting (Vps). These genes encode transport components that function at distinct stages of protein traffic between the Golgi complex and the vacuole. A subset of the Vps proteins, the class E Vps proteins, functions in the MVB sorting pathway (17, 53, 76). At present, 17 class E *VPS* genes have been identified (**Table 1**) (**Figure 1**). Class E *vps* mutants accumulate endosomal membranes and exhibit defects in the formation of MVB vesicles. The characterization of these proteins has resulted in the identification of three high-molecular-weight cytoplasmic protein complexes that function in the MVB sorting pathway. These complexes are called the ESCRT (endosomal sorting complex required for transport) complexes I, II, and III (41, 54, 73). The ESCRT machinery is required for the formation of MVBs. This process occurs at the late endosomal compartment,

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**Endosome:** an intracellular vesicle involved in transfer of proteins and lipids between different organelles

**Multivesicular body (MVB):** an endosome containing internal vesicles

**Vps:** vacuolar protein sorting

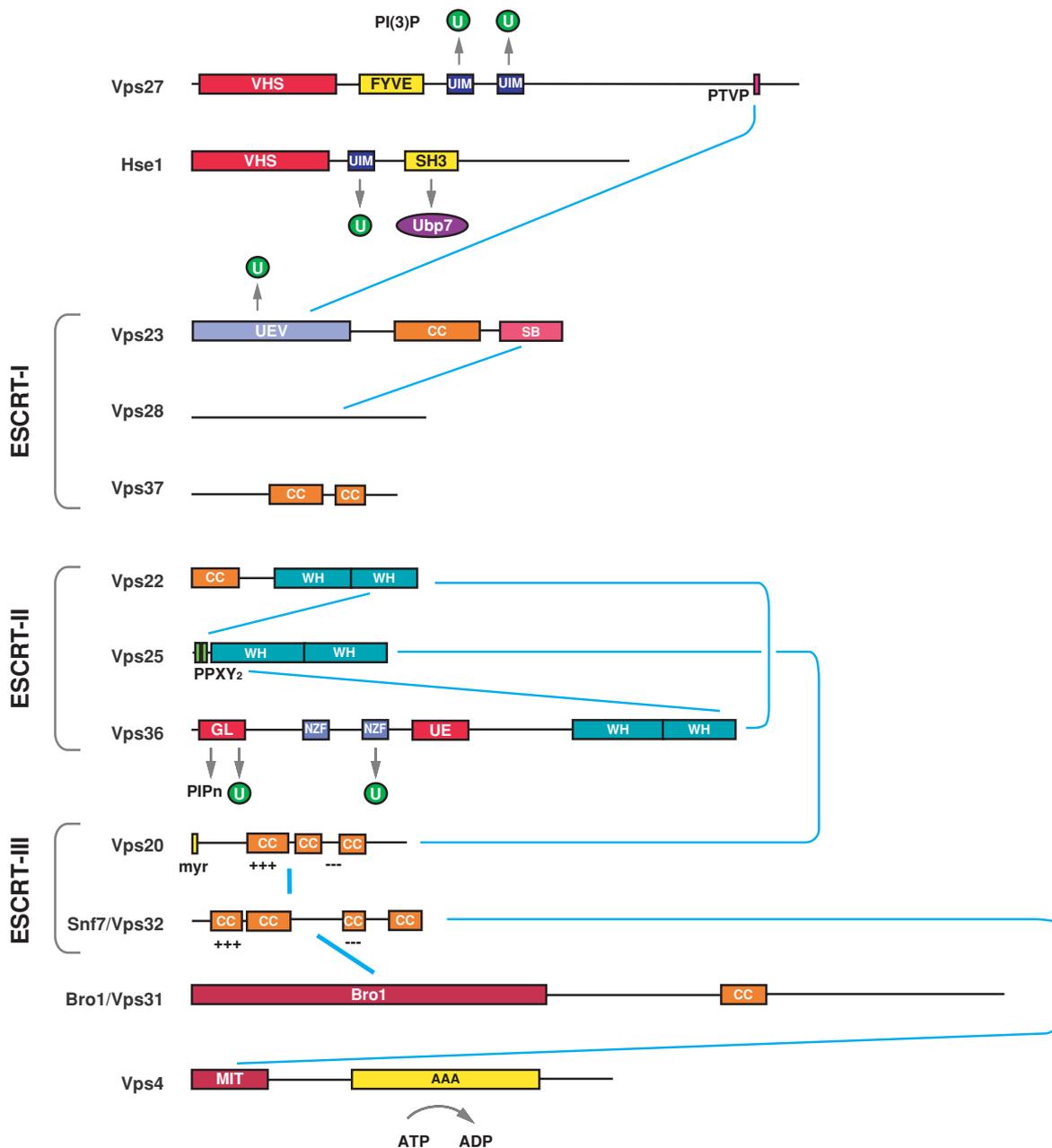
**Class E VPS:** phenotype manifesting alterations in multivesicular bodies

**ESCRT:** endosomal sorting complex required for transport

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**Table 1** Class E Vps proteins and complexes

Complex	Yeast protein	Human protein	Motifs	Binds to
Vps27-Hse1 complex	Vps27 Hse1	HRS STAM1, STAM2	UIM, FYVE, VHS UIM, VHS, SH3	Ubiquitin, PI(3)P, ESCRT-II (Vps23) Ubiquitin
ESCRT-I complex	Vps23 Vps28 Vps37	TSG101 VPS28 VPS37A, B, C, D	UEV  Coiled-coil	Ubiquitin, Vps27
ESCRT-II complex	Vps22 Vps25 Vps36	EAP30 EAP25 EAP45	Coiled-coil, WH PPXY, WH GLUE, NZF, WH	ESCRT-III (Vps20) Ubiquitin
ESCRT-III complex	Vps2/Did4 Vps20 Vps24 Snf7/Vps32	CHMP2A, B CHMP6 CHMP3 CHMP4A, B, C	Charged, coiled-coil Charged, coiled-coil Charged, coiled-coil Charged, coiled-coil	ESCRT-II (Vps25)
Vps4 complex	Vps4	VPS4A, B	AAA ATPase, MIT	ESCRT-III
Other MVB proteins	Bro1/Vps31 Vps60/Mos10 Fti1/Did2 Vta1 Vps44/Nhx1	ALIX/AIP1 CHMP5 CHMP1A, B LIP5 SLC9A6	Bro1 Charged, coiled-coil Charged, coiled-coil  Sodium/proton exchanger	LBPA, Doa4, ESCRT-III ESCRT-III ESCRT-III Vps4



**Figure 1**

Domain structure and interactions in the ESCRT network. Protein-protein interactions within the network are indicated by solid blue lines. Interactions with lipids, ubiquitin moieties, and other proteins are shown with gray arrows. For simplicity, only two of the ESCRT-III subunits, Vps20 and Snf7, are shown. The GLUE domain of human Vps36 binds to PIP<sub>3</sub>; the lipid specificity of the GLUE domain of yeast Vps36 has not been characterized.

where the limiting membrane invaginates and buds small vesicles into its lumen, giving rise to the characteristic morphology of numerous intraluminal vesicles within a larger membrane-enclosed endosome (28, 31, 76).

The yeast class E protein Vps27 and its mammalian homologue (yeast nomenclature is used throughout for simplicity; see **Table 1**) are required for protein sorting in the MVB pathway. Vps27 forms a complex with the Hse1 protein in the cytoplasm (5, 10, 12, 57, 84) and binds directly to monoubiquitinated cargo (10, 12, 84). ESCRT-I (Vps23, Vps28, and Vps37) also interacts with ubiquitinated cargo (14, 32, 53). Genetic studies indicate that ESCRT-II (Vps22, Vps25, and Vps36) acts downstream of ESCRT-I; however, the mechanism of this interaction is not yet known (6). ESCRT-III is composed of two major functional subcomplexes (Vps20/Snf7 and Vps2/Vps24 in yeast) that localize to endosomal membranes. ESCRT-III components fail to localize to endosomes in cells lacking ESCRT-II, suggesting a role for ESCRT-II in the recruitment and assembly of ESCRT-III at the endosome (6). Together, the ESCRT complexes appear to function in both cargo sorting and MVB vesicle formation. The genetic and biochemical data argue for an ordered reaction sequence; Vps27 recruits ESCRT-I, which in turn recruits ESCRT-II, which then recruits ESCRT-III to the endosome.

In addition to the complexes described above, there are several other important players. Vps4 is an AAA-type ATPase (8, 9) that plays a critical role in the MVB sorting pathway by catalyzing the dissociation of all three ESCRT complexes from the endosome (6, 9, 53). Inactivation of Vps4 results in the accumulation of the ESCRT machinery on the surface of the endosome (class E compartment). Purified Vps4 assembles into a homooligomer when loaded with ATP and is recruited to the endosome via the ESCRT-III complex (6, 9). The enzyme Doa4 (2, 6) deu-

biquitinates MVB cargoes prior to sorting into MVB vesicles and also is recruited to the ESCRT-III complex. Doa4 is recruited via an interaction with Bro1 (56, 63, 77), another class E Vps protein (Vps31).

## THE VPS27/HSE1 COMPLEX

The Vps27/Hse1 complex binds Ub via ubiquitin-interacting motifs (UIMs) in both subunits (12, 14, 52, 72, 81, 84, 97). Vps27 possesses a FYVE domain (for Fab1, YGL023, Vps27, and EEA1) that binds to the endosomal lipid phosphatidylinositol 3-phosphate (PI(3)P) (20, 33, 69, 78, 90, 100). Endosomal PI(3)P recruits the Vps27/Hse1 complex to the endosome. Vps27 is a docking site for the ESCRT-I complex and thereby initiates the MVB sorting reaction at the limiting membrane of the endosome. ESCRT-I physically interacts with membrane-bound Vps27/HRS through a motif in the COOH-terminal portion of Vps27 (4, 10, 13, 23, 55, 83). Therefore, the Vps27 protein appears to direct the compartment-specific activation of MVB sorting, and Vps27 function is regulated by specific interactions with both PI(3)P and ubiquitinated cargo at the late endosome. Hse1 and STAM (signal transducing adaptor molecule) contain an src homology-3 (SH3) domain. Vps27, Hse1, and their mammalian homologues contain predicted helical regions that are essential for formation of the complex (86).

The extreme C terminus of Vps27 contains the sequence LIEL, which binds to a groove in the clathrin N-terminal  $\beta$ -propeller domain (106). The human homologue of Vps27 also binds to clathrin via this motif (85). Planar clathrin lattices have been seen on endosomes containing Hrs (89). Clathrin may serve to cluster Vps27 and cargo at sites that will later invaginate to form the MVB vesicles. The human homologues of Vps27 and Hse1 are phosphorylated on Tyr residues, but the functional significance of this is unknown (57).

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**AAA:** ATPases associated with diverse cellular activities

**UIM:** ubiquitin-interacting motif

**PI(3)P:** phosphatidylinositol 3-phosphate

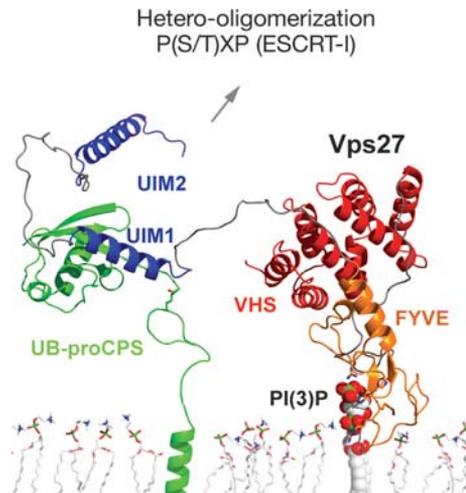
**FYVE:** Fab1, YOTB, Vac1, EEA1

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## Vps27 FYVE Domain and Membrane Localization

The FYVE domain that is responsible for localizing the Vps27/Hse1 complex is a compact 60-amino-acid  $Zn^{2+}$ -finger that selectively recognizes PI(3)P in preference to all other phosphoinositides. The crystal structure of the Vps27 FYVE domain (69) showed how such a small domain could selectively bind membrane-embedded PI(3)P (Figure 2). A basic RHHCR motif on the first  $\beta$ -strand provides all but one of the basic ligands for the acidic PI(3)P headgroup, as shown by the structure of the complex of the EEA1 FYVE domain with inositol (1,3)-bisphosphate [Ins(1,3)P<sub>2</sub>] (27, 70). The His residues with their relatively short side chains are proximal to the 3-phosphate group, and the Arg residues are more distal, allowing room for their longer side chains to reach the headgroup.

The FYVE domain, like most other membrane-lipid targeting domains (25, 46, 47, 61), is anchored to cell membranes by a combination of specific lipid binding and by less specific electrostatic and hydrophobic forces (26, 27, 58–60, 69). The Vps27 FYVE domain has low affinity ( $K_d = 90 \mu M$ ) for the soluble inositol (1,3)-bisphosphate, which corresponds to the headgroup of PI(3)P (15). The affinity rises to  $K_d = 30 nM$  when PI(3)P is presented in a realistic model of an endosomal membrane (15). This 3000-fold gain in affinity is explained by a hydrophobic protrusion (turret loop) of the FYVE domain that inserts into the hydrophobic core of the membrane. The depth and angle of membrane penetration dictate how the FYVE domain will be oriented relative to the bilayer and what other surfaces are available for interactions. These parameters have been analyzed by spectroscopy (59), computational simulations (26), and by structure-based modeling (27, 64, 69). While there is no consensus model for a single membrane-binding geometry for all FYVE domains, a consistent general picture has emerged for the best-studied



**Figure 2**

Structure of Vps27. Structures are shown where available for Vps27 proteins [FYVE (69), UIM1-ubiquitin complex (103), UIM1-UIM2 (103)], otherwise modeled on the basis of the closely related structure of the Hrs-VHS domain (64). Linker regions between the domains were generated arbitrarily and are shown only to indicate the length of the segment. The C-terminal putative Hse1-binding domain and the extended region of Vps27 are not shown. Ubiquitin is shown fused via an isopeptide bond between Gly-76 and Lys-8 of the prototypical cargo pro-carboxypeptidase S, and the transmembrane helix of pro-CPS was modeled as an ideal helix. Membrane docking of Vps27 is based on the computationally predicted optimal docking mode for the FYVE domain.

FYVE domains, including that of Vps27. For all PI(3)P-binding FYVE domains, the turret loop is buried in the membrane. The Vps27 FYVE domain is best described as binding PI(3)P in a “side-on” orientation (26, 69), while the EEA1 FYVE domain is tilted with its PI(3)P binding face close to the membrane in a partially “face-on” manner (26, 27). Modeling suggests that differences in the distribution of charged residues on the FYVE domain surface control differences in membrane docking. Given their variability, it seems likely that FYVE domains can wobble in situ and are not likely to be rigidly constrained by membrane forces alone. Such

flexibility might make FYVE domain proteins well adapted to function in dynamic protein networks, in which complexes are rapidly formed and broken down and the context of the domain's interactions is subject to constant change.

### VHS Domains in the Vps27/Hse1 Complex

Both subunits of the complex have N-terminal Vps27/Hrs/STAM (VHS) domains whose function is intriguing but still unknown. VHS domains are octahelical bundles (64, 68, 71, 95, 118) that are present only at the extreme N termini of the proteins that contain them. Although the VHS domains of the GGA trafficking adaptors bind directly to acidic cluster-dileucine motifs in cargo proteins in a groove between two helices of the VHS domain (68, 71, 95, 118), critical residues in this groove are altered in the VHS domains of the Vps27/Hse1 complex, and the ligands for these VHS domains are unknown. Some nonubiquitinated G-protein-coupled receptors are trafficked by the human homologue of Vps27 (43), and its VHS domain is one candidate for recognizing such cargo (38).

### UIMs and Recognition of Ubiquitinated Cargo

UIMs are short helical motifs first identified on the basis of homology to the Ub-recognition sequence in the proteasome subunit S5a (45). Hse1 has one UIM, and Vps27 has two. These UIMs bind monoubiquitin with low affinity, with  $K_d$  values in the range of 200  $\mu$ M to 2 mM (30). The second UIM of Vps27 has a  $\sim$ 10-fold-lower affinity for Ub than the first UIM (30). The structure of the second Vps27 UIM has been determined alone (30), showing that the UIM comprises a single helix. The structure of a Vps27 tandem UIM construct has been determined in complex with Ub by NMR (103). Only the first UIM (UIM-1) was found to participate

in complex formation in the structure, consistent with its higher affinity. The second UIM was found to flop about freely in solution. The 28-residue linker between the two UIMs is completely disordered, and the two UIMs appear to sample all accessible conformational space relative to each other.

The Vps27 UIM-1 binds to the Leu-8, Ile-44, Val-70 hydrophobic patch on the Ub surface. This is the same surface that is recognized by all other Ub-binding domains characterized to date. The UIM-1 contains a single six-residue hydrophobic strip that interacts with Ub. The signature C-terminal Ser of the UIM forms a hydrogen bond with the main chain of Ub, and conserved N-terminal Glu residues form salt bridges with Ub Arg-42 and Arg-72. The 400  $\text{\AA}^2$  surface area buried in the complex is smaller than that seen in most protein-protein complexes but is typical of what we have come to expect for low-affinity Ub-binding domain complexes.

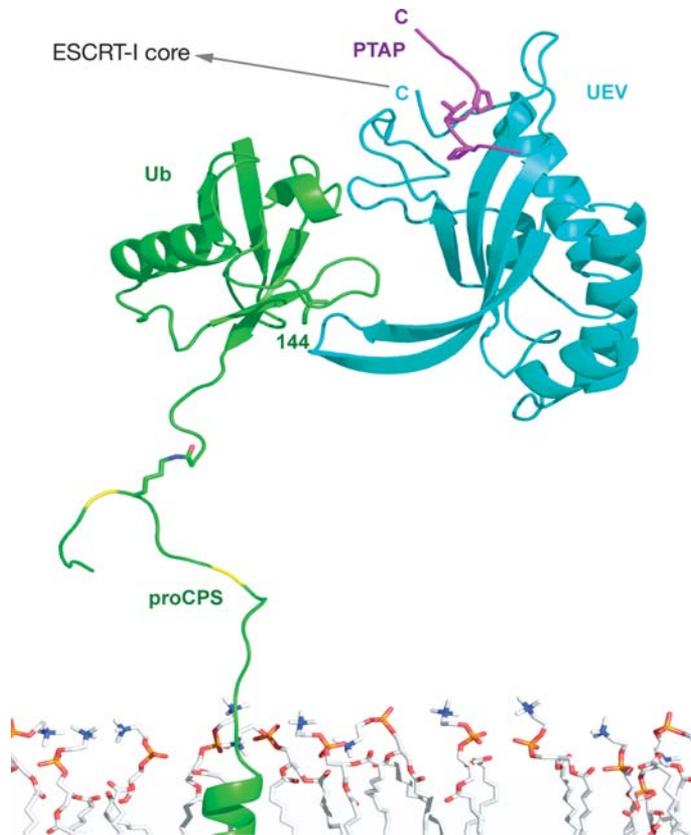
### SH3 Domain of Hse1 and STAM

The SH3 domain of STAM recruits the deubiquitinating enzyme ubiquitin isopeptidase Y (UBPY) (49). The biological rationale for a deubiquitination event at an early stage of entry into the ESCRT pathway is unclear, as the ESCRT proteins interact with ubiquitinated cargo. It seems possible that the STAM/UBPY interaction functions as an off switch to direct cargo out of the pathway or to inactivate components of the ESCRT machinery that are ubiquitinated. The SH3 domain of STAM recognizes a noncanonical SH3-binding motif within UBPY of the form PX(V/I)(D/N)RXXKP (48) with a relatively low affinity of 27  $\mu$ M, and the structure of this complex has been determined. The SH3 domain of Hse1 has been proposed to recruit Ubp7 on the basis of a large-scale proteomic study of yeast SH3 domains (107), although Ubp7 does not contain the PX(V/I)(D/N)RXXKP motif, and the interaction has yet to be confirmed.

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**VHS:** Vps27, Hrs, STAM

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**Figure 3**

Structure of ESCRT-I. Structures are shown for the UEV domain of human Vps23 in complex with ubiquitin (102) and the HIV-1 p6 PTAP-containing peptide (82). The remainder of the ESCRT-I structure is not yet available.

### THE ESCRT-I COMPLEX

The ESCRT-I complex binds directly to monoubiquitinated protein cargo through its UEV domain, a catalytically inactive variant of an Ub-conjugating enzyme (53). ESCRT-I interacts with Vps27 and a number of other cellular proteins, including the mammalian counterpart of Bro1 (101, 111) and the Ub ligase Tal, (3) via their P(S/T)XP sequences, as described above. Intense interest has centered on this motif since the discovery that HIV and certain other enveloped retroviruses contain this motif in their envelope proteins and use it to hijack the MVB sorting machinery to bud from host cells (24, 32, 65, 74, 109).

These P(S/T)XP sequences also bind to the ubiquitin E2 variant (UEV) domain. Tsg101 and Vps23 contain a C-terminal “steadiness box” (29) that is important for stability *in vivo*. The functions of the other domains and motifs within the ESCRT-I complex have yet to be worked out.

### The UEV Domain of Vps23

The cargo recruitment end of the ESCRT-I complex is the UEV domain, which is responsible for binding both to monoubiquitin moieties and to P(S/T)XP motifs (Figure 3). The structures of the UEV domains of Vps23 (105) and Tsg101 (102) have been determined in complex with Ub. In both structures, two different regions of the UEV domains contact Ub. The  $\beta 1$ - $\beta 2$  “tongue” contacts the Ile-44 hydrophobic patch of Ub, the same region involved in contacts with the Vps27 UIM and with other monoubiquitin binding domains. The loop between the  $\alpha 3$  and  $\alpha 3'$  helices forms a “lip” that contacts a hydrophilic site centered on Gln-62 of Ub (102, 105). In contrast to the Ile-44 site, the Gln-62 site does not participate in most other known Ub/Ub-binding domain interactions. Even though the UEV domain was discovered as a catalytically inactive homolog of Ub-conjugating enzyme, Ub binds to the UEV domain in a completely different manner.

The structure of the Tsg101 UEV domain in complex with the PTAP peptide of HIV-1 p6 shows how P(S/T)XP sequences are recognized by the ESCRT-I complex (82). The second Pro of the P(S/T)XP is a particularly critical element. The XP sequence, together with the first C-terminal flanking residues, forms one turn of a type II polyproline helix. This conformation is also seen in canonical SH3 and WW (tryptophan-tryptophan) domain complexes with Pro-based peptide motifs (117). The UEV domain recognizes the second Pro by using an aromatic pocket reminiscent of Pro-recognition pockets in SH3 and WW domains. The first Pro is in an extended conformation and binds in a shallow

**UEV:** ubiquitin E2 variant

pocket. The Thr hydroxyl appears to hydrogen bond to both main chain and side chain residues, although this was not fully defined in the NMR structure. The concept of using peptidomimetics directed at this site to block HIV release has attracted considerable interest.

## THE ESCRT-II COMPLEX

ESCRT-II forms a nexus between ubiquitinated cargo, the endosomal membrane, and the ESCRT-I and -III complexes (7). Its structural organization is currently the best understood of the three ESCRT complexes (Figure 4). The complex contains two Vps25 subunits, one Vps22 subunit, and one Vps36 subunit (42, 104). The N-terminal two thirds of Vps36 contain a series of domains [GLUE (99) and NZF (1)] that interact with membranes and cargo and perhaps have other func-

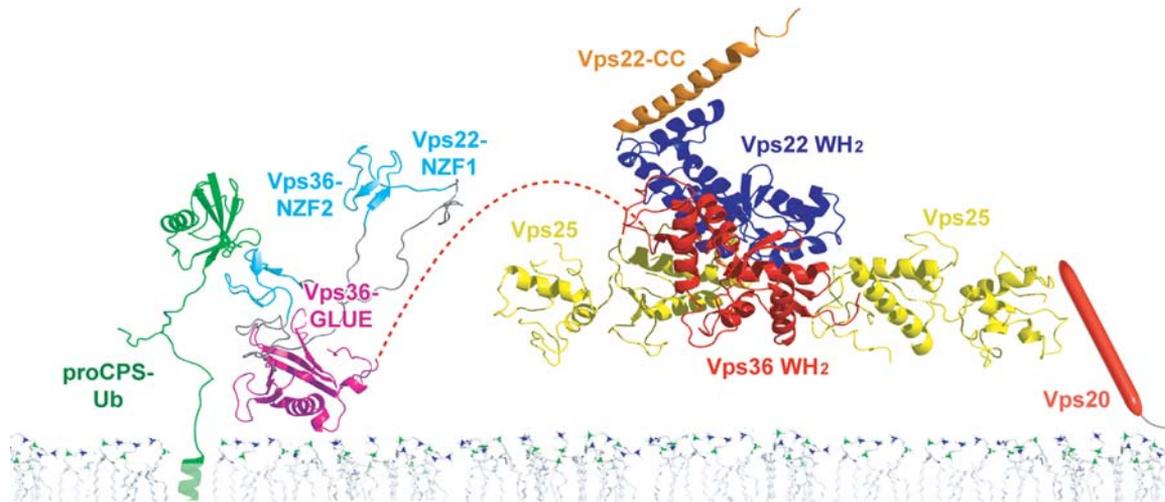
tions. Vps22, Vps25, and the C-terminal third of Vps36 form a tightly organized core that contains two binding sites for the ESCRT-III subunit Vps20 (104).

## The ESCRT-II Core

The ESCRT-II core is Y-shaped, with one Vps25 subunit forming the stalk. One of the branches is formed by the second Vps25 subunit, and the other branch is formed by the subcomplex consisting of Vps22 and the C-terminal third of Vps36 (42, 104). Although the three subunits do not have primary sequence homology to each other, each subunit consists of two repeats of a winged helix (WH) domain. WH domains are typically found in DNA-binding proteins, where the wing (a loop between two of the  $\beta$ -strands) contacts the nucleic acid. Vps22 and Vps36 form a tightly bound subcomplex. The second WH

**NZF:** Npl4 zinc finger

**WH:** winged helix



**Figure 4**

Structure of ESCRT-II. The structure of the ESCRT-II core (42) is shown docked to a membrane on the basis of the interaction between its Vps25 subunit and the membrane-bound ESCRT-III subunit Vps20 (104) (myristoyl group on Vps20 not shown for simplicity). The uncomplexed NZF1 domain and the NZF2-ubiquitin complex are modeled on the basis of the Npl4-NZF structure and ubiquitin complex (1). The GLUE domain (a variant of the GRAM domain, which is in turn a variant of the PH domain) is modeled and docked to the membrane on the basis of the GRAM domain of the lipid phosphatase MTMR2 (11). The GLUE domain of human Vps36 binds to  $PI(3,4,5)P_3$  and ubiquitin at sites that have yet to be determined (99), and are not shown. The binding properties of the yeast Vps36 GLUE domain have yet to be reported. The dashed line between the GLUE domain and Vps36 core winged helix (WH) region indicates residues 290–395 of Vps36, whose structure is unknown.

domains (WH2) of Vps22 and Vps36 bind to the Vps25 subunits through closely equivalent interactions. Vps22 and Vps36 present an aromatic cage to the Vps25 subunit. The N terminus of Vps25 contains two repeats of the sequence PPXY. Sequences of this type are better known for binding to WW domains (a polyproline-rich peptide motif binding domain that is unrelated to the WH domain, despite the similar abbreviation) in a conformation in which the Tyr side chain is exposed and presented to the WW domain. In Vps25, the Tyr side chain is buried, and the diPro sequence interacts with the aromatic cages in the WH2 motifs of Vps22 and Vps36. The Tyr of the PPXY motif of Vps25 is buried even in the isolated subunit unbound to the rest of the complex (113), suggesting it is unlikely to become exposed in a conformation available for WW domain binding. All of the intersubunit contacts are required for the complex to mediate carboxypeptidase S sorting in yeast (42).

### Vps36 NZF Domains

Yeast Vps36, but not its human counterpart, contains two Npl4 zinc finger (NZF) motifs in the region N-terminal to the core WH domains. The Npl4 NZF domain was first shown to bind Ub, and this interaction has been characterized structurally (1). The NZF domain binds to the same Ile-44 patch on Ub as do the UIM and UEV domains. Thus, none of these domains can bind to Ub simultaneously. The second NZF domain of Vps36 binds to Ub, and the Ub-binding site on the NZF domain is important for the sorting function of Vps36. The function of the first NZF domain is unknown.

### Vps36 GLUE Domain

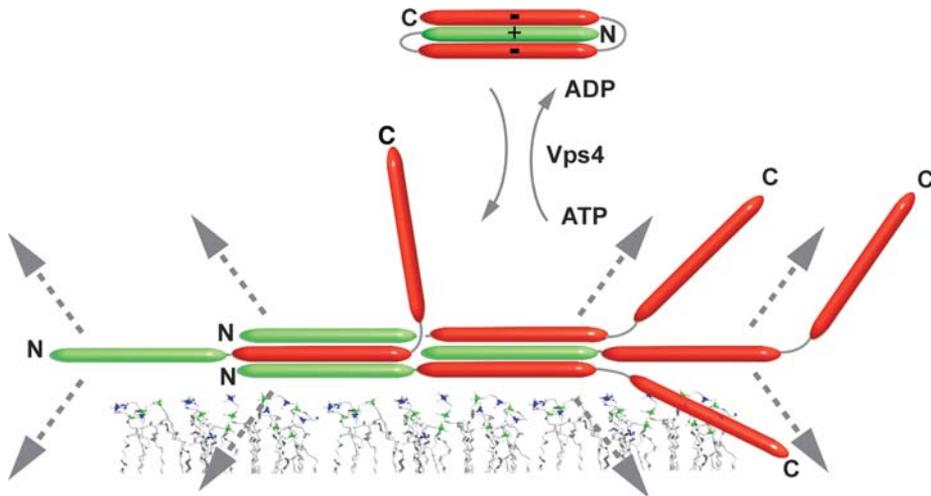
The absence of NZF or other known Ub-binding domains in human Vps36 and the other human ESCRT-II subunits led to a puzzle: How could the human ESCRT-II complex function in sorting ubiquitinated

cargo without a Ub-binding domain? This seeming paradox was resolved with the discovery of the GLUE (Gram-like ubiquitin) domain near the N terminus of the human protein (99). Vps36 also contains a GLUE domain, which is split into two segments by the first NZF domain. GLUE domains bind not only Ub but also the lipid phosphatidylinositol (3,4,5)-triphosphate (99). The structure of the GLUE domain has not been determined, but on the basis of distant sequence similarity it appears to be similar to the phospholipid-binding pleckstrin homology domain.

## THE ESCRT-III COMPLEX

The ESCRT-III complex consists of several subunits that are homologous to each other, highly charged, and contain predicted coiled-coil regions (6). Four subunits, Vps2, Vps20, Vps24, and Snf7, are essential for sorting function in yeast (6) (**Figure 4**). Two other proteins, Vps60 and Did2, share homology to the other ESCRT-III subunits but are less critical for function (6). Isolated ESCRT-III subunits are cytoplasmic in yeast (6) and soluble, at least at low concentrations, in vitro (56, 62). One of the subunits, Vps20, is myristoylated, but in isolation is nevertheless cytosolic. Thus the myristoyl group alone seems insufficient to drive it to the membrane. Upon association, the subunits form a large and tightly membrane-bound assembly of indeterminate stoichiometry (**Figure 5**). It is currently thought that this assembly comprises an oligomer of ESCRT-III complexes on the membrane surface.

The major link between the ESCRT-III complex and upstream complexes occurs via ESCRT-II in yeast (16). This interaction is conserved in humans (111). However, human Bro1 bridges Vps23 of ESCRT-I with Snf7 of ESCRT-III and provides a second link. The Vps20 subunit of ESCRT-III (16, 111) binds directly to Vps25 of yeast and human ESCRT-II (104, 116). The Vps2/Vps24 subcomplex of ESCRT-III recruits Vps4 in yeast (6). However, Snf7 and other ESCRT-III



**Figure 5**

Structure of ESCRT-III. The structure of ESCRT-III is unknown. This cartoon shows a tripartite variant of the bipartite model proposed by Hanson and colleagues (62). The C-terminal anionic region of ESCRT-III subunits is roughly twice as large as the N-terminal basic region and appears to have functions both in oligomerization of ESCRT-III on membranes and in binding to other proteins, such as Vps4. Gray arrows indicate directions in which the oligomeric array can grow.

proteins can also bind to Vps4 (16, 111), suggesting the ESCRT-III/Vps4 interaction is not stringently specific. Vps60 binds to the class E Vps protein Vta1 in yeast (16, 96) and humans (112). Human Vps24 has been proposed to bind to PI(3,5)P<sub>2</sub> (114). In summary, all ESCRT-III subunits have a conserved primary structure and interact with each other in a similar manner, whereas the individual subunits retain specific interactions of their own.

The N-terminal one third or so of ESCRT-III subunits are highly basic, whereas the C-terminal two thirds are acidic. Both regions contain predicted coiled-coils. The simple model for the monomeric form of the subunit is that the basic and acidic regions form an antiparallel coiled-coil pair with each other, stabilized by electrostatic interactions between the two halves. Experimental evidence for this model comes from a comparative analysis of human Snf7 and Vps24 and their fragments (62). The basic N-terminal regions bind tightly to membranes even in isolation (62). The C-terminal regions, in contrast, bind to human Vps4 (62, 94). There is

also evidence that the N-terminal basic regions can interact not only with membranes but also with other proteins (116). These data suggest that monomeric ESCRT-III subunits are in a closed conformation in which the N- and C-terminal portions are tightly associated with each other and are not available for interactions with membranes or other proteins.

What initiates formation of the insoluble ESCRT-III complex from its soluble subunits? In a working model, interactions with other proteins compete with the intraprotein interaction between the N- and C-terminal regions of ESCRT-III subunits and thereby liberate the N-terminal portion for interactions with membranes. This triggers the membrane localization of the subunit. In a working model, the membrane-bound form of the ESCRT-III subunit is in a more open conformation, more available for interactions with other subunits, and thus disposed to form a polymeric assembly on the membrane (**Figure 5**). The formation of the ESCRT-III complex is independent of Vps4 and ATP (6), and ESCRT-III-like aggregates of

isolated recombinant subunits have been observed to form spontaneously (62). In vivo formation of the ESCRT-III complex likely follows the binding of isolated ESCRT-III subunits to ESCRT-II, Bro1, or other factors that allosterically promote the complexation-competent conformation. These considerations point to factors such as ESCRT-II and Bro1 as the likely initiators of ESCRT-III complexation.

The membrane association of ESCRT-III is essentially irreversible in the absence of an energy input: Vps4 must hydrolyze ATP to solubilize the membrane-bound ESCRT-III assembly, once formed. In this respect, ESCRT-III proteins are analogous to the SNAREs of membrane fusion, which must be separated by the action of *N*-ethylmaleimide sensitive factor (NSF), once tight complexes have been formed. In contrast to ESCRT-III subunits, which cycle on and off membranes, SNAREs contain transmembrane regions and are permanently tethered to membranes (18).

## BRO1

Bro1 is a monomeric protein that is intimately associated with the ESCRT complexes (Figure 5). Bro1 is involved in deubiquitination of the general amino acid permease Gap1 (75) and is required for trafficking of carboxypeptidase S via the MVB pathway (77). Bro1 recruits Doa4, which deubiquitinates MVB cargo proteins (63). Bro1 is recruited to MVBs through its interactions with the ESCRT-III subunit Snf7, also known as Vps32 (16, 77).

The human homolog of Bro1 interacts with the human counterpart of Snf7 (50, 51, 101, 111); hence this key interaction is conserved from yeast to human. It contains a C-terminal Pro-rich region that interacts with the endocytic proteins SETA (22, 92), endophilins (21), and the human Vps23 subunit of ESCRT-I (101, 111). In contrast, Bro1 lacks a P(S/T)XP sequence and does not interact strongly with Vps23 (16). The human

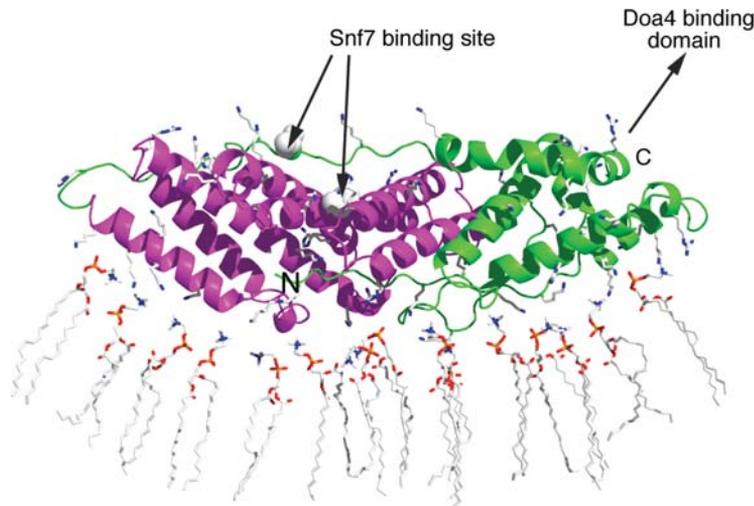
homologue of Bro1 is a key player in retroviral budding, because it interacts with HIV-1 and other retroviral proteins containing the sequence motif YPXL (34, 66, 101, 111). Little is known about the function of YPXL motif host proteins that bind to Bro1 homologues, although one protein, PacC, in *Aspergillus* has been described (110).

## Bro1 Domain

Bro1 and several other late endosomal proteins share a conserved N-terminal Bro1 domain. The Bro1 domain consists of roughly 370 residues and has a complex structure that is built around a core helical solenoid similar to tetratricopeptide repeat domains (56) (Figure 6). The Bro1 domain is necessary and sufficient for binding to the ESCRT-III subunit Snf7 and for the recruitment of Bro1 to late endosomes (56). Snf7 binds to a conserved hydrophobic patch on Bro1 that is required for protein complex formation and for the protein sorting function of Bro1 (56). The Bro1 domain of the Bro1 protein does not contain the binding site for Doa4 (56). A second conserved hydrophobic patch at one tip of the domain is not critical for sorting, and its function has yet to be determined. The structure resembles a boomerang with its concave face filled in, and it is tempting to speculate that the convex face could mediate the putative ability of the human Bro1 counterpart to sense negative curvature in invaginating luminal vesicles (67); however, this idea has not been tested for the Bro1 domain.

## VPS4

Vps4 is responsible for the ATP-dependent disassembly of the ESCRT complexes (8, 9). Vps4 is a homo-oligomer in yeast, and consists of an N-terminal MIT domain and a central AAA ATPase domain (Figure 7). Two Vps4 isoforms in human, Vps4A and Vps4B, can hetero-oligomerize with each other. AAA ATPases are ubiquitous disassembly machines



**Figure 6**

Structure of Bro1. The structure of the Bro1 domain (56), which comprises the N-terminal half of Bro1, is shown docked to a negatively curved membrane. The interaction of this domain with negatively curved membranes is suggested by the shape of the structure and by the properties of the full-length human Bro1 homologue; however, this interaction and docking mode are speculative and have yet to be directly tested.

that are ring hexamers and function in a wide range of cell processes (19). The structure of the AAA domain of Vps4 has been determined (93) and is similar in outline to the structures of other AAA ATPases such as NSF and p97. The N-terminal MIT domain [first called an ESP domain (79)] of Vps4 binds to the human ortholog of the ESCRT-III-like protein Did2 and to other ESCRT-III subunits as well (94, 115). The structure of a MIT domain from human Vps4 has been determined and has been shown to be a three-helix bundle (94) that contains the equivalent of 1.5 tetratricopeptide repeats, reminiscent of the Bro1 domain. The structure of the Vps4 monomer has been used to model a hexamer that assembles in the presence of ATP. This hexamer contains a central pore. In a working mechanism for ESCRT-III disassembly, supported by mutational analysis of the modeled pore, individual membrane-bound ESCRT-III subunits are fed through the pore into solution and so converted to their monomeric, soluble state.

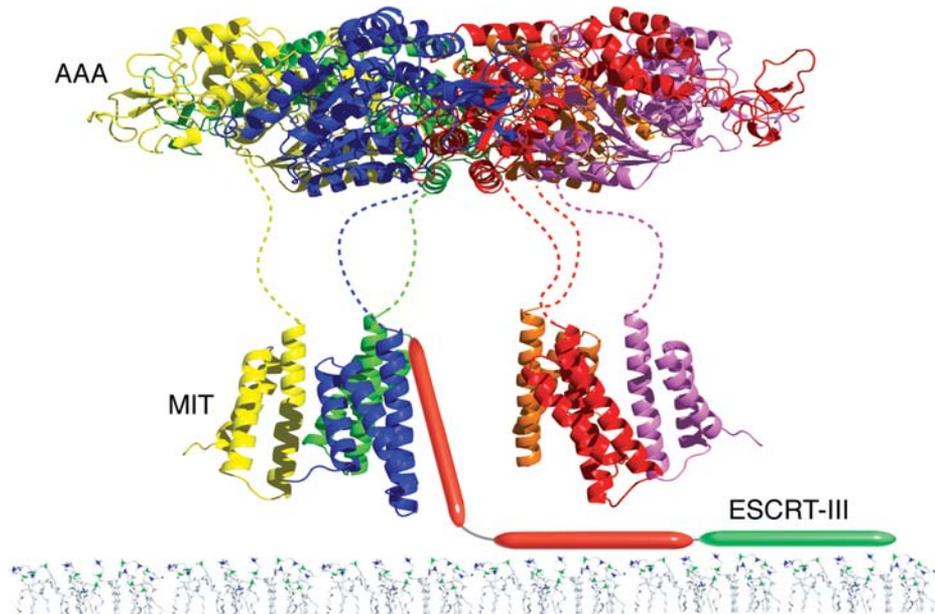
## CONCLUSIONS AND FUTURE PERSPECTIVES

In gross terms, the ESCRT systems have two major functions: the recruitment of cargo to MVBs, followed by its internalization via the invagination of luminal vesicles (**Figure 8**). Much has been learned in the past five years about the structures, interactions, and ordered assembly of the ESCRT machinery, and the mechanism of cargo recruitment. The presence of Ub-binding domains in Vps27/Hse1, ESCRT-I, and ESCRT-II has led to the suggestion that there is a serial handoff of ubiquitinated cargo from one complex to the next. Favoring this model, structural analysis shows that the Ub-binding domains interact with just one region of Ub, the Ile-44 patch. This offers an elegant mechanism to prevent more than one complex from interacting with Ub at a given time. However, no direct evidence of hand-off is available. In an alternative model, the presence of multiple Ub-binding sites in the Vps27/ESCRT-I/ESCRT-II “complex of complexes” offers an attractive mechanism for

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**MIT:** microtubule interacting and trafficking

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**Figure 7**

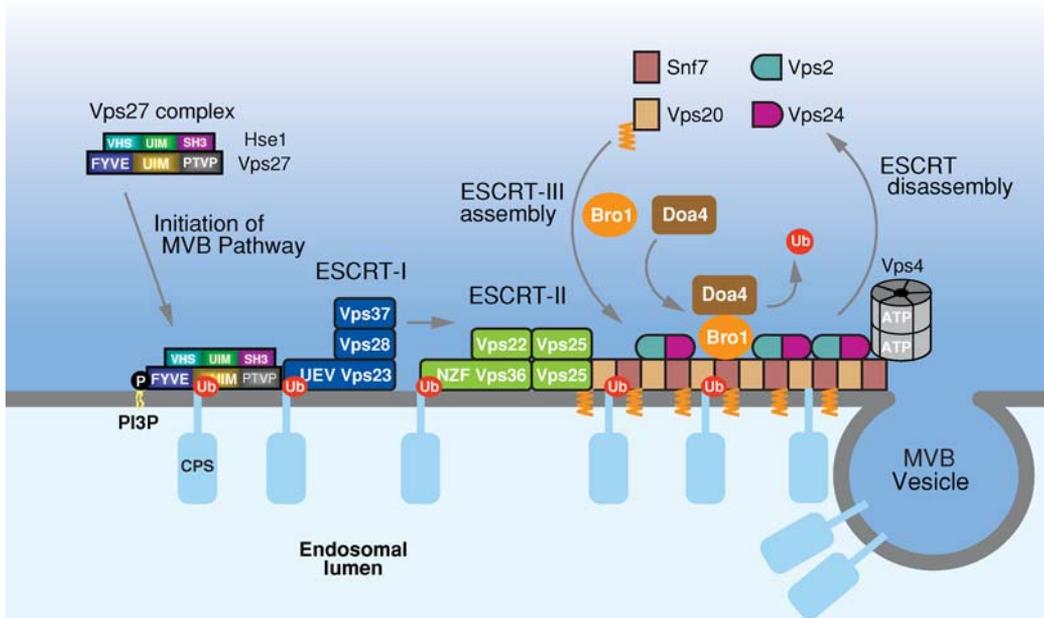
Structure of Vps4. The modeled structure of the Vps4 AAA domain hexamer was modeled (93) and is shown together with the corresponding six copies of the MIT domain (94). For simplicity, a single subunit of the ESCRT-III complex is shown engaged to a single MIT domain. The ESCRT-III subunit is thought to be fed through the central pore in the hexamer (not shown in this view) as ATP is hydrolyzed by the AAA domain.

receptor clustering by simultaneous binding. The presence of two Ub-binding domains in Vps27 and ESCRT-II seems more consistent with a clustering model than with a hand-off model, as there would be little reason to orchestrate hand-off within a single complex. The two models are not mutually exclusive, because hand-off might be operative at one stage in the pathway and clustering at another.

Despite many advances in other aspects of ESCRT function, the fundamental mechanism of vesicle budding remains a matter of conjecture. MVBs from mammalian cells have been reported to be rich in the phospholipid 2,2' lysobisphosphatidic acid (2,2' LBPA), which has been suggested to be a key driving force for membrane deformation leading to vesicle invagination (67). On the other hand, no evidence has surfaced for the presence of 2,2' LBPA in yeast, and no Vps pro-

teins have emerged as candidate regulators of LBPA levels. Given the high conservation of the protein machinery involved, it would be surprising if completely different mechanisms were responsible for the invagination of luminal vesicles in yeast and mammals. It seems likely that changes in vesicular pH and/or ionic strength are important. The  $\text{Na}^+/\text{H}^+$  antiporter Vps44 is the only transmembrane protein among the class E Vps proteins and remains the most mysterious in terms of its role in budding. It will be important to determine whether Vps44 drives pH or ionic strength changes in the lumen of the MVB and/or the budding vesicles and, if it does, what proteins act as the downstream effectors of these changes.

One of the major motivations for studying the ESCRT pathway is the discovery that this pathway is central to HIV budding, presenting an unprecedented number of new



**Figure 8**

The ESCRT complexes in MVB sorting. The Vps27 protein complex initiates the MVB-sorting process. It is targeted to endosomal membranes via its FYVE domain that binds PI(3)P, and its UIM domains, which bind ubiquitinated MVB cargo such as carboxypeptidase S (CPS). Vps27 subsequently recruits and activates the ESCRT-I complex via the P(S/T)XP motif in the C-terminal domain of Vps27 that interacts with the UEV domain of Vps23 in ESCRT-I. Ubiquitinated cargo is recognized by ESCRT-I (via the UEV domain of Vps23) and by ESCRT-II (via the NZF domain in Vps36). ESCRT-III is required for concentration of cargoes into MVB vesicles and coordinates the association of accessory factors such as Bro1 and the Doa4-deubiquitinating enzyme that removes ubiquitin from cargo. The AAA-type ATPase Vps4 plays a critical role in catalyzing the dissociation of the ESCRT complexes. Together, these proteins appear to direct MVB vesicle formation, cargo sorting into MVB vesicles, and vesicle fission. See text for further details.

potential therapeutic targets. A number of proteins in the pathway are essential for budding and are incorporated directly into HIV virions (101, 111). The usefulness of pathway members as targets depends on the relative sensitivity of virus and host to inhibition of the ESCRT pathway. The locus of HIV budding through this pathway remains controversial. The mechanism by which nascent virions avoid the fate of most other MVB cargo—destruction in the lysosome—remains unknown, and its identification would present an exceptionally interesting target.

The emerging picture of ESCRT assembly on endosomal membranes suggests the formation of arrays of inexact stoichiometry. Dynamic protein networks that assemble on membranes are central to signal transduction and subcellular trafficking. Their complexity, kinetic fragility, membrane localization, and inexact stoichiometry present great challenges to obtaining a precise structural and mechanistic understanding. The payoff will be equally great, perhaps providing a roadmap for analysis of many analogous membrane-bound signaling and trafficking systems.

## SUMMARY POINTS

1. The yeast class E *VPS* genes and their human homologues code for a network of proteins that comprise the ESCRT complexes and associated proteins.
2. The Vps27/Hse1, ESCRT-I, ESCRT-II, and ESCRT-III complexes are recruited to endosomal membranes in an ordered manner. Recruitment is initiated by the lipid PI(3)P and the presence of monoubiquitinated transmembrane proteins.
3. The role of the ESCRT network is to (a) recruit and cluster monoubiquitinated cargo, and (b) drive the formation, invagination, and fission of cargo-containing vesicles into the lumen of the MVB.
4. The structural basis for PI(3)P recruitment via FYVE domains and Ub recruitment via UIM, UEV, and NZF domains has been determined.
5. Vps27/Hse1, ESCRT-I, and ESCRT-II are soluble protein complexes. The structure of the ESCRT-II core complex has been determined.
6. The ESCRT-III complex is thought to be a tightly membrane-associated array. In concert with the ATPase Vps4, ESCRT-III may be involved in mechanical deformation of the membrane to drive the invagination and fission of intraluminal vesicles into MVBs.
7. The ESCRT network is essential for budding of the HIV virus, and structural studies of these complexes present potential targets for development of new anti-HIV therapeutics.
8. The mechanism of invagination is essentially unknown and presents a major challenge for mechanistic studies.

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## LITERATURE CITED

1. Alam SL, Sun J, Payne M, Welch BD, Blake BK, et al. 2004. Ubiquitin interactions of NZF zinc fingers. *EMBO J.* 23:1411–21
2. Amerik AY, Li SJ, Hochstrasser M. 2000. Analysis of the deubiquitinating enzymes of the yeast *Saccharomyces cerevisiae*. *Biol. Chem.* 381:981–92
3. Amit I, Yakir L, Katz M, Zwang Y, Marmor MD, et al. 2004. Tal, a Tsg101-specific E3 ubiquitin ligase, regulates receptor endocytosis and retrovirus budding. *Genes Dev.* 18:1737–52
4. Antonyak MA, McNeill CJ, Wakshlag JJ, Boehm JE, Cerione RA. 2003. Activation of the Ras-ERK pathway inhibits retinoic acid-induced stimulation of tissue transglutaminase expression in NIH3T3 cells. *J. Biol. Chem.* 278:15859–66
5. Asao H, Sasaki Y, Arita T, Tanaka N, Endo K, et al. 1997. Hrs is associated with STAM, a signal-transducing adaptor molecule: its suppressive effect on cytokine-induced cell growth. *J. Biol. Chem.* 272:32785–91

6. Babst M, Katzmann DJ, Estepa-Sabal EJ, Meerloo T, Emr SD. 2002. ESCRT-III: an endosome-associated heterooligomeric protein complex required for MVB sorting. *Dev. Cell* 3:271–82
7. Babst M, Katzmann DJ, Snyder WB, Wendland B, Emr SD. 2002. Endosome-associated complex, ESCRT-II, recruits transport machinery for protein sorting at the multivesicular body. *Dev. Cell* 3:283–89
8. Babst M, Sato TK, Banta LM, Emr SD. 1997. Endosomal transport function in yeast requires a novel AAA-type ATPase, Vps4p. *EMBO J.* 16:1820–31
9. Babst M, Wendland B, Estepa EJ, Emr SD. 1998. The Vps4p AAA ATPase regulates membrane association of a Vps protein complex required for normal endosome function. *EMBO J.* 17:2982–93
10. Bache KG, Brech A, Mehlum A, Stenmark H. 2003. Hrs regulates multivesicular body formation via ESCRT recruitment to endosomes. *J. Cell Biol.* 162:435–42
11. Begley MJ, Taylor GS, Kim SA, Veine DM, Dixon JE, Stuckey JA. 2003. Crystal structure of a phosphoinositide phosphatase, MTMR2: insights into myotubular myopathy and Charcot-Marie-Tooth syndrome. *Mol. Cell* 12:1391–402
12. Bilodeau PS, Urbanowski JL, Winistorfer SC, Piper RC. 2002. The Vps27p-Hse1p complex binds ubiquitin and mediates endosomal protein sorting. *Nat. Cell Biol.* 4:534–39
13. Bilodeau PS, Winistorfer SC, Kearney WR, Robertson AD, Piper RC. 2003. Vps27-Hse1 and ESCRT-I complexes cooperate to increase efficiency of sorting ubiquitinated proteins at the endosome. *J. Cell Biol.* 163:237–43
14. Bishop N, Horman A, Woodman P. 2002. Mammalian class E Vps proteins recognize ubiquitin and act in the removal of endosomal protein-ubiquitin conjugates. *J. Cell Biol.* 157:91–101
15. Blatner N, Stahelin RV, Diraviyam K, Hawkins PT, Hong W, et al. 2004. The molecular basis of the differential subcellular localization of FYVE domains. *J. Biol. Chem.* 279:53818–27
16. Bowers K, Lottridge J, Helliwell SB, Goldthwaite LM, Luzio JP, Stevens TH. 2004. Protein-protein interactions of ESCRT complexes in the yeast *Saccharomyces cerevisiae*. *Traffic* 5:194–210
17. Bowers K, Stevens TH. 2005. Protein transport from the late Golgi to the vacuole in the yeast *Saccharomyces cerevisiae*. *Biochim. Biophys. Acta* 1744:438–54
18. Brunger AT. 2001. Structure of proteins involved in synaptic vesicle fusion in neurons. *Annu. Rev. Biophys. Biomol. Struct.* 30:157–71
19. Brunger AT, DeLaBarre B. 2003. NSF and p97/VCP: similar at first, different at last. *FEBS Lett.* 555:126–33
20. Burd CG, Emr SD. 1998. Phosphatidylinositol(3)-phosphate signaling mediated by specific binding to RING FYVE domains. *Mol. Cell* 2:157–62
21. Chatellard-Cause C, Blot B, Cristina N, Torch S, Missotten M, Sadoul R. 2002. Alix (ALG-2-interacting protein X), a protein involved in apoptosis, binds to endophilins and induces cytoplasmic vacuolization. *J. Biol. Chem.* 277:29108–15
22. Chen B, Borinstein SC, Gillis J, Sykes VW, Bogler O. 2000. The glioma-associated protein SETA interacts with AIP1/Alix and AZIG-2 and modulates apoptosis in astrocytes. *J. Biol. Chem.* 275:19275–81
23. Davies BA, Topp JD, Sfeir AJ, Katzmann DJ, Carney DS, et al. 2003. Vps9p CUE domain ubiquitin binding is required for efficient endocytic protein traffic. *J. Biol. Chem.* 278:19826–33

24. Demirov DG, Ono A, Orenstein JM, Freed EO. 2002. Overexpression of the N-terminal domain of TSG101 inhibits HIV-1 budding by blocking late domain function. *Proc. Natl. Acad. Sci. USA* 99:955–60
25. DiNitto JP, Cronin TC, Lambright DG. 2003. Membrane recognition and targeting by lipid-binding domains. *Sci. STKE* re 16:1–15
26. Diraviyam K, Stahelin RV, Cho W, Murray D. 2003. Computer modeling of the membrane interaction of FYVE domains. *J. Mol. Biol.* 328:721–36
27. Dumas JJ, Merithew E, Sudharshan E, Rajamani D, Hayes S, et al. 2001. Multivalent endosome targeting by homodimeric EEA1. *Mol. Cell* 8:947–58
28. Felder S, Miller K, Moehren G, Ullrich A, Schlessinger J, Hopkins CR. 1990. Kinase activity controls the sorting of the epidermal growth factor receptor within the multivesicular body. *Cell* 61:623–34
29. Feng GH, Lih CJ, Cohen SN. 2000. TSG101 protein steady-state level is regulated posttranslationally by an evolutionarily conserved COOH-terminal sequence. *Cancer Res.* 60:1736–41
30. Fisher RD, Wang B, Alam SL, Higginson DS, Robinson H, et al. 2003. Structure and ubiquitin binding of the ubiquitin-interacting motif. *J. Biol. Chem.* 278:28976–84
31. Futter CE, Pearse A, Hewlett LJ, Hopkins CR. 1996. Multivesicular endosomes containing internalized EGF-EGF receptor complexes mature and then fuse directly with lysosomes. *J. Cell Biol.* 132:1011–23
32. Garrus JE, von Schwedler UK, Pornillos OW, Morham SG, Zavitz KH, et al. 2001. Tsg101 and the vacuolar protein sorting pathway are essential for HIV-1 budding. *Cell* 107:55–65
33. Gaullier JM, Simonsen A, D'Arrigo A, Bremnes B, Stenmark H, Aasland R. 1998. FYVE fingers bind Ptdins(3)P. *Nature* 394:432–33
34. Goila-Gaur R, Demirov DG, Orenstein JM, Ono A, Freed EO. 2003. Defects in human immunodeficiency virus budding and endosomal sorting induced by TSG101 overexpression. *J. Virol.* 77:6507–19
35. Gordon P, Carpentier JL, Cohen S, Orci L. 1978. Epidermal growth factor: morphological demonstration of binding, internalization, and lysosomal association in human fibroblasts. *Proc. Natl. Acad. Sci. USA* 75:5025–29
36. Haglund K, Di Fiore PP, Dikic I. 2003. Distinct monoubiquitin signals in receptor endocytosis. *Trends Biochem. Sci.* 28:598–603
37. Haigler HT, McKanna JA, Cohen S. 1979. Direct visualization of the binding and internalization of a ferritin conjugate of epidermal growth factor in human carcinoma cells A-431. *J. Cell Biol.* 81:382–95
38. Hanyaloglu AC, McCullagh E, von Zastrow M. 2005. Essential role of Hrs in a recycling mechanism mediating functional resensitization of cell signaling. *EMBO J.* 24:2265–83
39. Hershko A, Ciechanover A, Varshavsky A. 2000. The ubiquitin system. *Nat. Med.* 6:1073–81
40. Hicke L. 2001. Protein regulation by monoubiquitin. *Nat. Rev. Mol. Cell Biol.* 2:195–201
41. Hicke L, Dunn R. 2003. Regulation of membrane protein transport by ubiquitin and ubiquitin-binding proteins. *Annu. Rev. Cell. Dev. Biol.* 19:141–72
42. Hierro A, Sun J, Rusnak AS, Kim J, Prag G, et al. 2004. Structure of the ESCRT-II endosomal trafficking complex. *Nature* 431:221–25
43. Hislop JN, Marley A, von Zastrow M. 2004. Role of mammalian vacuolar protein-sorting proteins in endocytic trafficking of a non-ubiquitinated G protein-coupled receptor to lysosomes. *J. Biol. Chem.* 279:22522–31

44. Hochstrasser M. 2000. Evolution and function of ubiquitin-like protein-conjugation systems. *Nat. Cell Biol.* 2:E153–57
45. Hofmann K, Falquet L. 2001. A ubiquitin-interacting motif conserved in components of the proteasomal and lysosomal protein degradation systems. *Trends Biochem. Sci.* 26:347–50
46. Hurley JH, Meyer T. 2001. Subcellular targeting by membrane lipids. *Curr. Opin. Cell Biol.* 13:146–52
47. Hurley JH, Misra S. 2000. Signaling and subcellular targeting by membrane-binding domains. *Annu. Rev. Biophys. Biomol. Struct.* 29:49–79
48. Kaneko T, Kumasaka T, Ganbe T, Sato T, Miyazawa K, et al. 2003. Structural insight into modest binding of a non-PXXX ligand to the signal transducing adaptor molecule-2 Src homology 3 domain. *J. Biol. Chem.* 278:48162–68
49. Kato M, Miyazawa K, Kitamura N. 2000. A deubiquitinating enzyme UBPY interacts with the Src homology 3 domain of Hrs-binding protein via a novel binding motif PX(V/I)(D/N)RXXXKP. *J. Biol. Chem.* 275:37481–87
50. Katoh K, Shibata H, Hatta K, Maki M. 2004. CHMP4b is a major binding partner of the ALG-2-interacting protein Alix among the three CHMP4 isoforms. *Arch. Biochem. Biophys.* 421:159–65
51. Katoh K, Shibata H, Suzuki H, Nara A, Ishidoh K, et al. 2003. The ALG-2-interacting protein Alix associates with CHMP4b, a human homologue of yeast Snf7 that is involved in multivesicular body sorting. *J. Biol. Chem.* 278:39104–13
52. Katz M, Shtiegman K, Tal-Or P, Yakir L, Mosesson Y, et al. 2002. Ligand-independent degradation of epidermal growth factor receptor involves receptor ubiquitylation and hgs, an adaptor whose ubiquitin-interacting motif targets ubiquitylation by Nedd4. *Traffic* 3:740–51
53. Katzmann DJ, Babst M, Emr SD. 2001. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. *Cell* 106:145–55
54. Katzmann DJ, Odorizzi G, Emr SD. 2002. Receptor downregulation and multivesicular-body sorting. *Nat. Rev. Mol. Cell Biol.* 3:893–905
55. Katzmann DJ, Stefan CJ, Babst M, Emr SD. 2003. Vps27 recruits ESCRT machinery to endosomes during MVB sorting. *J. Cell Biol.* 162:413–23
56. Kim J, Sitaraman S, Hierro A, Beach BM, Odorizzi G, Hurley JH. 2005. Structural basis for endosomal targeting by the Bro1 domain. *Dev. Cell* 8:937–47
57. Komada M, Kitamura N. 2005. The Hrs/STAM complex in the downregulation of receptor tyrosine kinases. *J. Biochem.* 137:1–8
58. Kutateladze T, Overduin M. 2001. Structural mechanism of endosome docking by the FYVE domain. *Science* 291:1793–96
59. Kutateladze TG, Capelluto DGS, Ferguson CG, Cheever ML, Kutateladze AG, et al. 2004. Multivalent mechanism of membrane insertion by the FYVE domain. *J. Biol. Chem.* 279:3050–57
60. Kutateladze TG, Ogburn KD, Watson WT, de Beer T, Emr SD, et al. 1999. Phosphatidylinositol 3-phosphate recognition by the FYVE domain. *Mol. Cell* 3:805–11
61. Lemmon MA. 2003. Phosphoinositide recognition domains. *Traffic* 4:201–13
62. Lin Y, Kimpler LA, Naismith TV, Lauer JM, Hanson PI. 2005. Interaction of the mammalian endosomal sorting complex required for transport (ESCRT) III protein hSnf7-1 with itself, membranes, and the AAA+ ATPase SKD1. *J. Biol. Chem.* 280:12799–809
63. Luhtala N, Odorizzi G. 2004. Bro1 coordinates deubiquitination in the multivesicular body pathway by recruiting Doa4 to endosomes. *J. Cell Biol.* 166:717–29

64. Mao YX, Nickitenko A, Duan XQ, Lloyd TE, Wu MN, et al. 2000. Crystal structure of the VHS and FYVE tandem domains of Hrs, a protein involved in membrane trafficking and signal transduction. *Cell* 100:447–56
65. Martin-Serrano J, Zang T, Bieniasz PD. 2001. HIV-I and Ebola virus encode small peptide motifs that recruit Tsg101 to sites of particle assembly to facilitate egress. *Nat. Med.* 7:1313–19
66. Martin-Serrano J, Zang T, Bieniasz PD. 2003. Role of ESCRT-I in retroviral budding. *J. Virol.* 77:4794–804
67. Matsuo H, Chevallier J, Mayran N, Le Blanc I, Ferguson C, et al. 2004. Role of LBPA and Alix in multivesicular liposome formation and endosome organization. *Science* 303:531–34
68. Misra S, Beach BM, Hurley JH. 2000. Structure of the VHS domain of human Tom1 (target of myb 1): insights into interactions with proteins and membranes. *Biochemistry* 39:11282–90
69. Misra S, Hurley JH. 1999. Crystal structure of a phosphatidylinositol 3-phosphate-specific membrane-targeting motif, the FYVE domain of Vps27p. *Cell* 97:657–66
70. Misra S, Miller GJ, Hurley JH. 2001. Recognizing phosphatidylinositol 3-phosphate. *Cell* 107:559–62
71. Misra S, Puertollano R, Kato Y, Bonifacino JS, Hurley JH. 2002. Structural basis for acidic-cluster-dileucine sorting-signal recognition by VHS domains. *Nature* 415:933–37
72. Mizuno E, Kawahata K, Kato M, Kitamura N, Komada M. 2003. STAM proteins bind ubiquitinated proteins on the early endosome via the VHS domain and ubiquitin-interacting motif. *Mol. Biol. Cell* 14:3675–89
73. Morita E, Sundquist WI. 2004. Retrovirus budding. *Annu. Rev. Cell Dev. Biol.* 20:395–425
74. Myers EL, Allen JF. 2002. Tsg101, an inactive homologue of ubiquitin ligase E2, interacts specifically with human immunodeficiency virus type 2 Gag polyprotein and results in increased levels of ubiquitinated Gag. *J. Virol.* 76:11226–35
75. Nikko E, Marini AM, Andre B. 2003. Permease recycling and ubiquitination status reveal a particular role for Bro1 in the multivesicular body pathway. *J. Biol. Chem.* 278:50732–43
76. Odorizzi G, Babst M, Emr SD. 1998. Fab1p PtdIns(3)P 5-kinase function essential for protein sorting in the multivesicular body. *Cell* 95:847–58
77. Odorizzi G, Katzmann DJ, Babst M, Audhya A, Emr SD. 2003. Bro1 is an endosome-associated protein that functions in the MVB pathway in *Saccharomyces cerevisiae*. *J. Cell Sci.* 116:1893–903
78. Patki V, Lawe DC, Corvera S, Virbasius JV, Chawla A. 1998. A functional PtdIns(3)P-binding motif. *Nature* 394:433–34
79. Phillips SA, Barr VA, Haft DH, Taylor SI, Haft CR. 2001. Identification and characterization of SNX15, a novel sorting nexin involved in protein trafficking. *J. Biol. Chem.* 276:5074–84
80. Pickart CM. 2001. Mechanisms underlying ubiquitination. *Annu. Rev. Biochem.* 70:503–33
81. Polo S, Sigismund S, Faretta M, Guidi M, Capua MR, et al. 2002. A single motif responsible for ubiquitin recognition and monoubiquitination in endocytic proteins. *Nature* 416:451–55
82. Pornillos O, Alam SL, Davis DR, Sundquist WI. 2002. Structure of the Tsg101 UEV domain in complex with the PTAP motif of the HIV-1 p6 protein. *Nat. Struct. Biol.* 9:812–17
83. Pornillos O, Higginson DS, Stray KM, Fisher RD, Garrus JE, et al. 2003. HIV Gag mimics the Tsg101-recruiting activity of the human Hrs protein. *J. Cell Biol.* 162:425–34

84. Raiborg C, Bache KG, Gillooly DJ, Madshush IH, Stang E, Stenmark H. 2002. Hrs sorts ubiquitinated proteins into clathrin-coated microdomains of early endosomes. *Nat. Cell Biol.* 4:394–98
85. Raiborg C, Bache KG, Mehlum A, Stang E, Stenmark H. 2001. Hrs recruits clathrin to early endosomes. *EMBO J.* 20:5008–21
86. Raiborg C, Bremnes B, Mehlum A, Gillooly DJ, D'Arrigo A, et al. 2001. FYVE and coiled-coil domains determine the specific localisation of Hrs to early endosomes. *J. Cell Sci.* 114:2255–63
87. Raiborg C, Rusten TE, Stenmark H. 2003. Protein sorting into multivesicular endosomes. *Curr. Opin. Cell Biol.* 15:446–55
88. Reggiori F, Pelham HR. 2001. Sorting of proteins into multivesicular bodies: ubiquitin-dependent and -independent targeting. *EMBO J.* 20:5176–86
89. Sachse M, Strous GJ, Klumperman J. 2004. ATPase-deficient hVPS4 impairs formation of internal endosomal vesicles and stabilizes bilayered clathrin coats on endosomal vacuoles. *J. Cell Sci.* 117:1699–708
90. Sankaran VG, Klein DE, Sachdeva MM, Lemmon MA. 2001. High-affinity binding of a FYVE domain to phosphatidylinositol 3-phosphate requires intact phospholipid but not FYVE domain oligomerization. *Biochemistry* 40:8581–87
91. Scarlata S, Carter C. 2003. Role of HIV-1 Gag domains in viral assembly. *Biochem. Biophys. Acta* 1614:62–72
92. Schmidt MHH, Hoeller D, Yu JH, Furnari FB, Cavenee WK, et al. 2004. Alix/AIP1 antagonizes epidermal growth factor receptor downregulation by the Cbl-SETA/CIN85 complex. *Mol. Cell Biol.* 24:8981–93
93. Scott A, Chung H-Y, Gonciarz-Swiatek M, Hill GC, Whitby FG, et al. 2005. Structural and mechanistic studies of VPS4 proteins. *EMBO J.* 24:3658–69
94. Scott A, Gaspar J, Stuchell-Brereton M, Alam SL, Skalicky J, Sundquist WI. 2005. Structure and ESCRT-III protein interactions of the MIT domain of human Vps4A. *Proc. Natl. Acad. Sci. USA* 102:13813–18
95. Shiba T, Takatsu H, Nogi T, Matsugaki N, Kawasaki M, et al. 2002. Structural basis for recognition of acidic-cluster dileucine sequence by GGA1. *Nature* 415:937–41
96. Shiflett SL, Ward DM, Huynh D, Vaughn MB, Simmons JC, Kaplan J. 2004. Characterization of Vta1p, a class E Vps protein in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 279:10982–90
97. Shih SC, Katzmann DJ, Schnell JD, Sutanto M, Emr SD, Hicke L. 2002. Epsins and Vps27p/Hrs contain ubiquitin-binding domains that function in receptor endocytosis. *Nat. Cell Biol.* 4:389–93
98. Sigismund S, Polo S, Di Fiore PP. 2004. Signaling through monoubiquitination. *Curr. Top. Microbiol. Immunol.* 286:149–85
99. Slagsvold T, Aasland R, Hirano S, Bache KG, Raiborg C, et al. 2005. Eap45 in mammalian ESCRT-II binds ubiquitin via a phosphoinositide-interacting GLUE domain. *J. Biol. Chem.* 280:19600–6
100. Stahelin RV, Long F, Diraviyam K, Bruzik KS, Murray D, Cho W. 2002. Phosphatidylinositol 3-phosphate induces the membrane penetration of the FYVE domains of Vps27p and Hrs. *J. Biol. Chem.* 277:26379–88
101. Strack B, Calistri A, Craig S, Popova E, Gottlinger HG. 2003. AIP1/ALIX is a binding partner for HIV-1 p6 and EIAV p9 functioning in virus budding. *Cell* 114:689–99
102. Sundquist WI, Schubert HL, Kelly BN, Hill GC, Holton JM, Hill CP. 2004. Ubiquitin recognition by the human TSG101 protein. *Mol. Cell* 13:783–89

103. Swanson KA, Kang RS, Stamenova SD, Hicke L, Radhakrishnan I. 2003. Solution structure of Vps27 UIM-ubiquitin complex important for endosomal sorting and receptor downregulation. *EMBO J.* 22:4597–606
104. Teo H, Perisic O, Gonzalez B, Williams RL. 2004. ESCRT-II, an endosome-associated complex required for protein sorting: crystal structure and interactions with ESCRT-III and membranes. *Dev. Cell* 7:559–69
105. Teo H, Veprintsev DB, Williams RL. 2004. Structural insights into endosomal sorting complex required for transport (ESCRT-I) recognition of ubiquitinated proteins. *J. Biol. Chem.* 279:28689–96
106. ter Haar E, Harrison SC, Kirchhausen T. 2000. Peptide-in-groove interactions link target proteins to the beta-propeller of clathrin. *Proc. Natl. Acad. Sci. USA* 97:1096–100
107. Tong AHY, Drees B, Nardelli G, Bader GD, Brannetti B, et al. 2002. A combined experimental and computational strategy to define protein interaction networks for peptide recognition modules. *Science* 295:321–24
108. Urbanowski JL, Piper RC. 2001. Ubiquitin sorts proteins into the intraluminal degradative compartment of the late-endosome/vacuole. *Traffic* 2:622–30
109. VerPlank L, Bouamr F, LaGrassa TJ, Agresta B, Kikonyogo A, et al. 2001. Tsg101, a homologue of ubiquitin-conjugating (E2) enzymes, binds the L domain in HIV type 1 Pr55(Gag). *Proc. Natl. Acad. Sci. USA* 98:7724–29
110. Vincent O, Rainbow L, Tilburn J, Arst HN, Penalva MA. 2003. YPXL/I is a protein interaction motif recognized by *Aspergillus* PalA and its human homologue, AIP1/Alix. *Mol. Cell Biol.* 23:1647–55
111. von Schwedler UK, Stuchell M, Muller B, Ward DM, Chung HY, et al. 2003. The protein network of HIV budding. *Cell* 114:701–13
112. Ward DMV, Vaughn MB, Shiflett SL, White PL, Pollock AL, et al. 2005. The role of LIP5 and CHMP5 in multivesicular body formation and HIV-1 budding in mammalian cells. *J. Biol. Chem.* 280:10548–55
113. Wernimont AK, Weissenhorn W. 2004. Crystal structure of subunit Vps25 of the endosomal trafficking complex ESCRT-II. *BMC Struct. Biol.* 4:10
114. Whitley P, Reaves BJ, Hashimoto M, Riley AM, Potter BV, Holman GD. 2003. Identification of mammalian Vps24p as an effector of phosphatidylinositol 3,5-bisphosphate-dependent endosome compartmentalization. *J. Biol. Chem.* 278:38786–95
115. Yeo SCL, Xu LH, Ren JH, Boulton VJ, Wagle MD, et al. 2003. Vps20p and Vta1p interact with Vps4p and function in multivesicular body sorting and endosomal transport in *Saccharomyces cerevisiae*. *J. Cell Sci.* 116:3957–70
116. Yorikawa C, Shibata H, Waguri S, Hatta K, Horii M, et al. 2005. Human CHMP6, a myristoylated ESCRT-III protein, interacts directly with an ESCRT-II component EAP20 and regulates endosomal cargo sorting. *Biochem. J.* 387:17–26
117. Zarrinpar A, Bhattacharyya RP, Lim WA. 2003. The structure and function of proline recognition domains. *Sci STKE* DOI:10.1126/stke.2003.179.re8
118. Zhu GY, He XY, Zhai P, Terzyan S, Tang J, Zhang XJC. 2003. Crystal structure of GGA2 VHS domain and its implication in plasticity in the ligand binding pocket. *FEBS Lett.* 537:171–76