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REVIEW ARTICLE

The ESCRT complexes

James H. Hurley

Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD, USA

Abstract

The ESCRT machinery consists of the peripheral membrane protein complexes ESCRT-0, -I, -II, -III, and Vps4–Vta1, and the ALIX homodimer. The ESCRT system is required for degradation of unneeded or dangerous plasma membrane proteins; biogenesis of the lysosome and the yeast vacuole; the budding of most membrane enveloped viruses; the membrane abscission step in cytokinesis; macroautophagy; and several other processes. From their initial discovery in 2001–2002, the literature on ESCRTs has grown exponentially. This review will describe the structure and function of the six complexes noted above and summarize current knowledge of their mechanistic roles in cellular pathways and in disease.

Keywords: *ESCRT; ubiquitin; HIV-1; cytokinesis; protein structure; viral budding*

Introduction

Historical background

The discovery and history of the ESCRT complexes is intimately connected to the characterization of multivesicular bodies (MVBs) (Piper and Katzmann, 2007). The term MVB was first applied to features in electron micrographs of cells in the 1950s (Sotelo and Porter, 1959). The role of MVBs in the lysosomal degradation of activated signaling receptors became clear two decades later from electron microscopic analysis of the internalization of epidermal growth factor (Gorden *et al.*, 1978; Haigler *et al.*, 1979) (Figure 1). The power of yeast genetics to dissect intracellular transport pathways became clear at the start of the 1980s (Novick *et al.*, 1980). The vacuole is the yeast counterpart of the lysosome of animal cells. By the late 1990s, more than 60 yeast vacuolar protein sorting (*VPS*) genes involved in vacuole biogenesis had been identified (Bryant and Stevens, 1998). Of these genes 13 fell into the class E morphological subgroup, and were shown to be involved in the transport of proteins into the MVB en route to the vacuole. These genes code for most of the core subunits of the ESCRT complexes ESCRT-0 (Bilodeau *et al.*, 2002; Katzmann *et al.*, 2003), ESCRT-I (Katzmann *et al.*, 2001), ESCRT-II (Babst *et al.*, 2002b), ESCRT-III (Babst *et al.*, 2002a). Soon thereafter, similar functions in receptor sorting to lysosomes via the MVB pathway were demonstrated for the mammalian counterparts of the yeast ESCRT complexes (Raiborg *et al.*, 2002; Bache *et al.*, 2003a; 2003b; Lu *et al.*, 2003). In the past decade, the ESCRTs have been found to act in viral budding, cytokinesis, autophagy, and other pathways in addition to MVB biogenesis (Figure 2).

Scope of review

As we approach the 10-year anniversary of the discovery and naming of the ESCRT system, it seems appropriate to take a comprehensive look at what has been learned about the system. With a literature of over 500 publications that is growing at a rate of about three per week, this may also be one of the last opportunities to even attempt a comprehensive review. Even in a broad overview, it is impractical to cite and discuss every paper in the field. For more detailed information on the structure, function, and biochemistry of the ESCRTs, see Babst (2005), Hurley and Emr (2006), Alam and Sundquist (2007), Saksena *et al.* (2007), Williams and Urbe (2007), and Hurley (2008). The membrane mechanics of ESCRT-mediated budding and scission are reviewed by Hurley &

Address for Correspondence: James H. Hurley, Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, US Department of Health and Human Services, Bethesda, MD 20892, USA. E-mail: hurley@helix.nih.gov

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Hanson (2010). For more background on the biogenesis of multivesicular bodies, see Gruenberg and Stenmark (2004), Piper and Katzmann (2007), and Hanson *et al.* (2009). Further details on the sorting of ubiquitinated receptors and other cargo by the ESCRTs can be found in Katzmann *et al.* (2002), Piper and Luzio (2007), Hurley and Ren (2009), Luzio *et al.* (2009), and Raiborg and Stenmark (2009). For reviews on the role of ESCRTs in viral budding, see Morita and Sundquist (2004), Bieniasz (2006; 2009), Freed and Mouland (2006), Fujii *et al.* (2007), Chen and Lamb (2008), Carter and Ehrlich (2008), and Usami *et al.* (2009). The role of ESCRTs in cytokinesis is reviewed by Carlton and Martin-Serrano (2009), McDonald and Martin-Serrano (2009), Samson and Bell (2009), and Steigemann and Gerlich (2009). The possible roles of ESCRTs in autophagy are reviewed by Rusten and Stenmark (2009). The role of ESCRTs in disease are covered by Saksena and Emr (2009), and Stuffers *et al.* (2009).

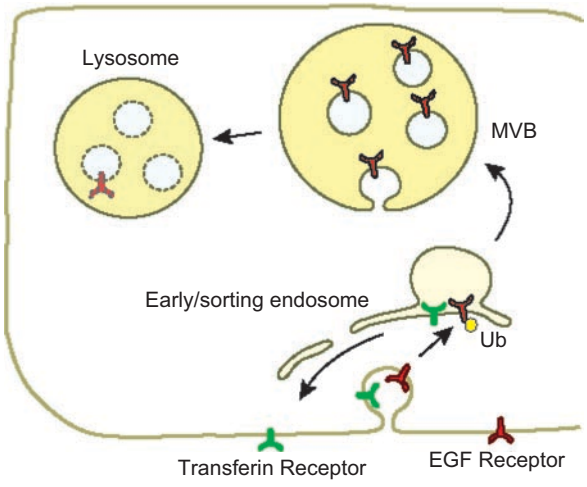

Figure 1. Multivesicular bodies.

Most of the newcomers to the ESCRT field are drawn in by discovering interactions between their favorite (usually metazoan) proteins and ESCRTs, or that the ESCRTs function in the metazoan cellular processes or human diseases that they study. One of the most confounding aspects for such newcomers is that much of the fundamental cell biology, biochemistry, and structural biology have focused on the yeast ESCRTs. Yeast and human ESCRT nomenclature differs. Unfortunately, ESCRT researchers have thus far been unable to reach agreement on a systematic nomenclature across species. Generally yeast-centric terminology will be used in this review, although an exception will be made for ALIX and for the subunits of ESCRT-0, for which the metazoan literature is substantially more extensive. See Table 1 for the conversion between yeast and metazoan nomenclature. While the main functions of the ESCRTs are conserved between yeast and humans, there are also important differences. One of the main goals of this review will be to highlight the similarities and differences between the most important aspects of the yeast and human ESCRTs.

Structure and function of the ESCRT complexes

ESCRT-0

The ESCRT-0 complex is required for sorting plasma membrane proteins into the MVB pathway in animal cells and for MVB biogenesis in yeast. ESCRT-0 binds to and clusters ubiquitinated cargo for delivery into MVBs, and

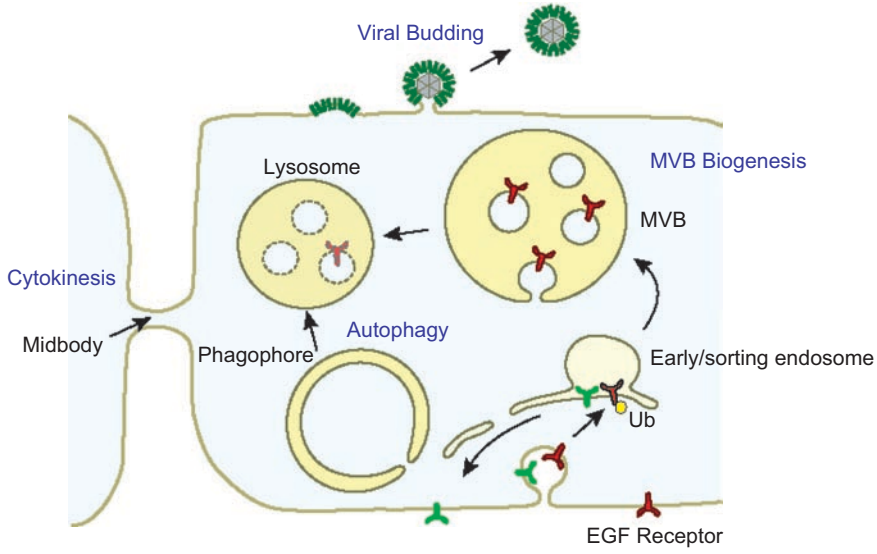

Figure 2. Major cellular functions of the ESCRTs.

recruits clathrin, ubiquitin ligases, and deubiquitinating enzymes, and almost certainly has other functions as well. ESCRT-0 functions as a 1:1 heterodimer (Ren *et al.*, 2009) of the subunits Vps27 and Hse1 (yeast; Figure 3A) (Bilodeau *et al.*, 2002) and Hrs and STAM (metazoa; Figure 3b) (Asao *et al.*, 1997; Bache *et al.*, 2003b). In humans there are two isoforms of the latter, STAM1 and STAM2. Both the Vps27 and Hse1 subunits contain N-terminal ubiquitin-binding VHS domains (Ren and Hurley, 2010). The VHS domain of Vps27 is followed by a FYVE domain (Burd and Emr, 1998; Gaullier *et al.*, 1998; Lohi and Lehto, 1998; Kutateladze *et al.*, 1999; Misra and Hurley, 1999; Misra *et al.*, 2001). The FYVE domains of Vps27 and Hrs bind phosphatidylinositol 3-phosphate with an affinity of 10s of nM (Stahelin *et al.*, 2002) and are responsible for targeting ESCRT-0 to early endosomes (Raiborg *et al.*, 2001b). Two ubiquitin-binding UIM motifs follow the FYVE domain of Vps27 (Bilodeau *et al.*, 2002; Shih *et al.*, 2002; Fisher *et al.*, 2003; Swanson *et al.*, 2003). The domain structure of human Hrs mirrors that of Vps27 in most respects, but the two UIMs of Vps27 are replaced by a single double-sided UIM (DUIM) in Hrs (Hirano *et al.*, 2006a) such that both the human and yeast subunits bind equal numbers of ubiquitin moieties, a total of five each. The multiplicity of ubiquitin binding domains allows ESCRT-0 to bind polyubiquitin chains with high avidity (Ren and Hurley, 2010) and cluster ubiquitinated cargo (Wollert and Hurley, 2010) *in vitro*. Hrs can undergo UIM-dependent autoubiquitination that is thought to result in autoinhibition of the protein's ability to bind ubiquitin (Hoeller *et al.*, 2006). Subsequent to the VHS domain, STAM (and Hse1) contain an SH3 domain that is involved in recruiting deubiquitinating enzymes such as UBPY (Kato *et al.*, 2000).

The two subunits of the ESCRT-0 heterodimerize through an elongated, rigid core consisting of an antiparallel coiled coil and two domain-swapped GAT domains (Prag *et al.*, 2007; Ren *et al.*, 2009). In HeLa cells all of the endogenous Hrs and STAM proteins are incorporated into the ESCRT-0 complex, leaving no pool of free subunits (Ren *et al.*, 2009). When taken out of the context of the assembled ESCRT-0 complex, free Hrs and STAM expose a large region of unpartnered coiled coil that binds promiscuously to many potentially non-physiological partners and leads to the formation of large non-physiological aggregates. As with subunits of ESCRT-I and -II, which are also constitutively assembled, caution must be exercised in drawing conclusions from studies of isolated subunits.

Table 1. ESCRT nomenclature

Complex	Yeast or generic protein	Selected synonyms (yeast)	Metazoan protein (yeast-centric terminology, this review)	Synonyms (metazoan)	Motifs and domains
ESCRT-0	Vps27		VPS27	Hrs,HRS, Hgs	VHS, FYVE, UIM (yeast), DUIM (metazoan), P(S/T)XP, GAT domain and coiled-coil core, clathrin-binding
	Hse1		HSE1	STAM1, 2	VHS, UIM, SH3, GAT domain and coiled-coil core
ESCRT-I	Vps23	Stp22	VPS23	TSG101	UEV, PRD,stalk, headpiece
	Vps28		VPS28		Headpiece, Vps28 CTD
	Vps37	Srn2	VPS37A,B,C,D		Basic helix, stalk, headpiece
	Mvb12		MVB12A,B	UBAP1, LOC390595	Stalk, headpiece ("UMA domain"), MAPB; UBAP1 and LOC290595 are the 3rd and 4th putative human MVB12 isoforms
ESCRT-II	Vps22	Snf8	VPS22	EAP30	Basic helix, winged-helix
	Vps25		VPS25	EAP20	Winged-helix
	Vps36		VPS36	EAP45	Winged-helix, GLUE, NZF1 (yeast), NZF2 (yeast)
ESCRT-III	Vps2	Did4Chm2	VPS2A,B	CHMP2A,B	MIM1
	Vps20	Chm6	VPS20	CHMP6	MIM2
	Vps24		VPS24	CHMP3	Weak MIM1
	Snf7	Vps32	SNF7A,B,C	CHMP4A, B, C	Weak MIM2
	Vps60	Chm5	VPS60	CHMP5	
	Did2	Chm1Vps46	DID2A, B CHMP7	CHMP1A, B	MIM1
	Ist1		IST1		MIM1, MIM2
Vps4–Vta1	V ps4		VPS4A,B	SKD1	MIT, AAA
	Vta1		VTA1	LIP5	MIT
Bro1/ALIX	Bro1	Vps31	ALIX	AIP1	Bro1, V, PRD

The GAT domains involved in heterodimerization do not bind ubiquitin and lack the consensus residues for ubiquitin binding (Prag *et al.*, 2007). The regions of ESCRT-0 subunits C-terminal to the core region are unstructured and contain a number of interaction motifs for other partners, although the function of most of these sequences (apart from their roles as spacers) is unknown. The best studied of these short motifs are P(S/T)XP motifs of Vps27 that bind to the Vps23 subunit of ESCRT-I (Bilodeau *et al.*, 2003; Katzmann *et al.*, 2003; Lu *et al.*, 2003; Pornillos *et al.*, 2003) and a clathrin-binding sequence at the C-terminus of Hrs (Raiborg *et al.*, 2001a). The C-terminal portion of Hse1 is involved in recruiting the ubiquitin ligase Rsp5 (Ren *et al.*, 2007) via an interaction whose counterpart in STAM is uncertain.

ESCRT-I

The ESCRT-I complex co-assembles with ESCRT-II on membranes (Kostelansky *et al.*, 2007) (Figure 4), and these two complexes appear to function as a 1:1 supercomplex (Gill *et al.*, 2007) to bud the limiting membrane of the MVB into its lumen (Wollert and Hurley, 2010). In HIV-1 budding and cytokinesis, where the ESCRTs are not needed for bud formation, ESCRT-I appears to be able act independently of ESCRT-II. In these latter pathways, ESCRT-I is probably important mainly for recruiting ESCRT-III. ESCRT-I is a heterotetramer of one copy each of the subunits Vps23, Vps28, Vps37 (Katzmann *et al.*, 2001) and Mvb12 (Chu *et al.*, 2006; Oestreich *et al.*, 2006b; Curtiss *et al.*, 2006; Audhya *et al.*, 2007; Kostelansky *et al.*, 2007; Morita *et al.*, 2007a). The subunits heterotetramerize through two contiguous but distinct core regions. Vps23, Vps37, and Mvb12 assemble into a 13 nm-long stalk that consists in part of an unusual antiparallel coiled-coil (Kostelansky *et al.*, 2007). Vps23, Vps28, and Vps37 form a headpiece region consisting of three pairs of antiparallel helices, one pair from each subunit, spread out in the shape of a fan (Kostelansky *et al.*, 2006; Teo *et al.*, 2006). Together the stalk and headpiece form a single rigid 18 nm-long structure.

Vps23 (known as TSG101 in humans) has an N-terminal ubiquitin E2 variant (UEV) domain responsible for binding ubiquitinated cargo (Katzmann *et al.*, 2001; Sundquist *et al.*, 2004; Teo *et al.*, 2004b) and P(S/T)XP motifs of ESCRT-0, viral proteins, and other proteins (Bilodeau *et al.*, 2003; Katzmann *et al.*, 2003; Lu *et al.*, 2003; Pornillos *et al.*, 2003). There is a Pro-rich linker region after the UEV domain with a GPPX$_3$Y motif that targets ESCRT-I to the midbody during cytokinesis (Carlton and Martin-Serrano, 2007; Morita *et al.*, 2007b; Lee *et al.*, 2008). The core region of Vps28 is followed by a short linker and a C-terminal four helix bundle domain (CTD) (Pineda-Molina *et al.*, 2006; Gill *et al.*, 2007). The yeast Vps28-CTD is primarily responsible for the 1:1 interaction

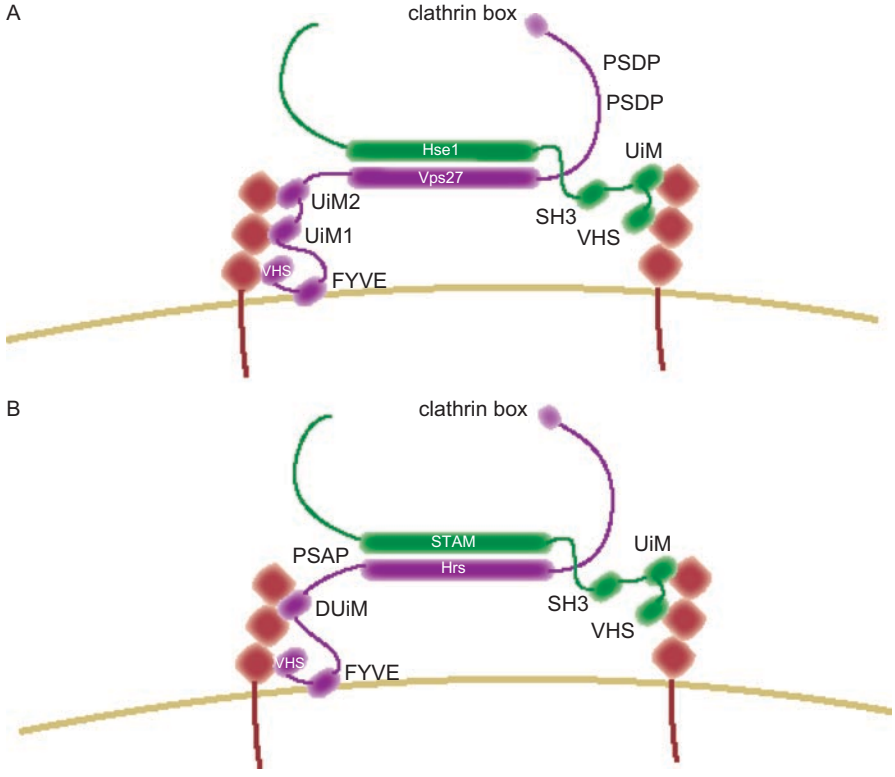

Figure 3. Schematic of the organization of ESCRT-0. (A) yeast; (B) human ESCRT-0 shown docked to a flat, cargo-bearing membrane.

with ESCRT-II (Kostelansky *et al.*, 2006; Gill *et al.*, 2007); the binding site on human ESCRT-I for ESCRT-II is unknown. The Vps28-CTD contains a conserved hydrophobic patch of unknown function; a possible role in interactions with the ESCRT-III subunit Vps20 has been suggested (Pineda-Molina *et al.*, 2006). The N-terminus of yeast Vps37 consists of a basic helix that contributes to membrane binding by ESCRT-I (Kostelansky *et al.*, 2007). Four VPS37 isoforms are present in the human proteome. In addition to two characterized MVB12 isoforms in humans and one in nematodes (Audhya *et al.*, 2007; Morita *et al.*, 2007a), two more putative human MVB12 isoforms have been proposed based on bioinformatics (de Souza and Aravind, 2010). Their sequence similarity to yeast Mvb12 is so low that their identification depended on proteomics and functional characterization, rather than homology. Despite a few clues, much remains to be learned about the functional significance of the multiple VPS37 and MVB12 isoforms in human cells.

ESCRT-II

The ESCRT-II complex is the essential partner of ESCRT-I in MVB biogenesis and bud formation (Babst *et al.*, 2002b; Wollert and Hurley, 2010). Indeed, ESCRT-II is probably the more important of the two complexes in MVB biogenesis, since overexpression of ESCRT-II can rescue deletions of ESCRT-I genes in yeast, but not vice versa (Babst *et al.*, 2002b). Despite its central role in MVB biogenesis and membrane budding, ESCRT-II appears to be non-essential for HIV-1 budding (Langelier *et al.*, 2006) and cytokinesis (Morita *et al.*, 2007b). In addition to playing a central role in membrane bud formation, like ESCRT-0 and -I, ESCRT-II binds ubiquitinated cargo, although only at one site (Alam *et al.*, 2004). ESCRT-II has a pivotal role in MVB biogenesis in bridging the upstream ubiquitin-binding ESCRT complexes to the downstream ESCRT-III machinery involved in membrane scission. ESCRT-II is probably responsible for connecting MVBs to microtubules via RILP, Rab7, and dynein (Progida *et al.*, 2007; Wang and Hong, 2006).

ESCRT-II is a Y-shaped 1:2:1 heterotetramer of the subunits Vps22, Vps25, and Vps36 (Hierro *et al.*, 2004; Teo *et al.*, 2004a; Im and Hurley, 2008) (Figure 4). Despite lacking sequence homology, the bulk of each of these subunits consists of two tandem winged-helix (WH) motifs. In other contexts, WH motifs are usually

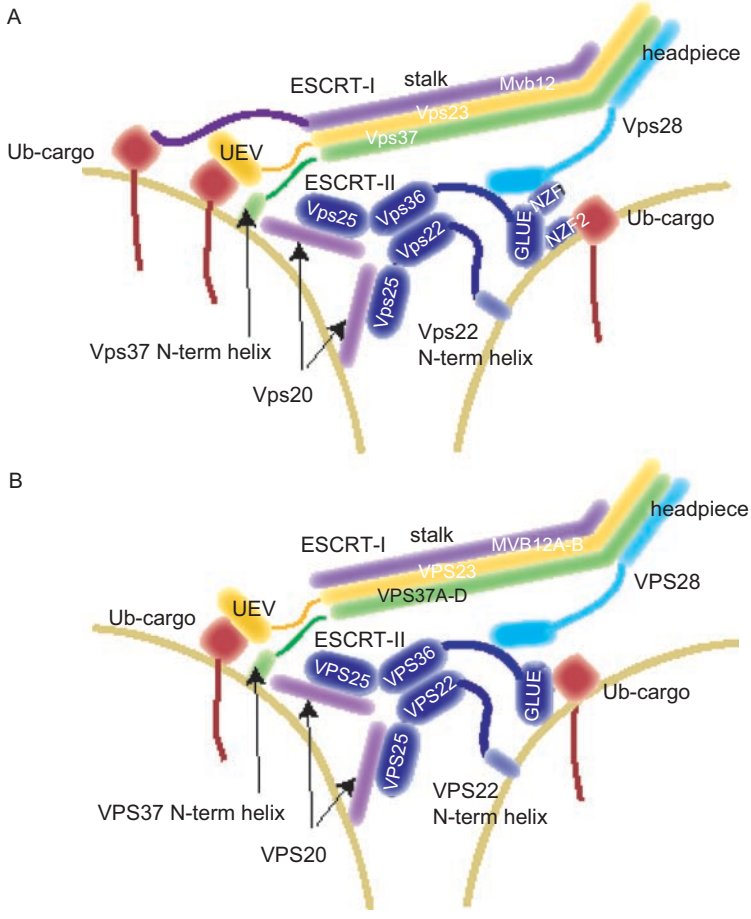

Figure 4. Schematic of the organization of ESCRT-I and –II. (A) yeast; (B) human ESCRT-I and -II depicted as a supercomplex assembled at a membrane neck.

involved in DNA binding, but there is no evidence that ESCRT-II binds DNA. ESCRT-II does bind RNA (Irion and St Johnston, 2007), but the RNA-binding activity, described below, does not involve the WH domains. The WH domain core regions of Vps22 and Vps36 form an extensive interface with one another, and these two subunits probably require one another for folding and stability. The two Vps25 subunits are more loosely associated with the Vps22–Vps36 subcomplex, and they do not contact each other. The tips of the second WH domain (WH2) of the Vps25 subunits are the locus for binding to the ESCRT-III subunit Vps20 (Im *et al.*, 2009). Both copies of Vps25 are essential for function (Hierro *et al.*, 2004; Teis *et al.*, 2010). Similar to Vps37 of ESCRT-I, the N-terminus of Vps22 consists of a basic helix that is important for membrane targeting (Im and Hurley, 2008), albeit without specificity for any particular endosomal lipid. The N-terminus of yeast Vps36 is complex. This region contains a variant pleckstrin homology (PH) domain that is referred to as a "GLUE" domain (Slagsvold *et al.*, 2005; Teo *et al.*, 2006). The GLUE domain binds preferentially to phosphatidylinositol 3-phosphate (Teo *et al.*, 2006), although it also binds to other phosphoinositides, as detailed below. Two Npl4 type zinc fingers, NZF1 and NZF2, are inserted within a loop in the yeast GLUE domain. NZF1 is the locus for binding to the ESCRT-I Vps28-CTD (Gill *et al.*, 2007), while NZF2 binds to a single ubiquitin moiety (Alam *et al.*, 2004). Mammalian VPS36 contains a GLUE domain as well, but without any zinc finger insertion. In mammalian VPS36, the GLUE domain itself binds directly to ubiquitin (Slagsvold *et al.*, 2005; Alam *et al.*, 2006; Hirano *et al.*, 2006b). In yeast Vps36, the GLUE-WH1 linker comprises a secondary binding site for ESCRT-I (Im and Hurley, 2008). This region is conserved in human VPS36 and could be the locus for binding to human ESCRT-I, although this has not been verified.

ESCRT-III

The ESCRT-III complex is the central membrane scission machine at the heart of the ESCRT system (Wollert *et al.*, 2009a). Of all the ESCRT complexes, only homologs of ESCRT-III and Vps4 (described below) are present in Archaeal proteomes, where they have a role in cell division (Lindas *et al.*, 2008; Samson *et al.*, 2008). In contrast to ESCRTs 0–II, ESCRT-III subunits contain no known ubiquitin binding domains, and exist in cytosol as inactive monomers (or possibly heterodimers) (Babst *et al.*, 2002a). The subunits polymerize into the active ESCRT-III complex only on the membrane (Babst *et al.*, 2002a). This assembly is detergent insoluble and relatively intractable to most biochemical methods.

In yeast, the core subunits required for function are Vps20, Snf7, Vps24, and Vps2, which assemble in that order (Teis *et al.*, 2008) (Figure 5). Vps20, Snf7, and Vps24 alone are sufficient for membrane scission (Wollert *et al.*, 2009a), while Vps2 is required for coupling to the Vps4 recycling machinery. The other yeast ESCRT-III subunits, Did2 (Nickerson *et al.*, 2006), Ist1 (Dimaano *et al.*, 2008; Rue *et al.*, 2008), and Vps60 are not strictly essential for function and appear to assemble with the rest of ESCRT-III at a late stage. Did2 (Nickerson *et al.*, 2006) and Vps60 help recruit and activate the Vps4–Vta1 complex for recycling, while Ist1 inhibits Vps4 activity (Dimaano *et al.*, 2008). Vps20–Snf7, Vps24–Vps2 (Babst *et al.*, 2002a), and Did2–Ist1 (Rue *et al.*, 2008; Xiao *et al.*, 2009) form preferential binary pairings with one another. Vps60 is the odd man out in that it binds more tightly to Vta1 (see below) than to any of its fellow ESCRT-III proteins (Azmi *et al.*, 2008; Bowers *et al.*, 2004; Shiflett *et al.*, 2004; Shim *et al.*, 2008). These pairings do not appear to dictate the stoichiometry of assembly. The precise stoichiometry of ESCRT-III is not known, and it is not clear whether or not it is strictly defined. At least in yeast, Snf7 is the dominant subunit, present at several-fold higher copy number than any other subunit, including Vps20 (Teis *et al.*, 2008). Certain ESCRT-III subunits either alone (Snf7) or in binary combinations (Vps24 + Vps2; Ist1 + Did2) form helical tubes of dimensions comparable to those of the necks of HIV-1 buds or the intralumenal vesicles (ILVs) of MVBs (Hanson *et al.*, 2008; Lata *et al.*, 2008b; Bajorek *et al.*, 2009b).

All ESCRT-III subunits contain an electrically polarized five-helix core (Muziol *et al.*, 2006). The first two helices are basic and bind strongly to acidic membranes, while helices 3–5 are acidic. More variable regions are found C-terminal to the five-helix core. In all cases studied the C-terminal region autoinhibits assembly of the ESCRT-III complex and helps maintain the soluble monomeric state (Zamborlini *et al.*, 2006; Shim *et al.*, 2007; Lata *et al.*, 2008a). Truncations of these regions strongly promote

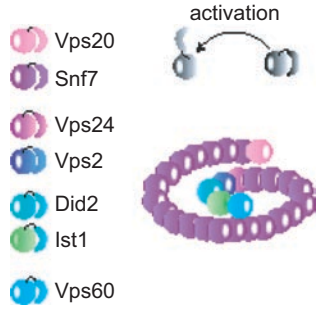

Figure 5. Speculative schematic of the organization of ESCRT-III. The assembly is depicted as a spiral on the basis of the EM images of (Hanson *et al.*, 2008) and for simplicity. The actual assembly is more likely dome-shaped rather than flat (Fabrikant *et al.*, 2009). The order of assembly of the first four subunits (beginning with Vps20 at the outside of the spiral), and the preponderance of Snf7 subunits shown is based on the observations of Teis *et al.* (2008).

complex assembly (Zamborlini *et al.*, 2006; Shim *et al.*, 2007), as does membrane binding and ESCRT-II binding to Vps20 (Saksena *et al.*, 2009). The extreme C-terminal regions of various subunits contain the MIT-domain-interacting motifs-1 and -2 (MIM1) (Vps24, Vps2, Did2, Ist1) and (MIM2) (Vps20, Snf7, Ist1) motifs that bind to two distinct faces of the Vps4 MIT domain. Human DID2B contains a specialized MIM1 variant that binds to both Vps4 and the MIT domain of the microtubule severing enzyme spastin. These regions also contain sequences that are less well defined at this point and bind a spectrum of MIT domains, described below. The C-terminus of Snf7 contains a motif that binds to the Bro1 domain of Bro1 (McCullough *et al.*, 2008).

Vps4–Vta1

Disassembly of the membrane-bound ESCRT-III complex is required to finish the ESCRT cycle and replenish the cytosolic pool of ESCRT-III subunits. The AAA ATPase Vps4 solubilizes ESCRT-III subunits at the cost of ATP hydrolysis (Figure 6). ATP consumption by Vps4 is the main thermodynamic driving force for the ESCRT cycle. The domain structure of Vps4 consists of an N-terminal ESCRT-III-binding MIT domain (Scott *et al.*, 2005b), a flexible linker, a large ATPase mixed-α/β domain, a small ATPase helical domain, a β-domain, and a C-terminal helix (Scott *et al.*, 2005a; Xiao *et al.*, 2007; Gonciarz *et al.*, 2008). Vps4 functions as an oligomer that under most conditions and by most reports is a dodecamer (Inoue *et al.*, 2008; Yu *et al.*, 2008; Landsberg *et al.*, 2009), although a tetradecameric form has been reported as well (Hartmann *et al.*, 2008). The dodecamer consists of two conformationally distinct hexameric rings (Yu *et al.*, 2008). The lower ring has a constricted pore and has been modeled based on the structure of the p97 D1 domain hexamer. The upper ring has a wider pore but its structure is not known in detail due to the limited resolution of the EM study and the lack of a closely related crystal structure. The central pore is required for function (Scott *et al.*, 2005a; Gonciarz *et al.*, 2008). ESCRT-III subunits physically contact pore residues during disassembly, but whether they pass all the way through the pore is uncertain.

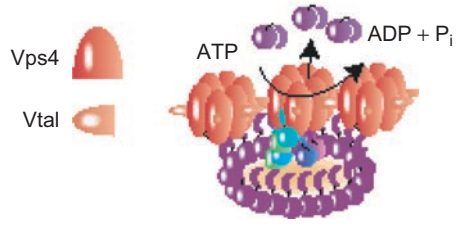

Figure 6. Schematic of the organization of Vps4-Vta1.

Vta1 is an ESCRT protein that binds to Vps4 and promotes its oligomerization, activity, and ESCRT-III binding (Yeo *et al.*, 2003; Shiflett *et al.*, 2004; Ward *et al.*, 2005; Lottridge *et al.*, 2006; Shestakova *et al.*, 2010). Although Vta1 is not constitutively associated with Vps4 in cytosol as are the subunits of ESCRT-0, -I, and -II, for practical purposes Vps4 and Vta1 function together as a complex in MVB biogenesis, HIV-1 budding, and probably other Vps4-dependent pathways. The deletion of Vta1 does not cause as severe of a defect in cargo sorting as deletion of Vps4 (Yeo *et al.*, 2003; Shiflett *et al.*, 2004; Lottridge *et al.*, 2006). Vta1 accelerates Vps4 ATPase activity (Azmi *et al.*, 2006; Lottridge *et al.*, 2006) and oligomerization *in vitro*, and promotes ESCRT-III disassembly in vitro (Azmi *et al.*, 2008). Vta1 binds to Vps4 via the β domain of the latter protein (Scott *et al.*, 2005a; Yang and Hurley, 2010). Vta1 contains two MIT domains at its N-terminus (Xiao *et al.*, 2008), both so divergent in sequence from other MIT domains that they were only identified after their crystal structures were solved. Vta1 is a dimer, with the subunits associating via the Vps4-binding VSL (Vps4, SBP1, LIP5) domain at the C-terminus of the protein (Azmi *et al.*, 2006; Xiao *et al.*, 2008). The Vta1 dimer appears to be tight and constitutive, and the dimer contact residues are required for its function. A long flexible linker connects the MIT domains to the VSL region. The Vta1 MIT domains interact with various ESCRT-III proteins, but especially strongly with the late-acting ESCRT-III protein Vps60 (Bowers *et al.*, 2004; Shiflett *et al.*, 2004; Azmi *et al.*, 2008; Shim *et al.*, 2008). Given that Vps60 appears to bind more tightly to Vta1 than to any of the ESCRT-III subunits, it seems reasonable to think of Vps60 as an adaptor for Vta1 to interact with the ESCRT-III assembly (Nickerson *et al.*, 2010).

The assembly of the Vps4–Vta1 complex has been intensively studied, yet it is still a source of mystery. A 37 Å resolution EM reconstruction shows that the two hexameric rings of Vps4 are non-equivalent, and that the lower ring surrounds a large pore (Yu *et al.,* 2008). Incorporation of Vta1 into the assembly leads to the appearance of additional densities at several regions around the structure, but it is not clear which densities belong to Vta1 itself and which reflect conformational changes in Vps4 induced by Vta1 binding. The β domain of Vps4, to which Vta1 binds, projects outward from the six tips of the lower ring of the hexamer, providing potentially up to six binding sites for Vta1 dimers at the outer edges of the two rings. The orientation of the β domain in the upper ring is not known. The reported 6:12 stoichiometry of Vta1 to Vps4 is hard to reconcile with current understanding of the symmetry of the two molecules and the number of binding sites. It has been suggested that Vta1 dimers could crosslink multiple Vps4 dodecamers in a lattice like arrangement (Yang and Hurley, 2010), but direct confirmation of this idea is lacking. Thus a higher

resolution structure of the complete Vps4–Vta1 assembly is urgently needed.

The mechanism of ESCRT-III disassembly is understood only in the most general outlines. The Vps4 MIT domains bind to the substrates. The high affinity interaction between the Vps2 MIM1 and the Vps4 MIT appears to be particularly critical. Recycling cannot be observed *in vitro* without either Vps2 (Lata *et al.*, 2008b; Wollert *et al.*, 2009a) or at least a chimeric Vps24 bearing a Vps2 MIM1 at its C-terminus (Ghazi-Tabatabai *et al.*, 2008). Even the very low affinity interactions of the Vps4 MIT domain with the Snf7 MIM2 are important for function (Shestakova *et al.*, 2010). After all, Snf7 is the most abundant ESCRT-III subunit in the lattice. Residues of the central pore of Vps4 are required for the disassembly function (Scott *et al.*, 2005a), but more direct evidence that ESCRT-III subunits physically occupy the pore at some point during disassembly is lacking. However it is hard to envision how the disassembly reaction could proceed without the bulk of the dodecamer acting as a rigid fulcrum to pry apart the ESCRT-III subunits. This might involve direct ESCRT-III pore interactions, or could be less direct if the MIT domain were physically in contact with both the pore and the ESCRT-III subunits simultaneously. The biochemical roles of the late-acting ESCRT-III subunits Did2 and Ist1 raise further questions. These two subunits preferentially associate, yet Did2 is a key activator of disassembly, while Ist1 seems to inhibit Vps4 via an unknown mechanism. Mutations in these subunits are among the few defects in the ESCRT pathway that perturb the size of ILVs (Nickerson *et al.*, 2006; 2010), and the mechanism behind their unusual phenotypes is obscure. Given these open questions, the ESCRT-III disassembly mechanism is likely to remain one of the most active areas of mechanistic ESCRT research in the next few years.

Bro1/ALIX

In yeast, Bro1 is not strictly required for MVB biogenesis, and seems to be mainly involved in promoting the recruitment and activity of the deubiquitinating enzyme Doa4 (Luhtala and Odorizzi, 2004) to the assembling ESCRT-III complex. By the same token, the mammalian counterpart of Bro1, ALIX, seems to be dispensable for the MVB targeting of the canonical cargo EGFR. However, ALIX has a central and required role in targeting the ESCRT machinery to the midbody for membrane abscission in cytokinesis, and is essential for the budding of certain viruses. ALIX was initially isolated based on its association with an apoptosis-related protein, ALG-2 (Missotten *et al.*, 1999; Vito *et al.*, 1999). It remains a mystery whether ALIX in some way serves to connect the apoptosis and MVB pathways. ALIX also has a range of interactions with endocytic and cytoskeletal components whose connection to the ESCRT machinery is unclear (Odorizzi, 2006). ALIX seems to be connected somehow to the lipid lysobisphosphatidic acid (LBPA), which is enriched in mammalian late endosomes and lysosomes (Matsuo *et al.*, 2004), although direct binding of ALIX and LBPA has not been shown.

ALIX has a tripartite domain structure consisting of an N-terminal Bro1 domain, a central V domain, and a flexible C-terminal proline-rich domain (PRD) (Figure 7). The Bro1 domain is shaped like a banana (Kim *et al.*, 2005), and binds to the C-terminal region of Snf7 through a conserved patch near the center of its concave face (Kim *et al.*, 2005; McCullough *et al.*, 2008). The V domain consists of two helical arms joined so as to form the shape of the letter "V". The V domain binds to YPXL motif sequences at a site on one of the arms facing into the crevice of the V-shape (Fisher *et al.*, 2007; Lee, S *et al.*, 2007; Zhai *et al.*, 2008). The isolated V domain undergoes a monomer–dimer equilibrium (Lee, S *et al.*, 2007) and is responsible for dimerizing the intact ALIX molecule (Pires *et al.*, 2009).

The Alix PRD comprises a remarkably concentrated zone of interaction motifs. The overlapping motifs that bind the midbody protein CEP55 (Lee *et al.*, 2008) and the apoptotic protein ALG-2 (Suzuki *et al.*, 2008) have been co-crystallized and visualized with the interaction partners. The Alix PRD contains a P(S/T)XP motif that interacts with ESCRT-I, but this motif is dispensable for function, at least in the setting of HIV-1 budding (Fisher *et al.*, 2007). The polyproline tract responsible for endophilin binding is also dispensable for the HIV-1 budding activity (Fisher *et al.*, 2007; Usami *et al.*, 2007). Finally, the PRD autoinhibits the ability of the intact protein to bind SNF7 (Zhou *et al.*, 2008).

ESCRT interactions and functional domains

ESCRT–ubiquitin interactions

By recent counts, roughly 20 classes of ubiquitin binding domains (UBDs) have been identified (Dikic *et al.*, 2009; Hurley *et al.*, 2006) in eukaryotic proteomes. The ESCRT system is a microcosm of this richness, making use of no fewer than six types of UBD: the VHS domain;

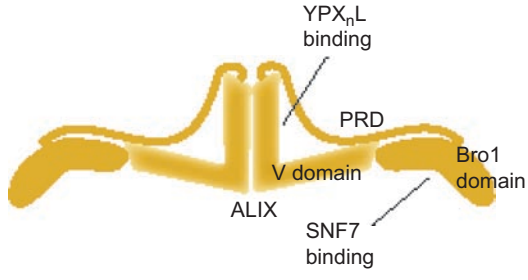

Figure 7. Schematic of the organization of Bro1.

the UIM and its variant the DUIM; the UEV domain; the Mvb12 C-terminal domain; the GLUE domain; and the NZF domain (Figure 8). All of these domains interact with low affinity (K_d > 100 μM) with monoubiquitin via Ile44 hydrophobic patch on the ubiquitin surface. This implies that the binding of any one ESCRT UBD to a ubiquitin moiety excludes the binding of that moiety to another ESCRT UBD. It also implies that the UBDs must cooperate either with one another or with other domains to increase their affinity for cargo, since ubiquitinated substrate proteins are present at bulk concentrations far lower than the 100s of μM in cells.

VHS domains are eight-helix bundles that contain a ubiquitin binding site formed by hydrophobic residues on helices 2 and 4 (Hong *et al.*, 2009). These residues, in particular a Leu and a Trp from helix 2, are conserved in the VHS domains of ESCRT-0 subunits from yeast to mammals (Ren and Hurley, 2010). ESCRT-0 VHS domain

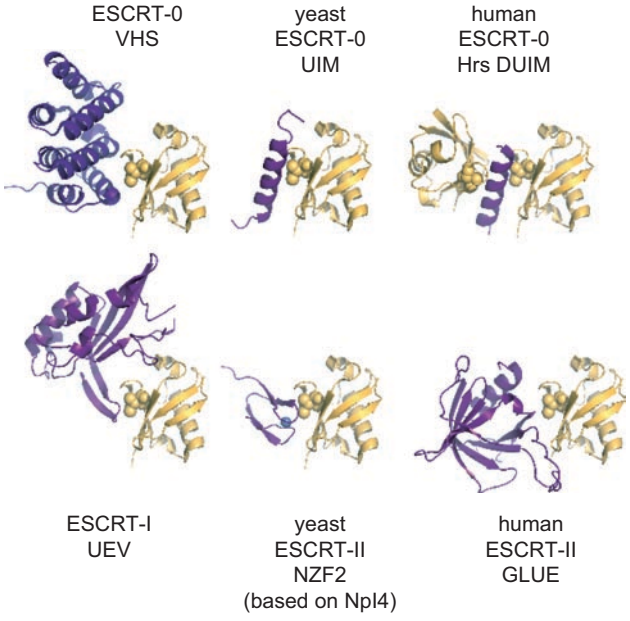

Figure 8. Ubiquitin binding domains. Ubiquitin is shown in yellow with Ile44 shown with space-filling spheres. UBDs are shown in blue. The VHS domain complex is shown for the human STAM subunit of ESCRT-0 (pdb entry 3LDZ) but is representative of all of the yeast and human ESCRT VHS domains. The UIM is shown for yeast ESCRT-0 subunit Vps27 (1Q0W) and is probably also representative of other "single" or conventional UIMs in the yeast ESCRT-0 Hse1 subunit and the human ESCRT-0 STAM subunit (Figure 3). The DUIM is shown for human ESCRT-0 subunit Hrs (2D3G). The UEV domain is shown for human ESCRT-I (1S1Q) and is also representative of the yeast UEV-ubiquitin complex. The structure of the yeast Mvb12 C-terminal UBD in complex with ubiquitin has not been reported. The structure of the NZF of Npl4 is shown (1Q5W), as a reasonable representation of the yeast ESCRT-II NZF2-ubiquitin interaction, as no structure is available for the latter. This domain is not present in human ESCRT-II. The structure of the GLUE domain of human ESCRT-II is shown (2HTH); the yeast GLUE domain does not bind ubiquitin.

binds ubiquitin at a single site with affinities of K_d = ~100 μM to ~2 mM. Yeast ESCRT-0 contains a total of three ubiquitin interacting motifs (UIMs). UIMs are among the simplest class of UBD, consisting of a single Leu and Ala-rich α-helix. The yeast ESCRT-0 UIMs bind ubiquitin with ~200 to 300 μM affinities (Fisher *et al.*, 2003). Human ESCRT-0 contains one conventional UIM and one double-sided UIM (DUIM). DUIMs contain two interlaced ubiquitin binding motifs that allow a single α-helix to bind two different ubiquitin moieties, one on each side of the helix (Hirano *et al.*, 2006a). Thus both yeast and human ESCRT-0 contain a total of five ubiquitin binding sites. None of the ESCRT-0 UBDs are individually essential for function, but inactivation of more than two out of the five cripples the sorting function of ESCRT-0 in yeast (Ren and Hurley, 2010).

ESCRT-I from all species contains a UEV domain at the N-terminus of the Vps23 subunit, which binds one ubiquitin moiety (Sundquist *et al.*, 2004; Teo *et al.*, 2004b). Yeast Mvb12 has a short C-terminal sequence that binds ubiquitin (Shields *et al.*, 2009), again in close proximity in three dimensions to the Vps23 UEV domain such that it could potentially cooperate in polyubiquitin binding. Finally, yeast and human ESCRT-II both contain a single ubiquitin-binding site, but they bind ubiquitin in different ways. The yeast Vps36 GLUE domain contains two Npl4 zinc fingers inserted within the β6–β7 loop. The second zinc finger (NZF2) of Vps36 binds a single ubiquitin moiety (Alam *et al.*, 2004). Human VPS36 has a simpler structure than its yeast ortholog, and is missing the two NZF domains. However, the GLUE domain of human VPS36 has acquired the ability to bind ubiquitin directly, with an affinity of 105 μM (Alam *et al.*, 2006), slightly tighter than that of the yeast NZF2. None of the UBDs of yeast ESCRT-I and -II is individually essential, but they contribute to cargo sorting in a cooperative manner, and multiple deletions shut down ubiquitinated cargo sorting (Shields *et al.*, 2009). The remaining ESCRT complexes ESCRT-III, Vps4–Vta1, and Bro1, are not known to bind ubiquitin.

The very low affinities of ESCRT UBDs for ubiquitin monomers raise the question of what there function is in sorting cargoes that are typically present in cells at nM concentrations. There is growing evidence that in many or most cases Lys63-linked polyubiquitination is involved in the trafficking of many or most cargoes into the ESCRT pathway. In yeast the vacuolar hydrolase Cps1 requires K63 polyubiquitination to transit from the Golgi into the MVB pathway, and the plasma membrane amino acid transporter Gap1 requires polyubiquitination for its sorting from early endosomes into MVBs (Lauwers *et al.*, 2009). Many other yeast transporters require polyubiquitination for their vacuolar sorting (Lauwers *et al.*, 2010), and the ESCRT-dependence of this process can be reasonably assumed, even if not demonstrated explicitly.

In human cells, the majority of ubiquitin attached to the ESCRT substrate EGFR occurs in the form of K63-linked chains (Huang *et al.*, 2006). While the K63-linked polyubiquitin dependence of ESCRT trafficking has only been rigorously demonstrated in a few cases, the observation of polyubiquitination of most of the relevant cargoes has led a general impression that K63 polyubiquitination is probably a major signal for cargo entry into the pathway. Typically chains lengths of 2–3 ubiquitin moieties have been observed (Lauwers *et al.*, 2010), but the chain length requirements have not been tested rigorously. ESCRT-0 binds to K63-linked di- and tetraubiquitin with affinities in the tens of μM (Ren and Hurley). The reduction of dimensionality from three to two on the membrane is probably a more compelling solution to the apparent paradox posed by the low affinity of ESCRT UBDs for ubiquitin in solution. When ubiquitin is tethered to membranes *in vitro*, ESCRT-0, -I, and -II can all be seen to colocalize with ubiquitin at the biologically plausible bulk concentrations of 65 nM for ubiquitin and 15 nM for ESCRTs (Wollert and Hurley, 2010).

ESCRT–lipid interactions

The endosomal lipid phosphatidylinositol 3-phosphate (PI(3)P) plays a pivotal role in the ESCRT pathway. PI(3)P binds to the FYVE domain of the Hrs/Vps27 subunit of ESCRT-0 with high affinity (Sankaran *et al.*, 2001; Stahelin *et al.*, 2002; Figure 9), and is essential for recruitment of ESCRT-0 to endosomes (Raiborg *et al.*, 2001b; Katzmann *et al.*, 2003). ESCRT-I has a low affinity for membranes on its own (Kostelansky *et al.*, 2007), and depends on protein–protein interactions to recruit it to and stabilize it at membranes. ESCRT-II binds preferentially to PI(3)P but – in contrast to the highly specific Hrs-PI(3)P interaction – also binds to other phosphoinositides via its GLUE domain (Slagsvold *et al.*, 2005; Teo *et al.*, 2006). The affinity of ESCRT-II for membranes is further enhanced by a non-specific but functionally important interaction between the basic N-terminal helix of the Vps22 subunit and acidic membranes lipids (Im and Hurley, 2008). ESCRT-III subunits bind to acidic membrane lipids with little or no specificity through a broad basic patch (Muziol *et al.*, 2006; Lata *et al.*, 2008b). There has been one report that Vps24 binds preferentially to $PI(3,5)P_2$ (Whitley *et al.*, 2003), but *in vitro*, this lipid has a minimal effect on the activity of Vps24 (Wollert *et al.*, 2009a).

MIT domains and MIT-interacting motifs

Microtubule-interacting and transport (MIT) domains were so named prior to the discovery of their main function in the ESCRT pathway: as modules that interact with C-terminal MIT domain-interacting motifs (MIMs) of ESCRT-III subunits (Obita *et al.*, 2007; Stuchell-Brereton *et al.*, 2007; Hurley and Yang, 2008; Kieffer *et al.*, 2008; Yang *et al.*, 2008). MIT domains allow the Vps4–Vta1 recycling machinery to engage with its substrate, the ESCRT-III complex. They also provide the basis for a host of ESCRT-III effectors to bind to activated, open-conformation ESCRT-III subunits. The effectors include microtubule severing enzymes (Yang *et al.*, 2008; Connell *et al.*, 2009), and deubiquitinating enzymes (Agromayor and Martin-Serrano, 2006; Tsang *et al.*, 2006; Ma *et al.*, 2007; Row *et al.*, 2007), among many others (Tsang *et al.*, 2006; Hurley and Yang, 2008). MIT domains are three-helix bundles (Scott *et al.*, 2005b) that bind to ESCRT-III MIMs via grooves between the helices (Obita *et al.*, 2007; Stuchell-Brereton *et al.*, 2007; Kieffer *et al.*, 2008; Yang *et al.*, 2008) (Figure 10). The MIT domain family is remarkably divergent in sequence space. Some MIT domains are so divergent in sequence that they have only been identified as such by three-dimensional structure determination (Xiao *et al.*, 2008) or by exceptionally sensitive bioinformatics analyses (Tsang *et al.*, 2006).

The MIT domain of Vps4 binds the helical MIM1 motif between helices α2 and α3 (Obita *et al.*, 2007; Stuchell-Brereton *et al.*, 2007) and the extended (non-helical) MIM2 motif between α1 and α3 (Kieffer *et al.*, 2008).

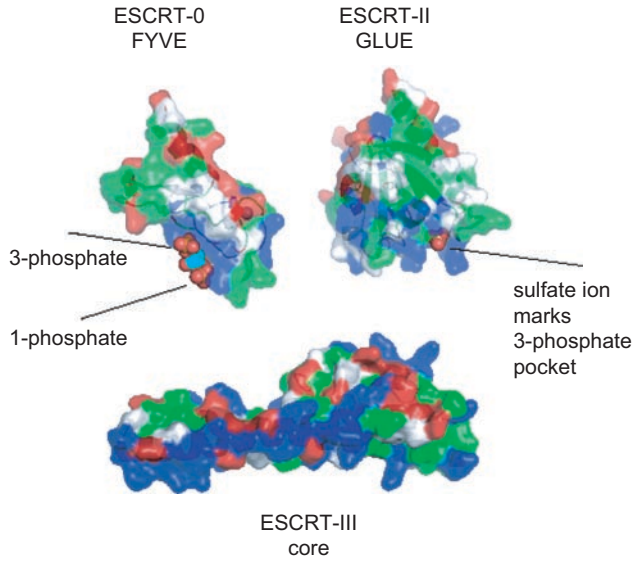

Figure 9. Membrane binding domains. Protein surfaces are colored green (hydrophobic residues), white (uncharged polar residues), red (acidic residues), and blue (basic residues). Structures are oriented such that the plane of the membrane is beneath each structure. The FYVE domain of ESCRT-0 (1VFY) targets phosphatidylinositol 3-phosphate, shown here as the soluble headgroup inositol 1,3-bisphosphate docked onto to the unliganded Vps27 FYVE domain structure from the liganded EEA1 FYVE structure (1JOC). The GLUE domain of yeast ESCRT-II (2CAY) is shown, with the non-canonical phosphoinositide binding pocket marked by a bound sulfate ion. The α1-α4 membrane binding core of the ESCRT-III subunit VPS24 (2GD5) is shown, with the autoinhibitory helix omitted.

The microtubule-severing enzyme spastin contains an N-terminal MIT domain that binds only to DID2B and IST1 (Yang *et al.*, 2008; Connell *et al.*, 2009). DID2B and IST1 contain MIM1 motifs that have an additional two turns of helix N-terminal to the canonical MIM1. These two turns contain several key small amino acid residues that are sterically compatible with a constricted binding site on the spastin-MIT domain. The extended MIM1 binding site on spastin-MIT is located between helices α1 and α3, like the Vps4-MIT MIM2 binding site. These differences show how hard it will be to predict the recognition specificity of MIT domains from sequence-gazing. Despite their small size in a structural sense, the substrate binding repertoire of the MIT domain family is remarkably rich. The large number of MIT domain-containing effector proteins in mammalian proteome explains, in part, the expansion of the number of ESCRT-III isoforms in mammalian cells. In terms of the details, most of the MIT-MIM recognition code, and its functional significance, remains to be elucidated.

Bro1 domains

Bro1 domains have several parallels to MIT domains. Structurally, they consist mainly of a tetratricopeptide repeat (TPR) domain (Kim *et al.*, 2005), although the Bro1 domain is far larger than the MIT domain, at ~370 residues versus ~90 residues for the MIT domain (Figure 11). They both bind to C-terminal helical motifs of ESCRT-III subunits (Kim *et al.*, 2005; McCullough *et al.*, 2008). In contrast to the diversity in structure, sequence, and specificity of MIT domains, the Bro1 domain family is well conserved and has only one known ESCRT-III interaction partner, Snf7. A hydrophobic patch surrounding a conserved Ile on the Bro1 domain (Kim *et al.*, 2005) contacts exposed hydrophobic residues from the C-terminal helix of Snf7 (McCullough *et al.*, 2008) (Figure 11). Bro1 domains have a second conserved patch centered on a Tyr that protrudes from one end of the structure (Kim *et al.*, 2005). The Tyr is phosphorylated in human cells, leading to the binding of this region to the SH2 domain of Src. Src phoshorylation of the PRD modulates interactions with endocytic and signaling proteins (Schmidt *et al.*, 2005), but whether it modulates the ESCRT pathway is unknown. Bro1 domains occur in two proteins in yeast, Bro1 and Rim20. The only identified function of Bro1 in yeast is to cooperate with Snf7 in the recruitment and activation of the deubiquitinating enzyme Doa4 to deubiquitinate cargo prior to ILV scission. The pH-sensing pathway in yeast converges with the ESCRT pathway at Snf7, which interacts with the Bro1 domain of Rim20 (Boysen and Mitchell, 2006). The human proteome includes the Bro1 domain proteins ALIX, discussed above, and rhophilin, Brox, and HD-PTP.

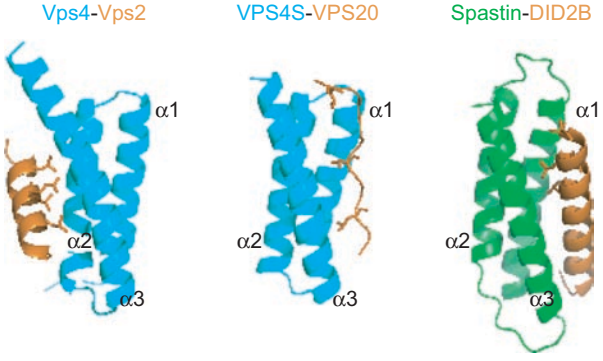

Figure 10. MIT domain-MIM complexes. The MIT domain of yeast Vps4(cyan) is shown bound to the MIM1 of Vps2 (2V6X). The MIT domain of human VPS4A (cyan) is shown in complex with the MIM2 of human VPS20 (2K3W). The MIT domain of human spastin (blue) is shown bound to the extended MIM1 of human DID2B (3EAB). MIMs are orange.

Cellular functions of the ESCRTs

ESCRTs sort ubiquitinated plasma membrane proteins to the lysosome

The central observation from the earliest analyses of ESCRTs is that all of the complexes, with the exception of ESCRT-III, contain ubiquitin-binding domains (Katzmann *et al.*, 2001; 2003; Bilodeau *et al.*, 2002; Raiborg *et al.*, 2002; Shih *et al.*, 2002; Bache *et al.*, 2003b; Mizuno *et al.*, 2003; Urbe *et al.*, 2003; Alam *et al.*, 2004). This binding provided a pivotal link between genes involved in transport through MVBs and the then-emerging role of ubiquitination as the major signal for sorting to the yeast vacuole or mammalian lysosome (Hicke, 2001). While animal cells appear to have multiple mechanisms for the formation of MVBs and MVB-like structures, some independent of the ESCRTs (Theos *et al.*, 2006; Trajkovic *et al.*, 2008), the only known route for lysosomal degradation of

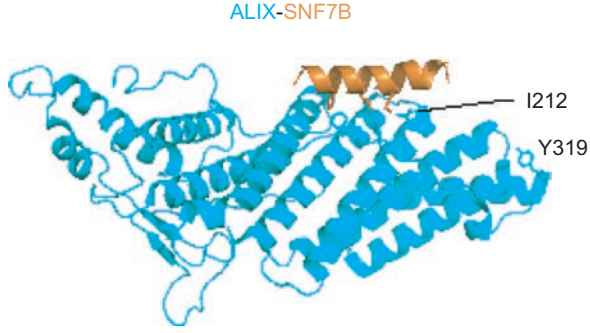

Figure 11. The Bro1 domain. The structure of the Bro1 domain of ALIX (cyan) is shown in complex with SNF7B (orange, 3C3Q). Conserved interaction residues are highlighted in stick models, and the Src binding site is also shown.

ubiquitinated plasma membrane proteins is the ESCRT pathway.

Historically, the initial observation that eventually led to the discovery of the ESCRTs was the ligand-dependent sorting of the EGF receptor (EGFR) in MVBs. Direct confirmation of a role for the ESCRTs in sorting EGFR to MVBs came from analysis of the human ESCRT-I subunit VP23 (aka TSG101, Table 1) (Babst *et al.*, 2000; Lu *et al.*, 2003) and VPS28 (Bishop *et al.*, 2002), and the ESCRT-0 subunit Hrs (Chin *et al.*, 2001; Bishop *et al.*, 2002; Lu *et al.*, 2003). Precise criteria for ESCRT cargoes have not been defined. The sorting of the best-characterized cargoes, exemplified by EGFR, has been examined by electron microscopy in the context of multiple cell types and the knockdown and overexpression of various ESCRT components. In many other cases, the turnover of the cargo is affected by depletion or overexpression of a handful of ESCRT proteins for which reagents have been widely disseminated, included Hrs, TSG101, and VPS4 in animal cells, or by deletion of class E *VPS* genes in yeast. For an even larger number of less well-characterized putative cargoes, ubiquitin-dependent turnover of plasma membrane proteins is reasonably assumed to signify ESCRT-dependent lysosomal degradation, since no other mechanism is known for the turnover of such proteins (Lauwers *et al.*, 2010). The range of plasma membrane proteins that are confirmed ESCRT cargoes spans receptor tyrosine kinases (RTKs) such as the EGF receptor, G-protein coupled receptors (GPCRs), ion channels, cadherins, permeases, gap junction proteins, and miscellaneous classes of receptors.

PDGF receptor was shown early on to be downregulated via the ESCRT-0 complex (Takata *et al.*, 2000). In *Drosophila*, mutation of the ESCRT-0 subunit Hrs impedes downregulation of the EGFR, PVR (PDGFR and VEGFR-related), and Torso RTKs (Lloyd *et al.*, 2002; Jekely and Rorth, 2003), as well as the non-RTK receptors Notch (Jekely and Rorth, 2003; Thompson *et al.*, 2005; Vaccari and Bilder, 2005; Herz *et al.*, 2006), Hedgehog receptor (Jekely and Rorth, 2003), and Dpp (TGF-β-related) receptor (Jekely and Rorth, 2003). The TGF-β receptor is an ESCRT substrate in human cells (Shim *et al.*, 2006). A role for ESCRT-0 in downregulating E-cadherin has been proposed (Toyoshima *et al.*, 2007). The interleukin-2 receptor is downregulated by ESCRTs via the β chain of the receptor (Yamashita *et al.*, 2008). The polycystin-1 and -2 proteins form a mechanosensory receptor–channel complex, and their *Caenorhabditis elegans* homologs are downregulated by ESCRT-0 (Hu *et al.*, 2007). The GABA(B) receptor is another example of a channel that has been reported to be downregulated via ESCRTs (Kantamneni *et al.*, 2008). The δ-opiod receptor (Hislop *et al.*, 2004), calcitonin-like receptor (Hasdemir *et al.*, 2007), protease-activated receptor-2 (Hasdemir *et al.*, 2007), and chemokine (C-X-C motif) receptor-4 (CXCR4) (Marchese *et al.*, 2003) are examples of human GPCRs that are downregulated through the ESCRT pathway. Transporters, such as ferroportin, are downregulated via the ESCRTs (De Domenico *et al.*, 2007). Virally ubiquitinated class I MHC is degraded via the ESCRTs (Hewitt *et al.*, 2002). The gap junction protein connexin-43 undergoes regulated turnover that is ESCRT-dependent (Worsdorfer *et al.*, 2008; Auth *et al.*, 2009). In yeast, the GPCRs Ste2 (Odorizzi *et al.*, 1998) and Ste3 (Piper *et al.*, 1995) are substrates of the ESCRT pathway. The yeast general amino acid permease Gap1 is an ESCRT substrate (Nikko *et al.*, 2003; Rubio-Texeira and Kaiser, 2006). The preceding are examples where a direct dependence of trafficking on ESCRT proteins has been shown, but the actual repertoire of ESCRT substrates is almost certainly far greater. Many other yeast proteins are sorted to the vacuole in a polyubiquitination-dependent manner, and are therefore presumed to be ESCRT substrates (Lauwers *et al.*, 2010). In plants, degradation of the PIN proteins and AUX1, which are plasma membrane proteins involved in transport of the hormone auxin, are regulated via the ESCRTs (Spitzer *et al.*, 2009).

Arrestin-like proteins have emerged as major players in selecting cell surface receptors for ubiquitination (Lin *et al.*, 2008), but their roles might go beyond this. The fungal PalF/Rim8 arrestin-like protein binds directly to the Vps23 subunit of ESCRT-I through an SXP motif that mimics the mode of binding of ESCRT-0 to ESCRT-I (Herrador *et al.*, 2010). Other arrestin-like proteins, Art1 and Art2, target ubiquitin ligases to plasma membrane receptors destined for downregulation. This suggests that PalF/Rim8 might be an adaptor that brings the heptahelical receptor PalH/Rim21 into the MVB pathway (Lin *et al.*, 2008).

ESCRT sort resident vacuolar and lysosomal proteins from the Golgi

The ESCRTs are involved in the sorting of newly synthesized degradative enzymes to the lysosome/vacuole. The mannose 6-phosphate receptors (MPRs) and their yeast counterpart Vps10 are not themselves ESCRT substrates and do not travel to the lysosome. These receptors deliver their cargo to the limiting membrane of the endosome, where their cargoes dissociate in the lumen and the receptors are recycled to the Golgi. MPR and Vps10 cargoes, notably yeast carboxypeptidase Y (Prc1), are mis-sorted in ESCRT mutant cells, and Prc1 secretion is a convenient diagnostic for ESCRT dysfunction in yeast (Raymond *et al.*, 1992; Marcusson *et al.*, 1994). MPR cargo mis-sorting comes about because the MPR and ESCRT pathways converge at the MVB, and defects in the ESCRT pathway inhibit the maturation of MVBs and their fusion with the vacuole/lysosome. Carboxypeptidase S (Cps1) is another yeast vacuolar hydrolase that depends on the ESCRTs for its proper localization (Odorizzi *et al.*, 1998).

In contrast, Cps1 is targeted by ubiquitination, which leads to its direct interaction with the ESCRTs. Similar to Cps1, the polyphosphatase Phm5 and the heme oxygenase Hmx1 appear to be ESCRT substrates based on their ubiquitination-dependent sorting to the vacuole (Reggiori and Pelham, 2001). The vacuolar protein Sna3 (Reggiori and Pelham, 2001; McNatt *et al.*, 2006; Oestreich *et al.*, 2006a; Stawiecka-Mirota *et al.*, 2007; Watson and Bonifacino, 2007) is an ESCRT substrate of unknown function, which by several accounts is ubiquitin-independent. However, the most recent data suggest that it traffics to the vacuole in a canonical ubiquitin-dependent manner (Stawiecka-Mirota *et al.*, 2007). The notion that Sna3 is an active participant in MVB biogenesis, as opposed to being a normal cargo, has been suggested (Piper and Katzmann, 2007). In human cells, lysosomal sorting of the resident protease cathepsin D depends on ESCRT function (Babst *et al.*, 2000).

ESCRTs are required for budding of most membrane enveloped viruses

Most, though not all, membrane enveloped viruses make use of the host ESCRT machinery to facilitate their budding from cells (Demirov and Freed, 2004; Morita and Sundquist, 2004; Chen and Lamb, 2008; Bieniasz, 2009; Carlton and Martin-Serrano, 2009; Usami *et al.*, 2009). Nearly all viruses that make use of this pathway encode proteins that contain one or more so-called late domains (Freed, 2002), short peptide sequences that interact with the ESCRTs or ESCRT-associated ubiquitin ligases (Table 2). The nuances of ESCRT function may vary somewhat for different viruses. Bearing in mind this variation, a broad working model for the main role of ESCRTs in viral budding posits that (1) viral buds are formed at the membrane by the assembly of viral proteins and do not require the ESCRTs; (2) one or more late domains in a viral protein recruit one or more of ESCRT-I, ALIX, and/or a WW-domain containing ubiquitin ligase; (3) ESCRT-I, ALIX, and/or the ubiquitin ligase initiate a pathway that leads to the assembly of ESCRT-III at the narrow neck connecting the viral bud to the host cell membrane; (4) ESCRT-III constricts the bud neck, leading to its severing; and (5) VPS4 recycles the ESCRT-III subunits, allowing for further rounds of budding. The three classes of late domain involved in ESCRT recruitment are of the form PPXY, P(S/T)XP, and YPX$_n$L. Despite some similarities in that all contain Pro residues and all are short, the cellular partners for each of these late domains are different.

PPXY late domains

PPXY sequences bind to WW domains (Kay *et al.*, 2000). WW domains are widespread in the human proteome, and bind to a range of PPXY sequences in human proteins as part of their normal function. The WW domain proteins most important in viral budding are a subset of HECT domain ubiquitin ligases (Martin-Serrano *et al.*, 2005). Knockdown experiments indicate that the presence of these ligases is required for the ESCRT-mediated release of PPXY-motif viruses. Despite considerable efforts, direct binding between one of the above-mentioned ubiquitin ligases and ESCRT complexes has not been demonstrated. The activity of the HECT domain is required for budding via these ligases (Martin-Serrano *et al.*, 2005), so it is possible that ubiquitination itself is enough to recruit ESCRT complexes to the sites of PPXY-directed virus budding. The ESCRT-I dependence of Mason-Pfizer monkey virus, which has a PPXY motif but not P(S/T)AP or YPX$_n$L, suggests that ESCRT-I could be targeted to budding sites by the ubiquitination of viral proteins (Chung *et al.*, 2008). Overexpression of HECT domain ubiquitin ligases can rescue budding of HIV-1 and Moloney murine leukemia virus mutants lacking late domains (Chung *et al.*, 2008; Usami *et al.*, 2008; Jadwin *et al.*, 2010), consistent with this concept. Direct fusion of ubiquitin to the C-terminus of a retroviral Gag protein lacking any late domain is sufficient to direct efficient virus release (Joshi *et al.*, 2008). On the other hand, the PPXY-dependent budding of prototypic foamy virus (PFV) can occur without ubiquitination of viral proteins (Zhadina *et al.*, 2007).

P(S/T)AP late domains

The P(S/T)AP motif has been intensively studied because interference with this motif blocks the budding of the HIV-1. The P(S/T)AP motif binds directly to the UEV domain of the VPS23 subunit of ESCRT-I. This is the same domain the binds to ubiquitin; however, the two sites are non-overlapping, and ubiquitin binding slightly enhances P(S/T)AP motif binding to the UEV domain. Part of the normal function of the VPS23 UEV domain is to bind to P(S/T)AP motifs in host proteins, of which the archetype is the Hrs subunit of ESCRT-0 (Lu *et al.*, 2003; Pornillos *et al.*, 2003). In contrast to humans, in yeast the Ala at the third position in the motif is not required. Other human P(S/T)AP proteins that interact with this site include the ubiquitin ligase Tal (Amit *et al.*, 2004), the GGA and Tom-like protein trafficking adaptors (Puertollano and Bonifacino, 2004; Puertollano, 2005), and ALIX, as described above. The structure of the PTAP motif of HIV-1 Gag in complex with the VPS23 UEV domain has been determined in solution by NMR and shows that the PTAP peptide binds in a groove between two loops and a small C-terminal β-sheet (Pornillos *et al.*, 2002). Hydrophobic contacts are made between the core PTAP residues (Figure 12) with Tyr63, Tyr69, and Met95 (Pornillos *et al.*, 2002). The HIV-1 PTAP motif peptide binds to the UEV domain with a higher affinity than host protein motifs such as that of Hrs, 20 μM vs. 150 μM (Pornillos *et al.*, 2003). The HIV-1 sequence contains

an additional Pro after the canonical tetrapeptide motif that contributes to this higher affinity. Library screens of peptide interactors with the human VPS23 UEV domain have shown that conservation of the Ala and the second Pro is critical; that Ser or Thr are equally favored at the second position; and that the first Pro is favored but not absolutely required for binding (Schlundt *et al.*, 2009).

The biggest mystery surrounding P(S/T)AP-mediated budding is how ESCRT-III proteins are recruited downstream of ESCRT-I. ESCRT-I has been reported to bind VPS20 (Pineda-Molina *et al.*, 2006) the complex also binds to IST1 via the VPS37 subunit (Bajorek *et al.*, 2009a). However, neither VPS20 nor IST1 have been shown to be required for HIV-1 budding. ESCRT-I binds to ALIX, again via its UEV domain, which in turn binds to SNF7 subunits of ESCRT-III that are critical for HIV-1 budding (Fisher *et al.*, 2007; Usami *et al.*, 2007). The ESCRT-I–ALIX interaction seems insufficient to explain how ESCRT-III is recruited, however, since knockdown of ALIX produces a much more modest effect on the efficiency of wild-type HIV-1 budding than knockdown of ESCRT-I or mutation of the HIV-1 PTAP motif. Knockdown of ESCRT-II also fails to impact HIV-1 budding efficiency (Langelier *et al.*, 2006). The effects of double knockdowns of ALIX and ESCRT-II subunits on HIV-1 budding have not been reported, however, and redundancy between these two factors (or additional factors such as Brox (Popov *et al.*, 2009)) cannot be ruled out. Other possibilities include that there may be direct interactions between ESCRT-I and ESCRT-III that have as yet evaded detection, or that as yet unidentified bridging partners are involved. There is considerable interest in the possibility of therapeutic interference with the HIV-1 PTAP/ESCRT-I interaction, and more insight into ESCRT-I-III connectivity is urgently needed.

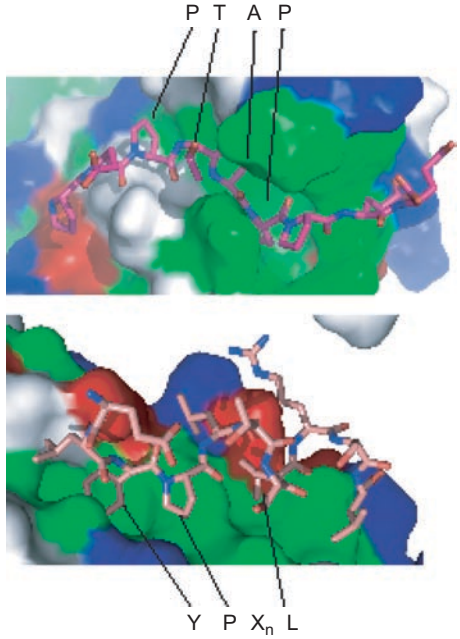

Figure 12. Viral late domains and ESCRTs. Top, the PTAP motif (stick model) of HIV-1 bound to the UEV domain of TSG101 (1M4Q). Bottom, the YPX_3L motif (stick model) of HIV-1 bound to the V domain of ALIX (2R02). Protein surfaces are colored green (hydrophobic residues), white (uncharged polar residues), red (acidic residues), and blue (basic residues).

YPXnL late domains

The YPX_nL class of late domains is less widespread than the other two, but its mechanism of action is arguably the best understood. YPX_nL late domains bind to one arm of the V domain of ALIX (Fisher *et al.*, 2007; Lee, S *et al.*, 2007; Munshi *et al.*, 2007) with ~5 μM affinity. YPX_nL peptides derived from different viruses contain n =1 or 3 residues. The motif from HIV-1 has n = 3, while that of equine infectious anemia virus (EIAV) has n = 1. For n = 3, the additional two residues fold into a helical conformation (Figure 12), which is not present for n = 1 (Zhai *et al.*, 2008). This allows the flanking Tyr and Leu residues to bind to the same sites on the V domain. There has been relatively little characterization of host YPX_nL motif proteins. YPX_nL-based late domains provide an elegant mechanism to support the release of viral buds because the Bro1 domain of ALIX directly binds to the SNF7 subunit of ESCRT-III. The SNF7 subunit is thought to be the most critical for membrane scission by analogy to the yeast proteins (Wollert *et al.*, 2009b). The SNF7-binding residues on the Bro1 domain are required for ALIX to support viral budding (Fisher *et al.*, 2007; Usami *et al.*, 2007).

Non-late domain recruitment of ESCRTs by viruses

The HIV-1 nucleocapsid (NC) is part of the Gag open reading frame, and is primarily responsible for RNA encapsidation. NC has a second function in binding to the Bro1 domain of ALIX and contributes to recruiting the ESCRT machinery for budding (Popov *et al.*, 2008; Dussupt *et al.*, 2009). This property is not limited to ALIX but is found among three other Bro1 domain-containing proteins, rhophilin, HD-PTP, and Brox (Popov *et al.*, 2009). The multiplicity of Bro1 domain proteins contributes redundancy to this mode of ESCRT recruitment, and may thus have masked the importance of ALIX in knockdown analyses. Bro1 domain-mediated recruitment of SNF7 now appears to be more central to HIV-1 budding than previously appreciated.

ESCRTs are required for the membrane abscission step in cytokinesis

ESCRTs localize to midbodies, the structure connecting two daughter cells just prior to the completion of cell division, as first visualized in studies of human VPS23

(Xie et *al.*, 1998). ESCRT mutants in *Arabidopsis* and *S. pombe* have impairments in cell division (Jin *et al.*, 2005; Spitzer *et al.*, 2006). The term "membrane abscission" refers to the cleavage of the narrow membrane neck connecting the two daughter cells. This is the final step in cytokinesis. These observations were developed into a mechanism for the role of the ESCRT machinery in the membrane abscission step in cytokinesis in 2007 by Carlton and Martin-Serrano (2007). This may be the most ancient role for the ESCRTs, as it is preserved even in a subset of the Archaea (Lindas *et al.*, 2008; Samson *et al.*, 2008). Curiously, however, ESCRT genes have not been reported as cell division mutants in yeast.

Localization of ESCRTs to the midbody requires the presence of the centrosomal and midbody protein CEP55 (Carlton and Martin-Serrano, 2007; Morita *et al.*, 2007b). CEP55 is a dimeric coiled coil protein that is recruited to midbodies and centrosomes downstream of the microtubule-associated protein MKLP1. The central portion of CEP55 comprises an unconventional coiled coil in which charged and bulky groups replaced the normal small hydrophobic residues at the core a and d position. This leads to local asymmetry in the dimer and the pushing apart of the two coils to create a single binding site for $GPPX_3Y$ motifs of ESCRT proteins (Figure 13) (Lee *et al.*, 2008). The UEV-stalk linker region of VPS23 and the PRD of ALIX both contain $GPPX_3Y$ motifs that bind to CEP55 with 1 μM affinity (Lee *et al.*, 2008). In a working model for cytokinetic membrane abscission by the ESCRTs, CEP55 recruits ESCRT-I and ALIX. ESCRT-I (directly or indirectly) and ALIX (directly via its Bro1 domain) recruit ESCRT-III, in particular SNF7 (Carlton *et al.*, 2008). Assembly of ESCRT-III leads to cleavage of the membrane neck by the same mechanism as in MVB biogenesis and detachment of viral buds.

Membrane trafficking and ubiquitination pathways have multiple roles in cytokinesis, and an important challenge is to understand the division of labor between them. A number of factors from the secretory pathway, including the exocyst complex, are required for cytokinesis (Gromley *et al.*, 2005). Components of the recycling endosome pathway are also required (Fielding *et al.*, 2005; Prekeris and Gould, 2008). In models of membrane abscission predating the discovery that ESCRTs were involved, it was postulated that fusion of secretory and/or recycling vesicles could lead to membrane abscission. It now appears that these pathways are individually necessary but not sufficient for membrane abscission. The ubiquitin ligase BRUCE is required for cytokinesis (Pohl and Jentsch, 2008). It is tempting to speculate that BRUCE-dependent ubiquitination could be important for the recruitment and activation of the ESCRTs in cytokinesis, but direct evidence is lacking. As in the endosome and MVB pathways, the lipid PI(3)P is important for ESCRT function in cytokinesis. A FYVE domain containing kinesin-binding centrosomal protein, FYVE-CENT (Sagona *et al.*, 2010) appears to link these molecules and plays a key role in delivering centrosomes to the site of midbody formation.

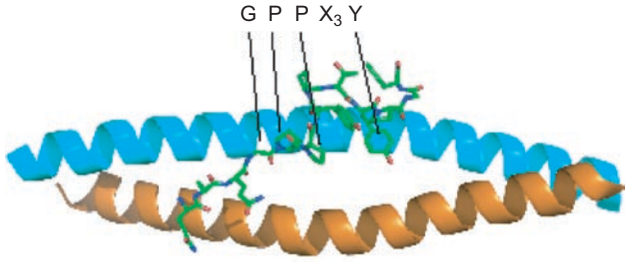

Figure 13. $GPPX_3Y$ motif targeting to midbodies. The two subunits of CEP55 are colored cyan and orange, and the $GPPX_3Y$ peptide of ALIX is shown in a stick model.

ESCRTs are required for autophagy

Macroautophagy, or autophagy for short, is the process whereby cells adapt to starvation by engulfing portions of their own cytosol for degradation, so as to replenish the pool of biosynthetic precursor molecules (Nakatogawa *et al.*, 2009). Beyond the starvation response, autophagy has a central role in signal downregulation, lipid catabolism, and degradation of damaged organelles, including peroxisomes and mitochondria (Levine and Kroemer, 2008). The autophagy pathway, in brief, consists of the formation of a preautophagosomal structure (PAS), the growth of an isolation membrane, the engulfment of cytosol within the isolation membrane (or phagophore), the closure of the phagophore into a double membraned autophagosome that is topologically equivalent to an MVB with a single ILV, and the fusion of the autophagosome with the lysosome/vacuole (Nakatogawa *et al.*, 2009). Like the MVB pathway, the autophagy pathway is initiated in conjunction with the synthesis of PI(3)P by class III PI 3-kinase (Nakatogawa *et al.*, 2009). In another parallel to the MVB pathway, ubiquitination can serve to target proteins into autophagosomes (Pankiv *et al.*, 2007; Kirkin *et al.*, 2009). Finally, the closure of the phagophore neck to form the autophagosome is topologically equivalent to the scission of endosomal bud necks to form ILVs.

Over the past three years it has become clear that the ESCRT machinery is required for autophagy in human cells (Rusten *et al.*, 2007; Rusten and Simonsen, 2008; Rusten and Stenmark, 2009). Stenmark and colleagues have made an excellent summary of the evidence that ESCRTs are involved in autophagy and the possible mechanisms that could be at work (Rusten and Stenmark, 2009). Most of the evidence is derived from EM studies showing autophagosome accumulation when ESCRT genes are silenced

Table 2. Viral late domains and ESCRT recruitment. This table is adapted and updated from one appearing in the comprehensive review of virus budding by Chen and Lamb (2008).

Virus	Reference	Notes
PTAP-TSG101 UEV		
HIV-1	(Demirov *et al.*, 2002; Garrus *et al.*, 2001; Martin-Serrano *et al.*, 2001; VerPlank *et al.*, 2001)	
Feline immunodeficiency virus	(Luttge *et al.*, 2008)	
Mason-Pfizer monkey virus	(Gottwein *et al.*, 2003)	
Moloney murine leukemia virus	(Segura-Morales *et al.*, 2005)	
HTLV-1	(Blot *et al.*, 2004; Bouamr *et al.*, 2003; Dorweiler *et al.*, 2006; Heidecker *et al.*, 2004; Wang *et al.*, 2004)	Minor/cell-type dependent role.
Prototypic foamy virus	(Patton *et al.*, 2005)	
Vesicular stomatitis virus	(Irie *et al.*, 2004)	
Ebola	(Licata *et al.*, 2003; Martin-Serrano *et al.*, 2001; Timmins *et al.*, 2003)	
Japanese encephalitis virus	(Chiou *et al.*, 2003)	PTAP motif not identified but TSG101 role is confirmed.
Lymphocytic choriomeningitis virus	(Perez *et al.*, 2003)	
Lassa virus	(Perez *et al.*, 2003; Urata *et al.*, 2006)	
Bluetongue virus	(Wirblich *et al.*, 2006)	
YPX$_n$L-ALIX-dependent		
HIV-1	(Strack *et al.*, 2003; von Schwedler *et al.*, 2003)	
Equine infectious anemia virus	(Martin-Serrano *et al.*, 2003; Strack *et al.*, 2003)	
Prototypic foamy virus	(Patton *et al.*, 2005; Stange *et al.*, 2005)	
Sendai virus	(Gosselin-Grenet *et al.*, 2007; Irie *et al.*, 2007; Sakaguchi *et al.*, 2005)	Consensus that ALIX interacts with viral YLDL sequence but Gosselin-Grenet *et al.* (2007) find no effect on budding of ALIX knockdown
Vaccinia virus	(Honeychurch *et al.*, 2007)	
Rous sarcoma virus	(Dilley *et al.*, 2010)	Secondary
PPXY-dependent		
Marburg	(Urata *et al.*, 2007)	Unconventional interaction with TSG101 is PTAP-independent but PPXY dependent.
Rous sarcoma virus	(Kikonyogo *et al.*, 2001; Medina *et al.*, 2005)	
Mason-Pfizer monkey virus	(Gottwein *et al.*, 2003)	
Moloney murine leukemia virus	(Segura-Morales *et al.*, 2005)	
HTLV-1	(Blot *et al.*, 2004; Bouamr *et al.*, 2003; Heidecker *et al.*, 2004; Wang *et al.*, 2004)	
Vesicular stomatitis virus	(Harty *et al.*, 1999)	
Rabies virus	(Harty *et al.*, 1999)	
Ebola virus	(Harty *et al.*, 2000; Licata *et al.*, 2003; Timmins *et al.*, 2003)	
Lymphocytic choriomeningitis virus	(Perez *et al.*, 2003)	
Lassa virus	(Perez *et al.*, 2003; Urata *et al.*, 2006)	
Bluetongue virus	(Wirblich *et al.*, 2006)	
ESCRTs involved – recruitment mechanism other or unknown		
Human cytomegalovirus	(Tandon *et al.*, 2009)	Note that Fraile-Ramos *et al.* (2007) find no effect of ESCRT knockdown.
Hepatitis C	(Corless *et al.*)	
Hepatitis B	(Lambert *et al.*, 2007)	
Herpes simplex type 1	(Pawliczek and Crump, 2009)	
Tomato bushy stunt virus	(Barajas *et al.*, 2009)	Ubiquitinated p33 protein binds to ESCRT-I and Alix

or knocked out in HeLa cells (Filimonenko *et al.*, 2007), *C. elegans* (Roudier *et al.*, 2005), *Drosophila* (Rusten *et al.*, 2007), and mouse neurons (Lee, JA *et al.*, 2007). Several possible mechanisms have been put forward (Rusten and Stenmark, 2009). First, in a parallel to the other roles for ESCRTs, ESCRT-III might be required to cleave the membrane neck of the phagophore to yield the closed double-membraned autophagosome. This is a difficult model to test directly as this neck is less amenable to visualization *in vivo* than the cytokinetic or viral bud necks. Reconstitution such as carried out for MVB biogenesis seems a still remote prospect. Second, ESCRT dysfunction could act indirectly by triggering signals that inhibit autophagy. Third, it might be important to have a pool of ESCRT-generated MVBs available for fusion with autophagosomes. In a variation on this model, the role of ESCRTs may be indirect in that they are required for the biogenesis of the lysosomes with which autophagosomes ultimately fuse. These possibilities are not mutually exclusive.

Other functions of the ESCRTs

Several other functions have been reported for ESCRTs, which may or may not ultimately prove to be connected to their well-characterized membrane remodeling and cargo sorting activities. In *Drosophila*, ESCRT-II, via its GLUE domain, binds to bicoid mRNA and establishes the mRNA gradient responsible for establishing polarity in the embryo (Irion and St Johnston, 2007). It is hard to rationalize why membrane trafficking machinery would be involved in setting up an mRNA gradient. One hypothesis is that the mRNA uses the microtubule-based transport of MVBs to "hitchhike" towards the minus end of microtubules (Piper and Luzio, 2007). The ESCRT-I subunit VPS23 was shown very recently to be required for the establishment of the cSMAC in the immunological synapse (Vardhana *et al.*, 2010). It is not yet clear whether or not cSMAC organization involves the membrane budding activity of ESCRT-I. ESCRT-II subunits in human cells were originally isolated and characterized as binding partners for the ELL proteins, elongation factors of RNA polymerase II (Kamura *et al.*, 2001), but the mechanistic implications of this have not been elucidated. The human ESCRT-III subunit DID2A was first cloned and named "CHMP1", for chromatin modifying protein 1, for a putative role in gene silencing, which has to date been neither refuted nor further elucidated.

ESCRT dysfunction and non-viral disease

ESCRTs and cancer

The human ESCRT-I subunit VPS23 was first isolated as a result of an antisense RNA screen for factors whose disruption promoted tumorigenesis, and named "tumor susceptibility gene 101" (Li and Cohen, 1996). Given what we now know about the role of ESCRT-I in downregulating proliferative receptor signaling, it is tempting to speculate that this could be connected to its tumor susceptibility phenotype. ESCRT functions in cytokinesis and autophagy could conceivably also mediate such a phenotype. Following some early reports that proved erroneous (Li *et al.*, 1998), there have been no confirmed reports of mutations in *TSG101* human tumors. A separate and compelling line of evidence mechanistically linking ESCRT dysfunction to cancer comes from observations in *Drosophila*. Mutation of *Drosophila* VPS23 leads to tumors (Moberg *et al.*, 2005). Mutations in the ESCRT-II subunit VPS25 also lead to cell overproliferation in *Drosophila* and induce tumor formation when apoptosis is blocked (Herz *et al.*, 2006; Thompson *et al.*, 2005; Vaccari and Bilder, 2005). The overproliferation is due at least in part to excess signaling by Notch, consistent with the model that ESCRTs antagonize proliferative signaling by downregulating proliferative receptors.

ESCRTs and neurological diseases

Two main lines of evidence connect the ESCRTs to neurological diseases. First, autosomal dominant mutations of the gene for the ESCRT-III subunit VPS2B lead to the neurodegenerative disorder frontotemporal dementia-3 (FTD3) (Lee, JA *et al.*, 2007). The mechanism of pathogenesis is thought to be linked to the role of VPS2B as a factor required for autophagy. Second, the hereditary spastic paraplegias (HSPs) are a group of inherited disorders (SPG1-46) characterized by a length dependent axonopathy of corticospinal motor neurons. Two of the proteins encoded by these genes, the SPG4 protein spastin (Yang *et al.*, 2008; Connell *et al.*, 2009) and the SPG15 protein FYVE-CENT (Sagona *et al.*, 2010), have functional interactions with ESCRT-III proteins. Impairment of these interactions leads to cytokinesis defects in cell culture. It is not clear how cytokinesis defects would lead selectively to impaired neuronal development. The midbody structure that coordinates cytokinetic abscission is closely linked to the centrosome, and these two structures have an overlapping protein composition. Indeed, ESCRTs have been visualized at both midbodies and centrosomes (Sagona *et al.*, 2010). It is unclear to what extent neurological pathogenesis in the HSPs is due to the ESCRT-SPG protein connection. The observation of ESCRT-associated protein localization to centrosomes (Sagona *et al.*, 2010) suggests a potential link to axon development.

Conclusion

The structure, function, and interactions of the central ESCRT machinery – that is, the portions conserved

from yeast to humans and preserved between different human subunit isoforms – are now relatively well understood. The reason for the diversity of complexes in human cells, with in principle 12 ESCRT-III and 16 ESCRT-I combinations possible, is only starting to be explored. Recent progress in visualizing ESCRT assemblies on membranes, reconstituting the ESCRT reaction *in vitro*, and analyzing the budding and scission mechanism computationally, have moved the field from hand-waving to a rough pictorial outline of the reaction with some biophysical understanding. The stage is now set for a deeper quantitative biophysical analysis of the mechanism. Connections between the ESCRTs and other major cellular machineries are only beginning to be understood. We have an initial view of how the ESCRTs could be linked the early endocytic apparatus and the cytoskeleton, but connections to other areas, such as the endosome-vacuole fusion machinery, need much more study. ESCRT–virus interactions may be appealing drug targets, but concerns about interference with normal physiological functions must be overcome. ESCRT dysfunction is linked to neurological diseases, but the mechanistic basis for the connection is largely a mystery. It is hoped that this review will serve as a one-stop sourcebook of information for newcomers who may go on to answer some of these questions.

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Declaration of interest

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Michael M. Cox