Insect Biochemistry and Molecular Biology 62 (2015) 211–225

Contents lists available at ScienceDirect

Insect Biochemistry and Molecular Biology

journal homepage: www.elsevier.com/locate/ibmb

The vacuolar protein sorting genes in insects: A comparative genome view

Zhaofei Li [a, *], Gary Blissard [b]

[a] State Key Laboratory of Crop Stress Biology for Arid Areas, Key Laboratory of Northwest Loess Plateau Crop Pest Management of Ministry of Agriculture, College of Plant Protection, Northwest A&F University, Taicheng Road, Yangling, Shaanxi 712100, China
[b] Boyce Thompson Institute, Cornell University, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form
6 November 2014
Accepted 21 November 2014
Available online 5 December 2014

Keywords:
VPS
ESCRT
Retromer
VPS-C
GARP
PI3K-III

ABSTRACT

In eukaryotic cells, regulated vesicular trafficking is critical for directing protein transport and for recycling and degradation of membrane lipids and proteins. Through carefully regulated transport vesicles, the endomembrane system performs a large and important array of dynamic cellular functions while maintaining the integrity of the cellular membrane system. Genetic studies in yeast *Saccharomyces cerevisiae* have identified approximately 50 vacuolar protein sorting (VPS) genes involved in vesicle trafficking, and most of these genes are also characterized in mammals. The VPS proteins form distinct functional complexes, which include complexes known as ESCRT, retromer, CORVET, HOPS, GARP, and PI3K-III. Little is known about the orthologs of VPS proteins in insects. Here, with the newly annotated *Manduca sexta* genome, we carried out genomic comparative analysis of VPS proteins in yeast, humans, and 13 sequenced insect genomes representing the Orders Hymenoptera, Diptera, Hemiptera, Phthiraptera, Lepidoptera, and Coleoptera. Amino acid sequence alignments and domain/motif structure analyses reveal that most of the components of ESCRT, retromer, CORVET, HOPS, GARP, and PI3K-III are evolutionarily conserved across yeast, insects, and humans. However, in contrast to the VPS gene expansions observed in the human genome, only four VPS genes (VPS13, VPS16, VPS33, and VPS37) were expanded in the six insect Orders. Additionally, VPS2 was expanded only in species from Phthiraptera, Lepidoptera, and Coleoptera. These studies provide a baseline for understanding the evolution of vesicular trafficking across yeast, insect, and human genomes, and also provide a basis for further addressing specific functional roles of VPS proteins in insects.

1. Introduction

In eukaryotic cells, the endomembrane network consists of morphologically distinct organelles with different functions in cargo sorting and degradation. Constant communication between these organelles is usually through the exchange of trafficking vesicles. Transport of lipids and protein cargos between the intracellular compartments, including transport to and from the plasma membrane, is precisely regulated to maintain the integrity and dynamics of the cell and its organelles (Brighouse et al., 2010; Campelo and Malhotra, 2012).

In the yeast *Saccharomyces cerevisiae*, the vacuole is a prominent organelle that shares functional characteristics with lysosomes from other eukaryotic groups such as vertebrates and invertebrates. The vacuole and lysosomes play vital roles in hydrolysis of proteins, carbohydrates, and lipids. Vacuolar proteins are usually segregated from secretory proteins, and are packaged into transport vesicles and trafficked specifically to the vacuole. Genetic screening studies have identified about 50 vacuolar protein sorting (VPS) genes that affect protein sorting and trafficking to the vacuole in yeast (Arlt et al., 2011; Hedman et al., 2007). Based on the vacuolar morphology observed in cells expressing various VPS mutants, VPS genes were grouped into seven classes, i.e. classes A–F and mutants with tubule-vesicular vacuoles (Banta et al., 1988; Hedman et al., 2007; Raymond et al., 1992). Class A includes VPS8, VPS10, VPS13, VPS29, VPS30, VPS35, VPS38, VPS44, VPS46, VPS51, VPS52, VPS53, VPS54, VPS55, and VPS74. This type of mutant showed wild-type vacuolar morphology, with vacuoles present as 3–10 subcompartments. These proteins may affect the delivery of soluble vacuolar proteins to the vacuole, but have little effect on the

* Corresponding author. Tel.: +86 18792598371.
E-mail address: zhaofeili73@outlook.com (Z. Li).

http://dx.doi.org/10.1016/j.ibmb.2014.11.007
0965-1748/

delivery of membrane proteins to the vacuole. Class B mutants include VPS5, VPS17, VPS39, VPS41, and VPS43, which lack large vacuolar structures and typically contain more than 20 "fragmented" vacuole-like compartments per cell. These small compartments are not functional as targeting sites for the VPS system. Class C VPS mutants contain no identifiable vacuole and the members of this group include VPS11, VPS16, VPS18, VPS33. Dysfunction of these proteins results in the accumulation of abnormal membrane-enclosed structures, which may be a mixture of vesicle-like organelles and lipid droplets. Class D VPS mutants are characterized as defective for vacuolar segregation, and a slightly enlarged vacuole structure. This group includes VPS3, VPS6, VPS9, VPS15, VPS21, VPS34, and VPS45. Class E mutants comprise another large group that includes VPS2, VPS4, VPS20, VPS22, VPS23, VPS24, VPS25, VPS27, VPS28, VPS31, VPS32, VPS36, and VPS37. Mutations in these genes result in the formation of a smaller organelle, distinct from the vacuole, which appears to be an exaggerated form of a prevacuolar compartment (PVC). Finally, a small group of VPS genes have been characterized as Class F, and these include VPS1 and VPS26. Class F mutants usually have a large central vacuole surrounded by class B-like fragmented small vacuolar structures (Banta et al., 1988; Bonangelino et al., 2002; Hedman et al., 2007; Raymond et al., 1992). An abundance of data has shown that VPS proteins function as distinct complexes that are directly or indirectly involved in vesicle formation, transport, tethering, and fusion with target membranes. These complexes include: 1) the endosomal sorting complex required for transport (ESCRT) (Henne et al., 2011; Hurley, 2010; Raiborg and Stenmark, 2009; Rusten et al., 2012; Schuh and Audhya, 2014); 2) the tethering complexes CORVET (class C core vacuole/endosome tethering), HOPS (homotypic fusion and protein sorting) (Balderhaar and Ungermann, 2013; Nickerson et al., 2009; Solinger and Spang, 2013), and GARP (Golgi-associated retrograde protein) (Bonifacino and Hierro, 2011; Brocker et al., 2010); 3) the retrieval complex, Retromer (Attar and Cullen, 2010; Bonifacino and Hurley, 2008; Seaman, 2012); and 4) the class III PI3K (phosphoinositide 3-kinase) complexes (Vanhaesebroeck et al., 2010).

Although most of the above complexes are well-studied in yeast, the detailed mechanisms of their functions in higher eukaryotes remain incompletely understood. Remarkably, the orthologs of these VPS genes in insects have not been identified or studied in any great detail, even though several of these genes have been characterized in Drosophila (Moberg et al., 2005; Vaccari et al., 2009). In the current study, we carried out a comparative genomic analysis to identify and characterize the orthologs of VPS genes in 13 sequenced insect genomes and we compared gene expansions in insects with those in the human genome. In addition we also analyzed and report conservation of signature motifs associated with most VPS protein groups. Combined these studies present a global view of the conservation and divergence of VPS proteins in insects and their relationships to orthologous human protein families.

2. Methods

2.1. Mining insect genomes for VPS genes

VPS genes of the yeast *S. cerevisiae* and the orthologs from the human genome were used for searches of insect genomes using BLASTP and TBLASTN programs. BLAST searches were performed using databases of sequenced insect genomes from 6 Orders, including Hymenoptera (*Apis mellifera*, *Nasonia vitripennis*, and *Harpegnathos saltator*; http://hymenopteragenome.org), Diptera (*Drosophila melanogaster*, *Aedes aegypti*, *Anopheles gambiae*, and *Culex quinquefasciatus*; http://flybase.org and http://www.vectorbase.org), Hemiptera (*Acyrthosiphon pisum*; http://www.aphidbase.com), Phthiraptera (*Pediculus humanus corporis*; http://www.vectorbase.org), Lepidoptera (*Bombyx mori*, *Danaus plexippus*, and *Manduca sexta*; http://silkworm.genomics.org.cn, http://monarchbase.umassmed.edu, and http://agripestbase/manduca, and http://agripestbase/manduca), and Coleoptera (*Tribolium castaneum*; http://beetlebase.org). Specific BLAST searches were also carried out at the National Center for Biotechnology Information (NCBI).

2.2. Alignments and protein structure analysis

Amino acid sequences were aligned using Clustal W2 at the EMBL-EBI server (http://www.ebi.ac.uk/services). Alignments were edited with GeneDoc software (http://www.nrbsc.org/gfx/genedoc/). Protein domain and motif analyses were performed with the web-based tools InterProScan (Jones et al., 2014) at EMBL-EBI (http://www.ebi.ac.uk/interpro), COILS (Lupas et al., 1991) at the ExPASy server (http://www.expasy.org/proteomics), and Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan). Protein molecular weights (MW) and isoelectric points (pI) were calculated with Compute pI/Mw program at the ExPASy server.

2.3. Phylogenetic analysis

The phylogenetic reconstruction was conducted in MEGA6 (Tamura et al., 2013) using the Neighbor-joining statistical method (Saitou and Nei, 1987). The evolutionary distances were computed with the Jones–Taylor–Thornton (JTT) matrix-based method (Jones et al., 1992). The rate variation among amino acid sites was modeled with a gamma distribution. A distance-based bootstrap test with 1000 replicates set was used to analyze the phylogenetic trees (Felsenstein, 1985).

3. Results and discussion

3.1. The ESCRT pathway

The majority of the core components of the ESCRT complexes are from the class E VPS morphological group (Hurley, 2010). The ESCRT machinery was originally found to be required for the maturation of multivesicular bodies (MVBs) and the biogenesis of vacuolar lysosomes. The ESCRT pathway is essential for sorting ubiquitinated membrane proteins into MVBs by deforming the endosomal-limiting membrane inward, followed by scission to release the newly formed vesicles (Hurley, 2010; Hurley and Hanson, 2010). Recent data indicate that, beyond MVB formation, ESCRT complexes have crucial functions in cytokinesis, viral budding, and autophagy (Caballe and Martin-Serrano, 2011; Raiborg and Stenmark, 2009; Rusten et al., 2012; Votteler and Sundquist, 2013). Most recently, it was found that ESCRTs are also necessary for plasma membrane repair (Jimenez et al., 2014). In yeast and humans, the ESCRT pathway is composed of five distinct subcomplexes: ESCRT-0, -I, -II, and -III, and VPS4-Vta1, as well as some accessory proteins, such as Alix (apoptosis-linked gene 2-interacting protein X) (Henne et al., 2011; Hurley, 2010). Our analysis revealed that these components of the ESCRT pathway are highly conserved in yeast, insects, and humans (Table 1, Fig. S1). In addition, some gene expansions that occurred in the human genome lineage are not present across insect genomes, which appear to be generally uniform in their content of core ESCRT pathway complexes.

Table 1
Properties of the ESCRT system.

Complex	Subunits			Functional perspectives			
	Yeast	Human	Insects[a]	Functional roles	Lipid binding	Motif/domain	Inter-complex interactions
ESCRT-0	VPS27	Hrs	+	Cargo sorting	PtdIns3P/FYVE domain on VPS27	VHS, FYVE UIM, GAT, CB	VPS27–VPS23
	Hse1	STAM1 STAM2	+			VHS, UIM SH3, GAT	
ESCRT-I	VPS23	Tsg101	+	Bud formation	Electrostatics on N-terminus of VPS37	UEV, AAA CC, PRD	VPS23–VPS27
	VPS28	VPS28	+			CTD	VPS28–VPS36
	VPS37	VPS37A	+			NTH, Mod(r), PRD	
		VPS37B	+			Mod(r), PRD	
		VPS37C				Mod(r), PRD	
		VPS37D				Mod(r), PRD	
	MVB12	MVB12A	+[1]			MABP, UMA	
		MVB12B isoform1				MABP, UMA	
		isoform2				MABP	
		UBAP1				UMA, UBA	
ESCRT-II	VPS22	EAP30	+	Bud formation	PtdIns3P/GLUE domain on VPS36	ND, WH1, WH2	
	VPS25	EAP20	+			WH1, WH2	VPS25–VPS20
	VPS36	EAP45	+			GLUE/NZF, WH1, WH2	VPS36–VPS28
ESCRT-III	VPS2	CHMP2A	+	Membrane scission	Myristoylation of VPS20; electrostatics on helix-1 of CHMP3	Helix core, MIM	VPS2–VPS4
		CHMP2B	+[2]			Helix core, MIM	
	VPS20	CHMP6	+			Helix core, MIM	VPS20–VPS25
		CHMP3	+				
	VPS24	isoform1				Helix core, MIM	VPS24–VPS4
		isoform2				Helix core, MIM	
		isoform3				Helix core, MIM	
	VPS32 (Snf7)	CHMP4A	+			Helix core, MIM	Snf7-VPS4
		CHMP4B				Helix core, MIM	Snf7-Alix
		CHMP4C				Helix core, MIM	
	VPS46	CHMP1A	+			Helix core, MIM	VPS46-Vta1
		CHMP1B				Helix core, MIM	
	VPS60	CHMP5	+			Helix core, MIM	VPS60-Vta1
VPS4-Vta1	VPS4	VPS4A	+	Disassembly and recycling of ESCRT-III	–	MIT, AAA, CC	VPS4-ESCRT-III
		VPS4B				MIT, VSL	
	Vta1	LIP5	+				Vta1-VPS60 Vta1-CHMP1
Accessory	VPS31	Alix	+[3]	Cytokinesis, viral budding	–	Bro1, V-domain, PRD	Alix-Snf7

+[1]: absent in *Culex quinquefasciatus*, +[2]: absent in *Acyrthosiphon pisum*, *Aedes aegypti*, *Anopheles gambiae*, *Apis mellifera*, *Culex quinquefasciatus*, *Harpegnathos saltator*, *Nasonia vitripennis*, +[3]: absent in *Nasonia vitripennis*.

[a] Insects represent sequenced insect genomes, including those of *Acyrthosiphon pisum*, *Aedes aegypti*, *Anopheles gambiae*, *Apis mellifera*, *Bombyx mori*, *Culex quinquefasciatus*, *Danaus plexippus*, *Drosophila melanogaster*, *Harpegnathos saltator*, *Manduca sexta*, *Nasonia vitripennis*, *Pediculus humans corporis*, and *Tribolium castaneum*.

3.1.1. ESCRT-0

The ESCRT-0 complex is required for recognizing and sorting ubiquitinated membrane proteins. ESCRT-0 is a heterodimer composed of Vps27 and Hse1 (in yeast), and Hrs and STAM (in mammals) (Hurley, 2010; Raiborg and Stenmark, 2009; Ren and Hurley, 2011). Both subunits can bind ubiquitin and thereby select ubiquitinated cargo for transport. In addition, VPS27/Hrs also bridges ESCRT-0 and ESCRT-I through the interaction of the VPS27/Hrs P(S/T)XP motif and the UEV (ubiquitin E2 variant) domain of VPS23/Tsg101 (Henne et al., 2011; Hurley, 2010; Ren and Hurley, 2011). **Hse1/STAM**. There are two isoforms of STAM (STAM1 and STAM2) in humans. They share 55.8% amino acid identity. Only one ortholog of STAM was found in sequenced insect genomes (Fig. 2B; Table 1). Compared with yeast and humans, the N-terminal 400 amino acids (aa) of STAM are also highly conserved in insects. This conserved region contains domains VHS (VPS27, HRS, STAM domain), UIM (ubiquitin-interacting motif), SH3 (SRC homology 3 domain), and GAT (GGA and Tom1), which serve functions in binding ubiquitin, binding proline-rich peptide, and heterodimerization, respectively. In contrast to the N-terminal region, the C-terminal region of approximately 140–200 aa of Hse1/STAM is highly variable, especially those from Diptera and Lepidoptera. The STAM proteins of Dipteran species are extended at the C-terminus by approximately 80–180 aa, whereas the STAM proteins of Lepidopteran species (*B. mori*, *D. plexippus*, and *M. sexta*) contain a short C-terminus, that is 60–120 aa shorter than the other species (Fig. S1, Hse1/STAM). The high rates of amino acid substitutions within the C-terminal region of Hse1/STAM may explain confident bootstrap support for grouping STAM of *D. melanogaster* with those from Lepidopteran species on the phylogenetic tree (Fig. 2B). **VPS27/Hrs**. Similar conservation was also observed for VPS27/Hrs. The N-terminal region of approximately 500 aa of VPS27/Hrs contains VHS, UIM, GAT, and FYVE (*F*ab-1, *Y*GL023, *V*PS27, and *E*EA1) domains. The FYVE domain interacts with phosphatidylinositol 3-phosphate (PtdIns(3)P or PI3P) to target ESCRT-0 to endosome membranes. This N-terminal region of VPS27/Hrs is also highly

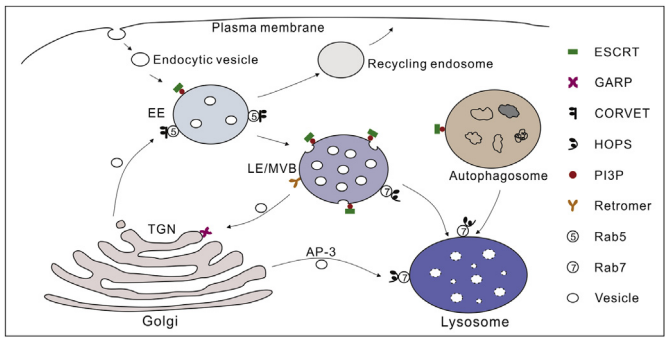

Fig. 1. Schematic representation of complexes associated with vesicular transport and its regulation in eukaryotic cells. Abbreviations: AP-3, adapter complex protein 3; CORVET, class C core vacuole/endosome tethering; EE, early endosome; ESCRT, endosomal sorting complex required for transport; GARP, Golgi-associated retrograde protein; HOPS, homotypic fusion and protein sorting; LE, late endosome; MVB, multivesicular bodies; PI3P, phosphatidylinositol 3-phosphate; Rab, Ras-like GTP-binding protein; TGN, trans-Golgi network.

conserved from yeast to insects and humans (Fig. 2). Although the C-terminal regions of VPS27/Hrs proteins are highly variable in length, a clathrin-box (CB) motif is conserved in position at the extreme C-terminus (Fig. 2).

The C-termini of Hse1/STAM and VPS27/Hrs can interact with other proteins, but the biological significance of those interactions is not known (Hurley, 2010). Since ESCRT-0 is the initial cargo-sorting complex of the ESCRT pathway, these C-terminal variations may reflect a species-specificity related to intracellular cargo sorting. Additionally, compared with the other insect species, the N-terminus of Hrs from *B. mori* contains an additional 280 aa, and the middle regions of *B. mori* and *D. plexippus* have 460 or 477 aa insertions (Fig. S1, VPS27/Hrs). Considering the high degree of conservation among the N-terminal regions of VPS27/Hrs proteins, these additional insertion regions in Hrs of *B. mori* and *D. plexippus* might be caused by frame shifts and/or additional unidentified introns in available genome assemblies.

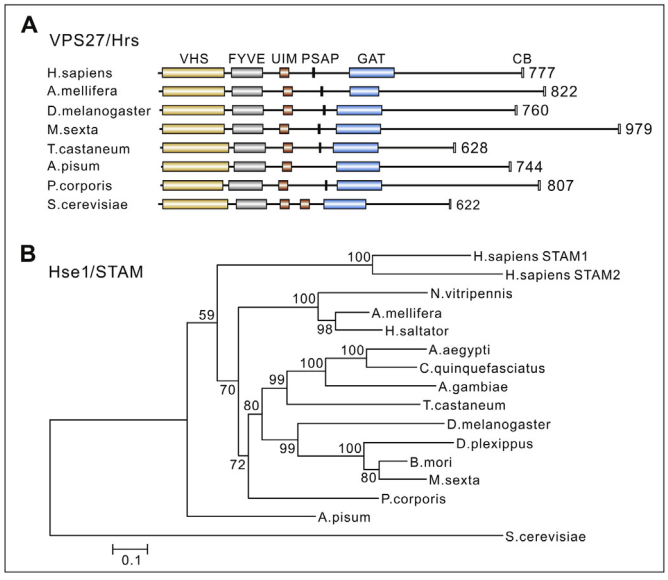

Fig. 2. Structural similarities and phylogenetic analysis of components of the ESCRT-0 complex. (A) Domain organization of VPS27/Hrs. The diagram shows the approximate sizes and the relative positions of predicted domains/motifs of VPS27/Hrs. Abbreviations: VHS, (VPS27, HRS, STAM) domain; UIM, ubiquitin-interacting motif; FYVE, Fab1/YOTP/Vac1/EEA1; GAT, GGA and Tom1; CB, clathrin box. (B) A neighbor-joining phylogenetic tree was generated from Hse1/STAM sequences from the indicated species. Bootstrap confidence values are indicated on the branches. The accession numbers for amino acid sequences are listed in Supplemental Table S1.

3.1.2. *ESCRT-I*

ESCRT-I assembles with ESCRT-II as a super complex to mediate budding into the limiting membrane of the endosome (Henne et al., 2011; Hurley, 2010). ESCRT-I is a heterotetramer composed of VPS23 (Tsg101 in mammals), VPS28, VPS37, and MVB12 (multivesicular body sorting factor of 12 KDa). The ESCRT-I core stalk contains the C-terminal coiled-coil (CC) and steadiness box (SB) of VPS23/Tsg101, and one helix each from VPS37 and MVB12. The headpiece of ESCRT-I consists of the N-terminal UEV domain of VPS23/Tsg101, the N-terminal helical region (NTH) of VPS37, and the C-terminal ubiquitin interacting domain, UMA (ubiquitin associated protein-1-MVB12 associated), of MVB12 (Gill et al., 2007; Hurley, 2010; Kostelansky et al., 2007; Morita et al., 2007). The UEV domain of VPS23/Tsg101 can bind ubiquitinated cargo (Katzmann et al., 2001) and the P(S/T)XP motif of VPS27/Hrs, Alix, and viral proteins (Katzmann et al., 2003; Votteler and Sundquist, 2013). The proline-rich region (PRD) following the UEV domain of VPS23/Tsg101 can interact with midbody protein CEP55 (Lee et al., 2008). The N-terminus of VPS37 forms weak electrostatic interactions with phospholipids and targets ESCRT-I to the endosome membrane (Gill et al., 2007; Kostelansky et al., 2007). The N-terminal core region of VPS28 is involved in ESCRT-I headpiece formation, while the C-terminal four helix bundle domain (CTD) is extended out as adapter module for interacting with VPS36, a component of ESCRT-II (Gill et al., 2007; Kostelansky et al., 2007). Among the genes encoding ESCRT-I components, VPS37 and MVB12 show expansions in insects and humans. In the human genome, there are four VPS37 isoforms (VPS37A–D) and four MVB12 isoforms (MVB12A, MVB12B isoform1 and isoform2, and UBAP1) (Kostelansky et al., 2007; Morita et al., 2007; Stefani et al., 2011) (Table 1, Fig. 3). **VPS37**. All of the VPS37 isoforms contain one

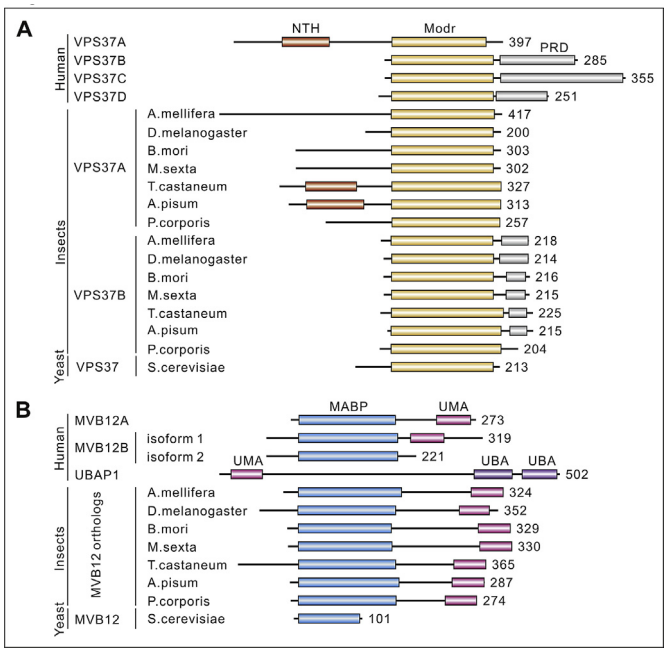

Fig. 3. Domain organization of the ESCRT-I components VPS37 (A) and MVB12 (B). The illustration shows the approximate sizes and the predicted domains/motifs of VPS37 and MVB12. The species and group names are indicated on the left. Accession numbers for amino acid sequences are listed in Supplemental Table S1. Abbreviations: MABP, MVB12-associated beta-prism; Modr, modifier of rudimentary; NTH, N-terminal helical region; PRD, proline-rich domain; UBA, ubiquitin-associated/translation elongation factor EF1B; UMA, ubiquitin associated protein-1-MVB12 associated.

Mod(r) (modifier of rudimentary) domain of approximately 150 aa. Among the human VPS37 genes, the N-terminus of VPS37A contains the NTH but no proline-rich region in the C-terminal region. In contrast, the C-termini of VPS37B, C, D each contain a proline-rich domain (PRD), but of varying lengths (70–180 aa), but no NTH domain in the N-terminal region (Fig. 3A, Human). In insect genomes, two homologs of VPS37 were identified (Table 1, Figs. 3A and 4A). Unlike human VPS37A, no NTH domain is present at the N-termini of insect VPS37A homologs, except for those of *T. castaneum* and *A. pisum*. Generally, the length of the N-terminal region of insect VPS37A proteins is substantially shorter than that of human VPS37A, except for that from the Hymenoptera (Figs. 3A and S1). The domain structure of insect VPS37B is similar to that of human VPS37B, C, D, although the predicted C-terminal PRD domain of insect VPS37B is reduced in size, to only approximately 20–40 aa (Fig. 3A). Based on the conserved Mod(r) domain sequences, phylogenetic analysis showed that VPS37 forms two distinct groups. Group I consists of insect VPS37A, while group II includes human VPS37 proteins and insect VPS37B (Fig. 4A). Insect VPS37A and VPS37B are distantly related. Amino acid sequence identities between insect VPS37A and VPS37B from the same species are relatively low, ranging from 18 to 28%, while the identities between insect VPS37B vs. human VPS37B, C, and D range from 23 to 32%. **MVB12**. We identified only one ortholog of MVB12 among all of the sequenced insect genomes examined. The insect MVB12 orthologs share a similar domain architecture with yeast and humans MVB12. Each insect MVB12 contains an N-terminal MABP (MVB12-associated beta-prism) domain and a C-terminal UMA domain (Fig. 3B). In contrast, human UBAP1, which was recently characterized as a new component of ESCRT-I (Stefani et al., 2011), contains a UMA and two UBA (Ubiquitin-associated/translation elongation factor EF1B) domains (Fig. 3B, UBAP1). Recent studies indicate that the MABP domain of MVB12 may be involved in binding of ESCRT-I to acidic phospholipids and proteins (Boura and Hurley, 2012). Notably, we failed to identify an ortholog of MVB12 in *C. quinquefasciatus*. Given the high conservation, this might be due to an absence of the gene from the available genome assembly. The sequence identities of predicted human and insect MVB12 proteins to that of yeast are relatively low, only about 9–19%. In addition, yeast MVB12 contains only a partial MABP domain, and lacks the C-terminal UMA domain (Fig. 3B). Phylogenetic analysis showed that insect MVB12 represents a lineage distinct from that of humans. The amino acid sequence variation between MABP and UMA domains resulted in low confidence bootstrap values for several nodes on the MVB12 gene family phylogeny (Fig. 4B). The other two subunits of ESCRT-I, VPS23/Tsg101 and VPS28, are very highly conserved among yeast, most insects, and humans (Fig. S1), sharing similar predicted domain structures. Divergent sequences were observed for Tsg101 from *B. mori* and *D. plexippus*. In the predicted Tsg101 proteins of both *B. mori* and *D. plexippus*, approximately 90 N-terminal amino acids are absent, and both contain only a partial UEV domain. In contrast, the Tsg101 from *M. sexta* is highly similar in domain structure to those proteins from other insects and humans (Fig. S1, VPS23/Tsg101).

3.1.3. ESCRT-II

ESCRT-II cooperates with ESCRT-I to promote membrane budding in the biogenesis of MVBs, but the ESCRT-II complex may not be required for cytokinesis and viral budding (Henne et al., 2011; Hurley, 2010; McCullough et al., 2013). In some well-studied cases of viral budding, ESCRT-I independently recruits ESCRT-III, the membrane scission machine, to the sites of viral budding and scission of the newly formed virus particle (Hurley and Hanson, 2010; Votteler and Sundquist, 2013). Also, recent data suggest that ESCRT-II is involved in HIV-1 genomic RNA

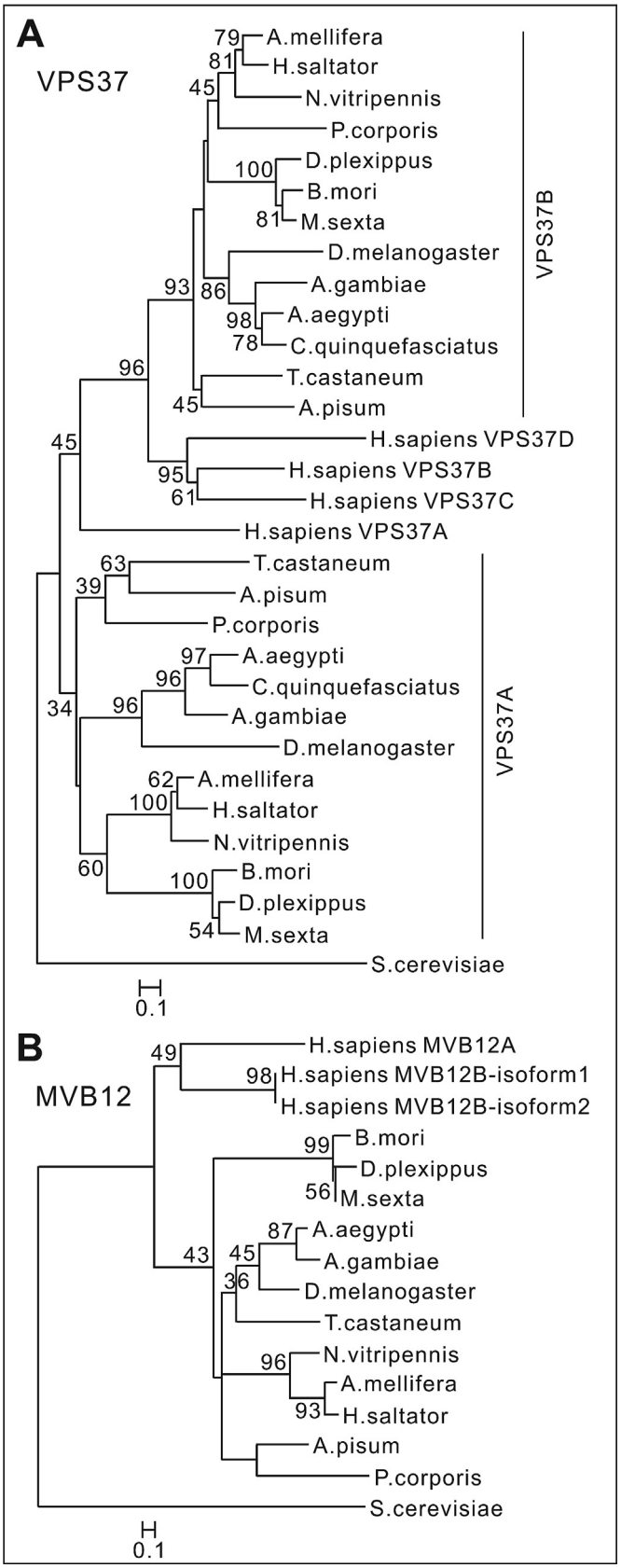

Fig. 4. Neighbor-joining phylogenetic trees of the ESCRT-I components VPS37 (A) and MVB12 (B). The rooted tree of VPS37 was constructed with the conserved Modr domain as shown in Fig. 3. Bootstrap confidence values greater than 30% are indicated on the branches. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

trafficking and assembly (Ghoujal et al., 2012). ESCRT-II is a Y-shaped heterotetramer consisting of a 1:2:1 ratio of VPS22 (ELL-associated protein of 30 kDa, or EAP30 in mammals), VPS25 (EAP20), and VPS36 (EAP45) (Hierro et al., 2004; Teo et al., 2004). Two subunits of VPS25 comprise the extended arms of Y-shaped ESCRT-II, while VPS22 interacts with VPS36 to form the base of the Y (Henne et al., 2011; Hurley, 2010; Teo et al., 2004). All three components of ESCRT-II from yeast, insects, and humans, are extremely well-conserved (Figs. 5 and S1). One exception is VPS36 from *Pediculus corporis*, which appears to be truncated by 135 aa at the N-terminus (containing no GLUE domain) and with an additional 148 aa at the C-terminus (Fig. 5, VPS36; Fig. S1). The common feature of ESCRT-II components is that they contain two tandem winged-helix (WH) motifs in the C-terminal region (Fig. 5). The exact role(s) of the WH motifs are not clear. The WH motifs likely are involved in interactions of subunits or in mediating ESCRT-II interactions with other ESCRT complexes, as it was previously demonstrated that the WH2 motif of VPS25 is the binding site of ESCRT-III subunit VPS20 (Teo et al., 2004). VPS22 also contains an N-terminal helical domain (ND) (Fig. 5), but the function of that domain is not clear (Hierro et al., 2004; Teo et al., 2004). The N-terminal region of about 140 aa in VPS36 from yeast, insects, and humans, consists of a "GLUE" (GRAM-like ubiquitin-binding in EAP45) domain, which is responsible for interaction with ubiquitin and for binding of PI3P (Hierro et al., 2004; Teo et al., 2006, 2004). The latter electrostatic interaction recruits ESCRT-II to the endosome membrane (Hierro et al., 2004; Teo et al., 2004). In yeast, two NZF (Npl4-type zinc finger) motifs (NZF1, NZF2) are inserted into the GLUE domain of VPS36. NZF1 directs the interaction of VPS36 with VPS28, while NZF2 binds with ubiquitin (Gill et al., 2007; Teo et al., 2006). The NZF motifs are absent in the orthologs of VPS36 from insects and humans (Fig. 5). However, as mentioned above, the GLUE domain of human VPS36 retains a ubiquitin-binding activity and also functions in cargo recognition (Teo et al., 2006).

3.1.4. ESCRT-III

ESCRT-III is a dynamic complex, which functions as a "scissor" required for membrane scission and vesicle release (Henne et al., 2011; Hurley, 2010; Hurley and Hanson, 2010; Peel et al., 2011; Saksena et al., 2009; Wollert et al., 2009). In yeast, the core subunits of ESCRT-III are VPS2, VPS20, VPS24, and VPS32/Snf7 (Babst et al., 2002; Saksena et al., 2009; Wollert et al., 2009). The orthologs of these components in mammals are called charged multivesicular body proteins (CHMPs) and are named CHMP2, CHMP6, CHMP3, and CHMP4, respectively (Henne et al., 2011; Hurley, 2010; Saksena et al., 2009; Wollert et al., 2009). Multiple isoforms of these proteins were identified in humans, which includes two isoforms of CHMP2 (CHMP2A, B), three isoforms of CHMP4 (CHMP4A, B, and C), and three isoforms of CHMP3 (isoforms 1–3) (Fig. 6A–C, Table 1). In insect genomes, gene expansion was only observed for CHMP2 (Table 1). While all insect species examined had an ortholog of human CHMP2A, we identified an ortholog of human CHMP2B in only a few insect species, which includes the 3 Lepidopteran species plus *P. corporis*, *T. castaneum*, and *D. melanogaster* (Table 1; Fig. S1). Sequence analysis indicated that the core components of ESCRT-III are extremely well-conserved in insects (Fig. S1). Amino acid sequence identities for ESCRT III subunits among the insect species were 74–98% (CHMP2A), 53–95% (CHMP2B), 45–86% (VPS20/CHMP6), 60–92% (VPS24/CHMP3), and 62–96% (Snf7/CHMP4) (Fig. S1). Further phylogenetic analysis showed that in insects and humans VPS2A/CHMP2A genes are closely related to VPS2 of *S. cerevisiae*. They form a distinct group from VPS2B/CHMP2B (Fig. 6A). Even though VPS2 orthologs come from an unknown common ancestral gene, they may have evolved independently and have divergent functional role(s). The three isoforms of human CHMP3 are grouped on the same branch with the VPS24 ortholog of *P. corporis* (Fig. 6B). In addition, phylogenetic analysis indicated that insect orthologs of VPS32/Snf7 are closely related to human CHMP4B, C (Fig. 6C). ESCRT-III core components contain a five-helix core at their N-terminus. The first two helixes are basic and responsible for membrane binding, and the following three helixes are acidic. After the helix core domain, there is a variable region, which is involved in autoinhibition of ESCRT-III subunits (Bajorek et al., 2009; Hurley, 2010; Lata et al., 2008). At the C-terminus, the core components contain a conserved MIM (MIT-interacting motif), which is critical for VPS4 binding (Bajorek et al., 2009; Stuchell-Brereton et al., 2007). Recent data demonstrate that VPS20, VPS24, and Snf7 consist of the membrane scission core, while VPS2 mediates coupling the scission complex to VPS4 (Hurley, 2010; Saksena et al., 2009). The high sequence and domain structure conservation of ESCRT-III core components in yeast, insects, and humans (Fig. S1), reflect the importance of the membrane scission role of ESCRT-III and demonstrate its conservation during evolution. However, the existence of multiple isoforms for these core components in humans could imply divergent species- and/or tissue-specific functions of ESCRT-III in mammals. In addition to the core components, ESCRT-III also includes the accessory proteins VPS46 and VPS60 (CHMP1 and CHMP5 in mammals, respectively) (Shestakova et al., 2010). In prior studies, depletion of VPS46 and VPS60 did not show a severe defect in cargo sorting and vesicle trafficking (Raymond et al., 1992) and both proteins are known to interact with Vta1 and ESCRT-III. Thus, they may function in recruiting and activating the VPS4-Vta1 complex, and promoting disassembly of ESCRT-III, but perhaps in a non-essential manner (Lottridge et al., 2006; Yang et al., 2012). In Drosophila, CHMP1 was found to be essential for the regulation of epidermal growth factor receptor (EGFR) signaling (Valentine et al., 2014). Only a single VPS60 (CHMP5) is found in yeast, and single highly conserved orthologs were identified in insects and humans (Table 1, Fig. S1). Two isoforms of VPS46 exist in humans (CHMP1A and CHMP1B, Table 1) and the functional difference between these isoforms is not clear. In insects, only a single ortholog of CHMP1 was found (Table 1). Insect CHMP1 shares 42–44% amino acid identity with VPS46 of yeast, and 47–51% and 68–76% with CHMP1A and

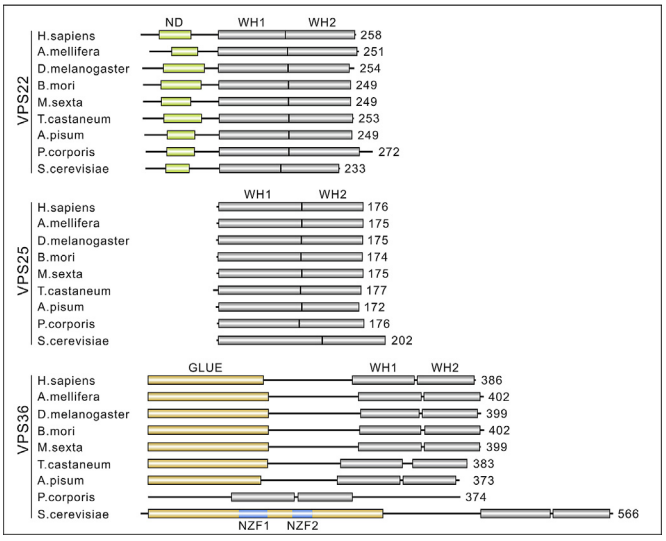

Fig. 5. Domain organization of ESCRT-II components VPS22, VPS25, and VPS36. The illustration shows the approximate sizes and the predicted domains/motifs found in VPS22, VPS25 and VPS36 proteins. Abbreviations: GLUE, GRAM-like ubiquitin-binding in EAP45; ND, N-terminal helical domain; NZF, Npl4-type zinc finger; WH, winged helix. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

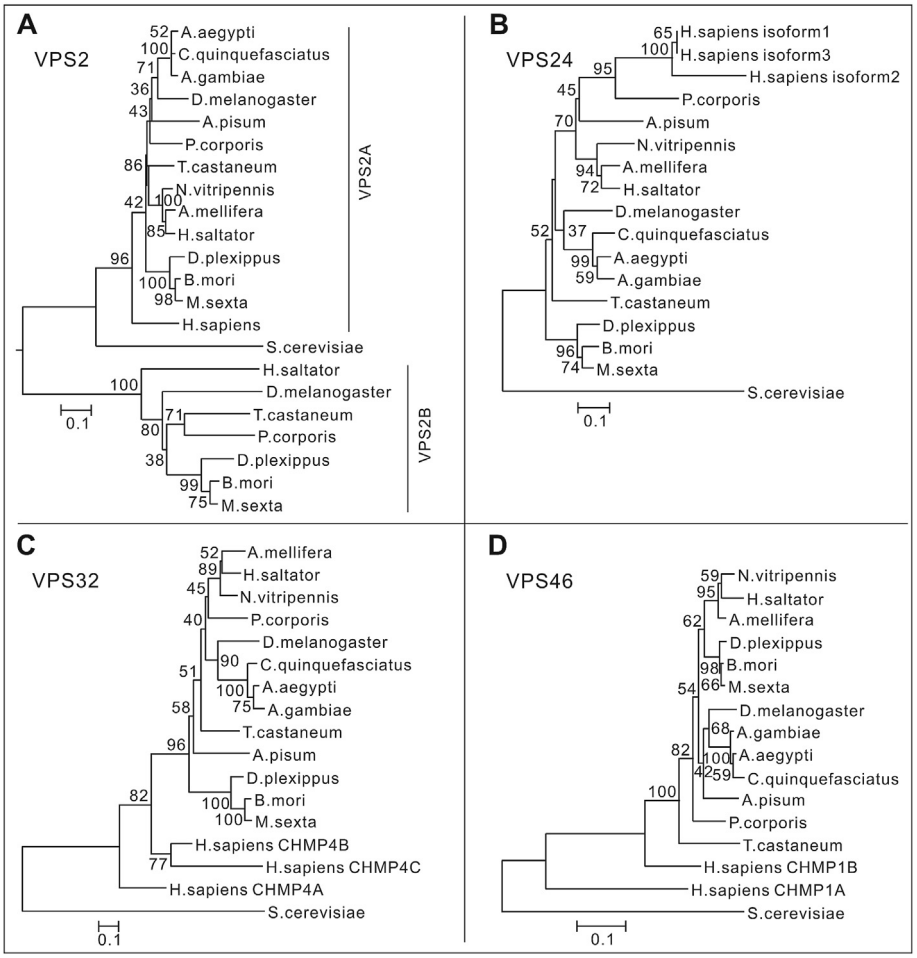

Fig. 6. The neighbor-joining phylogenetic trees of the ESCRT-III components VPS2 (A), VPS24 (B), VPS32 (C), and VPS46 (D). Bootstrap confidence values greater than 30% are indicated on the branches. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

CHMP1B of humans, respectively. Phylogenetic analysis also suggested that insect CHMP1(VPS46) is more closely related to human CHMP1B than to CHMP1A (Fig. 6D).

3.1.5. *VPS4–Vta1 complex*

The VPS4–Vta1 complex functions as a recycling machine to disassemble ESCRT-III (Lata et al., 2008; Stuchell-Brereton et al., 2007). In the VPS4–Vta1 complex, VPS4 is a class I AAA (ATPase associated with various cellular activities) ATPase (Babst et al., 1997). In its active state, VPS4 assembles into a stable dodecamer of two hexameric rings and then complexes with Vta1 (Monroe et al., 2014; Yang and Hurley, 2010). VPS4 captures ESCRT-III through the interaction of its N-terminal MIT (microtubule-interacting and transport) domain with MIM motifs of the core subunits of ESCRT-III (Lata et al., 2008; Stuchell-Brereton et al., 2007). There is a single VPS4 gene in yeast and insects, but two isoforms of VPS4 exist in humans. The two human VPS4 proteins (VPS4A and B) are 81% identical and share about 62% and 75–79% amino acid identity with that of yeast and humans, respectively. Disruption of VPS4 function caused tumor growth in Drosophila (Rodahl et al., 2009). Expression of hydrolysis mutant VPS4 E231Q in Lepidopteran *Spodoptera frugiperda* Sf9 cells resulted in the similar class E phenotype as observed in yeast and mammalian cells (Li and Blissard, 2012). Vta1 (LIP5 in mammals) is an active regulator of VPS4 (Azmi et al., 2008). Vta1 binds to VPS4 through its C-terminal VSL domain (Skalicky et al., 2012), and binds to ESCRT-III through its N-terminal MIT domain (Shim et al., 2008; Skalicky et al., 2012). Although deletion of Vta1 in yeast did not cause a severe defect in vesicle trafficking or morphological change of vacuole (Azmi et al., 2006), Vta1 was found to promote VPS4 oligomerization, activate VPS4 ATPase, and accelerate ESCRT-III disassembly (Azmi et al., 2006; Hurley, 2010; Lottridge et al., 2006). Vta1 is also highly conserved in yeast, insects, and humans, including its N-terminal two MIT domains and the C-terminal VSL domain (Fig. S1, Vta1).

3.1.6. *Bro1/Alix*

In addition to the essential subcomplexes described above, the ESCRT system also includes the accessory protein Bro1 (Alix in mammals) (Bissig and Gruenberg, 2014; Henne et al., 2011; Hurley, 2010). In yeast, Bro1 was originally identified as a class E VPS protein (VPS31). Yeast Bro1 shows 17–21% identity with Alix of insects and humans, while the orthologs of Alix of insects are 39–46% identical to that of humans. Bro1 and Alix share a similar domain structure organization. Both proteins contain a Bro1 domain at their N-terminus, a central coiled-coil motif that forms a V-shaped domain, and a C-terminal proline-rich (PR) domain (Fig. S1, VPS31/Alix). The Bro1 domain is involved in the interaction of Bro1/Alix with the ESCRT-III subunit Snf7/CHMP4 (McCullough et al., 2008). Functional differences between Bro1 and Alix were identified in yeast and humans. Bro1 is required for ESCRT-dependent ubiquitinated cargo sorting and MVB biogenesis, whereas Alix is mainly involved in cytokinesis (Bissig and

Gruenberg, 2014). Additionally, in many cases Alix is also involved in viral budding from the plasma membrane. The PR domain of Alix is responsible for interaction with midbody protein CEP55 (Lee et al., 2008), apoptotic protein ALG-2 (Missotten et al., 1999), and the ESCRT-I subunit Tsg101 (Falguieres et al., 2008). Therefore, these interactions link Alix with cell abscission, apoptosis, and probably with intracellular vesicle formation (Bissig and Gruenberg, 2014; Hurley, 2010). In the case of viral budding, the V-domain of Alix is known to interact with the so-called "late-budding motif" YPXnL ($n < 4$) in viral proteins to promote virus budding, by recruiting ESCRT-III to virus budding sites (Fujii et al., 2007; Votteler and Sundquist, 2013).

3.2. VPS-C complexes: CORVET and HOPS

The VPS-C tethering complexes CORVET and HOPS are mainly associated with endosomes and vacuolar lysosomes (Fig. 1) (Nickerson et al., 2009). Both CORVET and HOPS contain the VPS-C core subcomplex, which is composed of class C VPS proteins VPS11, VPS16, VPS18, and VPS33. In addition to the four shared proteins, CORVET contains two VPS21/Rab5-binding subunits, VPS3 and VPS8, whereas HOPS contains two Ypt7/Rab7-binding subunits, VPS39 and VPS41 (Balderhaar and Ungermann, 2013; Brocker et al., 2012; Kummel and Ungermann, 2014; Nickerson et al., 2009; Solinger and Spang, 2013). Additionally, a low abundance of intermediate complexes of CORVET and HOPS (which consist of VPS-C core and VPS39-VPS8 and VPS3-VPS41) have also been isolated (Peplowska et al., 2007).

By binding to activated VPS21/Rab5-GTP, CORVET is recruited to the endosome membrane, where it binds to SNARE proteins pep12 and others through VPS33 and VPS41 subunits (Balderhaar and Ungermann, 2013; Peplowska et al., 2007). Through collaboration with the SNARE complex, CORVET promotes the fusion of endocytic and Golgi-derived vesicles with the endosome (Balderhaar et al., 2013; Balderhaar and Ungermann, 2013; Hong and Lev, 2014; Nickerson et al., 2009). However, details of the interactions between CORVET and SNARE complex proteins are not clear. Currently, concepts on the functions of VPS-C tethering complexes are derived from studies of HOPS complexes (Balderhaar and Ungermann, 2013). Through its interaction with Ypt7/Rab7, HOPS is recruited to endosome and lysosome membranes, where HOPS specifically interacts with vacuolar SNARE components consisting of Vam3, Vam7, Vti1 and Nyv1 via VPS-C core subunits VPS16, VPS18, and VPS33 (Brocker et al., 2012; Kramer and Ungermann, 2011; Lobingier and Merz, 2012). By promoting vacuolar SNARE complex assembly, HOPS protects the trans-SNARE complex from disassembly by Sec17/α-SNAP and Sec18/NSF, and accelerates endosome-vacuolar lysosome fusion (Hong and Lev, 2014; Lobingier et al., 2014; Xu et al., 2010). It was also found that VPS41 interacts with API5, a subunit of AP-3 complex. This interaction and other evidence suggest that the HOPS complex is required for AP-3-dependent vesicle transport from Golgi to the vacuole (Angers and Merz, 2009). Additionally, HOPS interacts with Syntaxin17 (Syn17), an autophagosome SNARE protein, to promote the fusion of autophagosomes with lysosomes in *Drosophila* (Takats et al., 2014).

The components of CORVET and HOPS are evolutionary conserved. The VPS-C core subunits VPS11 and VPS18 have orthologs in insects and humans (Table 2, Fig. S1). They share 19–45% and 34–42% amino acid identity between insects and humans, respectively. Two isoforms of VPS16 (VPS16A and VPS16B/SPE-39) and VPS33 (VPS33A and VPS33B) were identified in insects and humans (Table 2). It was found that VPS33A interacts with VPS16A and functions in endolysosomal fusion, whereas the interaction of VPS33B and VPS16B is required for phagocytosis and early endosome fusion (Akbar et al., 2009; Zhu et al., 2009). The corresponding orthologs of VPS8 and VPS41, were also found in insects and humans (Table 2, Fig. S1). In addition, two VPS39 homologs were reported in mammals (VPS39/Vam6 and TRAP-1, transforming growth factor-beta receptor-associated protein 1) (Nickerson et al., 2009). However, only a single ortholog of VPS39/Vam6 was identified in insects (Table 2). In addition, we did not identify an ortholog of VPS3 in insects and humans. A VPS3 ortholog is also absent from the *Caenorhabditis elegans* genome (Nickerson et al., 2009). Presently, it is not yet clear whether TRAP-1 may be a functional homolog of VPS3, or whether a homolog of

Table 2
Properties of the tethering complexes.

Complex	Subunits			Functional perspectives			
	Yeast	Human	Insects	Functional roles	Membrane binding	Motif/domain	Interactions
VPS-C core	VPS11	VPS11	+	VPS-C core	Through interaction with the adapter complexes	α-solenoid, β-propeller Ring finger	VPS11–VPS18 VPS11–VPS16 VPS11–VPS3
	VPS16	VPS16A	+			α-solenoid, β-propeller	VPS16–VPS33
		VPS16B	+				
	VPS18	VPS18	+			α-solenoid, β-propeller Ring finger	VPS18–VPS8
	VPS33	VPS33A	+			SM	VPS33-SNAREs
		VPS33B	+				
CONVET	VPS3	?	?	Adapter complex	Through interaction with Rab5-GTP	α-solenoid, β-propeller	VPS3-Rab5
	VPS8	VPS8	+			α-solenoid, β-propeller Ring finger	VPS8-Rab5
HOPS	VPS39	VPS39 TRAP-1	+	Adapter complex	Through interaction with Rab7-GTP	α-solenoid, β-propeller	VPS39-Rab7
	VPS41	VPS41	+			α-solenoid, β-propeller	VPS41-Rab7
GARP	VPS51	Ang2	+		Through interaction of Ypt6/Rab6 with	CC	
	VPS52	Are1	+		VPS52, Arl1 with VPS53	CC	VPS52-Ypt6/Rab6
	VPS53	HCCS1	+			CC	VPS53-Arl1
	VPS54	HCC8	+			CC	

? unknown ortholog in insects and humans.

VPS3 with low sequence identity has yet to be identified. Except for VPS33, the components of CORVET and HOPS are similar in their secondary structures, containing a predicted β-propeller at their N-termini and α-solenoid in the C-terminal region. Additionally, VPS8, VPS11, and VPS18 also contain a Ring finger motif at their extreme C-terminus, which may function as an E3 ubiquitin ligase domain (Table 2, Fig. S1) (Nickerson et al., 2009; Plemel et al., 2011). VPS33 is a Sec1/Munc18 (SM) family member (Lobingier et al., 2014). Both of the VPS33 isoforms (VPS33A and VPS33B) contain two predicted Sec1 (secretory blood group 1) domains (Fig. 7). Some insect species have only partial sequences of orthologs of CORVET and HOPS components. For example, the VPS39 ortholog of *D. plexippus* lacks approximately 130 N-terminal amino acids and approximately 80 C-terminal amino acids. Similarly, VPS39 of *A. aegypti* lacks approximately 380 aa from the N-terminus. VPS18 of *C. quinquefasciatus* also lacks approximately 430 aa from the N-terminus. Future studies should reveal whether these observed differences represent functional adaptations, or whether they may simply result from frame shift or other errors in the available genome assemblies.

3.3. The retromer complex

The retromer complex was originally identified in yeast as an endosome-to-Golgi retrieval machinery. Retromer is essential to retrieve vacuolar hydrolase receptors from endosome to Golgi and maintain the homeostasis of hydrolase receptors in the trans-Golgi network (TGN) (Fig. 1) (Attar and Cullen, 2010). Available data suggest that retromer also mainly functions in retrieving cargo proteins from endosome-to-TGN in mammalian cells, but this complex is also required for endosome-to plasma membrane sorting and cell signaling regulation (Attar and Cullen, 2010; Seaman, 2012). Recently, retromer was also found to be required for recruiting the WASH complex, which mediates formation of branched actin patches providing the sorting platform in endosomes (Seaman et al., 2013).

In yeast, retromer is a hetero-pentameric complex consisting of five VPS proteins (VPS5, VPS17, VPS26, VPS29, and VPS35) (Attar and Cullen, 2010; Seaman, 2012) (Table 3). Biochemically and phenotypically, retromer can be dissected into two subcomplexes: cargo-selective trimer (VPS26, VPS29, and VPS35) and membrane-deforming heterodimer (VPS5 and VPS17) (Seaman, 2012). The components of the cargo-selection complex are highly conserved in yeast, insects and humans (Fig. S1, Table 3). They share 43–75% (VPS26), 47–87% (VPS29), and 34–70% (VPS35) amino acid identity among different kingdoms, respectively (Fig. S1). In humans, two VPS26 proteins (VPS26A, VPS26B) encoded by separate genes exist (Kerr et al., 2005). Both VPS26 proteins function as part of the cargo-selection trimer, and produce two cargo-selection subcomplex variants (VPS26A-VPS35-VPS29, VPS26B-VPS35-VPS29) (Bugarcic et al., 2011). Tissue-specific function and distinct intracellular distribution of VPS26 isoforms in mammals suggest that the VPS26 gene expansion diversifies the function of retromer (Bugarcic et al., 2011). Our analysis indicated that there is only a single VPS26 in *M. sexta* and in insects in general. The VPS26 orthologs contain a highly conserved N-terminal arrestin domain, but VPS26 proteins from different species contain a variable C-terminus (Fig. S1, VPS26). Distinguished from the other VPS26 proteins, the C-termini of VPS26 proteins from Dipteran and Lepidopteran insects are extended by 70–290 aa (Fig. S1, VPS26). It is known that VPS26 interacts with VPS35, which is the cargo recognizing subunit (Seaman, 2012). Recent studies also demonstrate that VPS26 can bind to retromer cargo protein SorLA, implying that VPS26 is also involved in cargo sorting (Fjorback et al., 2012). It is not clear whether the sequence variations at the C-terminus of VPS26 are related to the cargo binding specificity or capacity. VPS29 interacts with VPS5 and VPS35 to bridge the cargo-selection and membrane-bending subcomplexes of retromer (Seaman, 2012). VPS35 is the core of the cargo-selection complex and is directly involved in cargo binding (Attar and Cullen, 2010; Seaman, 2012). VPS29 and VPS35 are well-conserved among yeast, insects, and humans, including the extremely conserved VPS26 interaction motif "PRLYL" within the N-terminus of VPS35 (Fig. S1, VPS35). Compared with VPS29 and VPS35 from the other species, the orthologs from *H. saltator*, *A. mellifera*, and *D. plexippus* contain substantial variations in sequence length in various portions of these predicted proteins (Fig. S1). Because of the critically important functions of these proteins and the high degree of conservation from yeast to insects to humans, it is not clear whether these are true variations in the proteins or simply result from sequence assembly issues.

The components of the membrane-deforming subcomplex are also highly conserved, but are more variable than that of the cargo-selection subcomplex. In humans, two orthologs each of yeast VPS5 and VPS17 exist as SNX1 (sorting nexin 1) and SNX2, SNX5 and SNX6, respectively. However, in insects, we identified one ortholog each of SNX1/SNX2 and SNX5/SNX6 (Table 3, Fig. 8). VPS5, VPS17, and SNX proteins are members of the sorting nexin family (Attar and Cullen, 2010; Koumandou et al., 2011) and they all contain a PX (Phox homology) domain and a C-terminal BAR (Bin, Amphiphysin, Rvs) domain. However, SNX1 and SNX2 of humans also contain an SNX-N (sorting nexin N-terminal) domain, which has an unknown function (Attar and Cullen, 2010). Domain structure analysis shows that the SNX-N domain is absent in yeast VPS5 and its orthologs in insects. In addition, the N-terminal length of insect SNX is generally shorter than that of human SNX1/SNX2, by approximately 20–60 aa (Figs. 8A and S1).

Phylogenetic analyses showed that insect orthologs of VPS5, VPS17, and VPS26 have similar evolutionary relationships to human orthologs: insect orthologs all form a clade on the phylogenetic tree, distinct from that of humans (Figs. 8C, D and 9).

3.4. GARP complex

GARP is a heterotetramer tethering complex, which is predominantly associated with the trans-golgi network (TGN, Fig. 1), where it cooperates with SNARE (soluble NSF attachment protein receptor) complexes to promote the fusion of endosome-derived vesicles to TGN (Bonifacino and Hierro, 2011; Brocker et al., 2010). The retrieval function of GARP is essential and required for recycling receptors for vacuolar/lysosomal hydrolase precursors, transmembrane proteins, and SNARE proteins (Bonifacino and Hierro, 2011). Recent data demonstrate that the GARP complex is also involved in microRNA-mediated gene regulation (Vasquez-Rifo et al., 2013). In yeast, GARP is composed of VPS51, VPS52, VPS53, and VPS54 (Bonifacino and Hierro, 2011). Except for VPS51, these

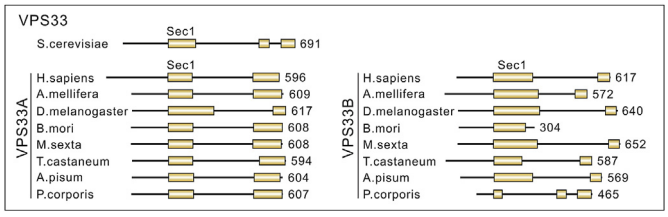

Fig. 7. Domain organization of the VPS-C complex core component VPS33. The scheme shows the approximate sizes and the predicted domains/motifs of VPS33. Abbreviations: Sec1, secretory blood group 1. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

Table 3
Properties of the retromer system.

Complex	Subunits			Functional perspectives			
	Yeast	Human	Insects	Functional roles	Lipid binding	Motif/domain	Subunits interactions
Membrane-bending complex	VPS5	SNX1 SNX2	+	Membrane deformation	with PtdIns3P via PX domain	PX, BAR SNX-N	VPS5–VPS17 SNX1–SNX5/6
	VPS17	SNX5 SNX6	+			PX, BAR	SNX2–SNX5/6
Cargo selection complex	VPS26	VPS26A VPS26B	+	Cargo recognition and selection	Through interactions with Rab7 and/or SNX3	Arrestin	VPS26–VPS35
	VPS29	VPS29	+			Calcineurin-like phosphoesterase	VPS29–VPS35
	VPS35	VPS35	+			PRLYL	

components, are highly conserved in yeast, insects, and humans (Fig. S1). VPS51 orthologs from insects and humans are more distantly related but have close similarities in the N-terminal region (Figs. 10 and S1). A common feature for GARP components is the presence of predicted coiled-coil (CC) motifs within the N-terminal 300 aa region (Fig. 10). These CC motifs may be involved in the interactions of GARP subunits (Bonifacino and Hierro, 2011). In addition, it was shown that the amino-terminal regions of VPS53 and VPS54 also bind to SNARE proteins Syn6, Syn16, and Vamp4 (Perez-Victoria and Bonifacino, 2009). Compared with the other species examined, VPS51 of *B. mori* and *M. sexta* contain one additional CC motif at their C-terminus. In the case of VPS53, the other species examined contained 2–4 CC motifs, whereas *B. mori* and *M. sexta* had only a single CC motif (Fig. 10). The observation of the variations in CC motifs among the different GARP subunits from different species is curious and will require future experimental analysis to determine whether there are significant functional implications associated with these differences.

3.5. Class III PI3K complex

PI3K enzymes phosphorylate position 3 within specific phosphoinositides (PtdIns). They are essential components of intracellular signaling pathways and are involved in many different biological processes. In higher eukaryotes, PI3K is divided in three classes (I, II, III) based on the lipid substrate specificity. Among them, class III PI3K (PI3K-III) has a substrate specificity that is limited to PtdIns, generating PtdIns(3)P. PtdIns(3)P functions as

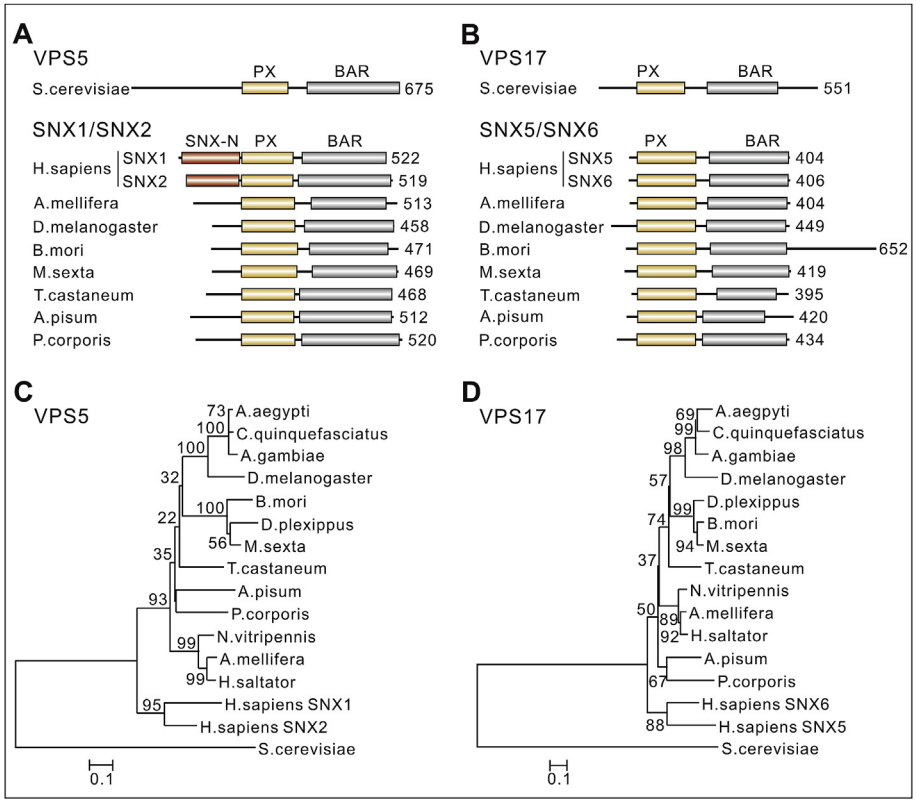

Fig. 8. Domain organization (A, B) and the neighbor-joining phylogenetic trees (C, D) of the components of retromer membrane-deforming subcomplex. (A, B) The scheme shows the approximate sizes and the predicted domains/motifs of VPS5 (A) and VPS17 (B). (C, D) Bootstrap confidence values greater than 30% are indicated on the branches. Abbreviations: BAR, BIN-Amphiphysin-Rvs domain; PX, Phox homology; SNX, sorting nexin; SNX-N, sorting nexin N-terminal domain. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

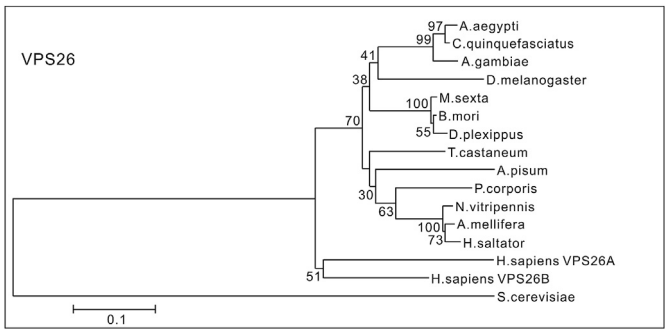

Fig. 9. The neighbor-joining phylogenetic tree of the Retromer core component VPS26. Bootstrap confidence values greater than 30% are indicated on the branches. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

targeting sites on the endomembrane for many proteins that are involved in vesicle trafficking (Vanhaesebroeck et al., 2010, 1997). Yeast have only class III PI3Ks, and they can be functionally distinguished as two complexes (I, II). Both of the complexes contain the PI3K-III core components VPS15, VPS34, and VPS30. VPS15 is the regulatory subunit and forms a heterodimer with VPS34, the catalytic subunit (Vanhaesebroeck et al., 2010). VPS30 is the adapter subunit that promotes VPS15–VPS34 core assembly, and also provides a binding platform for other proteins, such as Atg14 (autophagy-related protein 14) (in PI3K-III complex I) and VPS38 (in PI3K-III complex II). PI3K-III complex I plays essential roles in autophagy, whereas complex II is involved in vacuole protein sorting (Vanhaesebroeck et al., 2010). Similarly, mammalian PI3K-III contains the VPS15–VPS34 core complex, but also contains more adapter/regulatory subunits that permits it to carry out a variety of functions. Recent data suggest that the

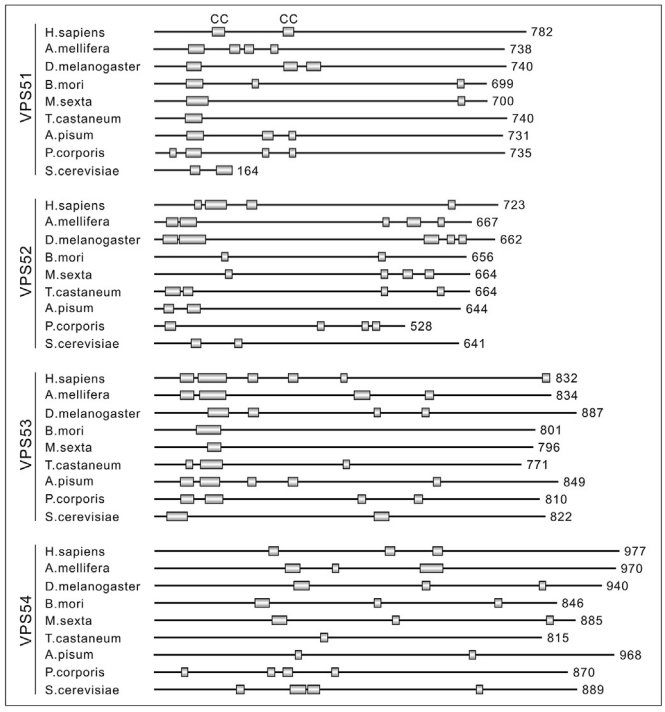

Fig. 10. Domain organization of the components of the GARP complex. The illustration shows the approximate sizes and the predicted coiled-coil (CC) motifs of VPS51, VPS52, VPS53 and VPS54. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

VPS15–VPS34-UVRAG (ultraviolet irradiation resistance-associated gene, a putative ortholog of yeast VPS38) complex is mainly involved in endosomal vesicle trafficking, while complexes containing the VPS15–VPS34 core plus other proteins, such as Beclin 1 and Atg14L (homologs of VPS30 and Atg14 in yeast, respectively) function in regulating autophagy (Thoresen et al., 2010; Vanhaesebroeck et al., 2010). Our analysis revealed that the PI3K-III core VPS15–VPS34 and the essential adapter/regulator proteins VPS30/Beclin1 are also highly conserved in insects (Fig. S1, VPS30/Beclin1), even though some insect sequences contained substantial gaps, possibly representing genome assembly errors. However, VPS38/UVRAG, a key regulator of endosomal trafficking and autophagy (Pirooz et al., 2014), shows a high level of divergence between yeast, insects, and humans (Fig. S1, VPS38/UVRAG). The amino acid identity of VPS38/UVRAG between insects and yeast is only 9–13%, and between insects and humans is 13–21%. The amino acid sequence identity of VPS38/UVRAG even within insect species is only 15–67%. Recent data indicate that PI3K-III plays critical roles in regulation of membrane protein location in Drosophila wing cells (Abe et al., 2009).

3.6. Other characterized VPS genes

In addition to the above mentioned complexes composed of VPS proteins, several additional VPS proteins have been well characterized. One is VPS1 and its ortholog, Dynamin in humans. VPS1/Dynamin is a GTPase associated with microtubules, that is required for the scission of clathrin-coated vesicles from the plasma membrane (Ferguson and De Camilli, 2012; Morlot and Roux, 2013; Vater et al., 1992). VPS1/Dynamin consists of a GTPase domain, the stalk, a pleckstrin homology (PH) domain, a GTPase effector domain, and the C-terminal proline-rich domain. These domains hydrolyze GTP, transmit conformation and mediate oligomerization, bind to membranes, and interact with regulatory proteins (Faelber et al., 2012; Ferguson and De Camilli, 2012; Morlot and Roux, 2013). In mammals, three Dynamin isoforms are encoded by different genes. Dynamin 1 and 3 are highly expressed in neurons and brain, respectively, while Dynamin 2 is expressed ubiquitously. Only a single dynamin gene was identified in invertebrates (Ferguson and De Camilli, 2012; van der Bliek and Meyerowitz, 1991) and our analysis showed that insect genomes contain only one ortholog of Dynamin. Insect Dynamin shares a high level of conservation with yeast VPS1 and human Dynamin proteins (Fig. S1, VPS1/Dynamin). As expected, phylogenetic analysis indicates that insect orthologs of Dynamin form a lineage that is distinct from that of humans (Fig. 11A).

VPS6 and VPS43 and their mammalian orthologs Syntaxin-7 and SNAP-25 are members of the SNARE membrane fusion system. Both proteins are essential and required for fusion of endocytic trafficking vesicles with the prevacuolar compartment/late endosome and/or the vacuole/lysosome (Becherer et al., 1996; Nakamura et al., 2000; Ungermann and Wickner, 1998). Amino acid sequence alignments revealed that VPS6/Syntaxin-7 and VPS43/SNAP-25 are highly conserved in yeast, insects, and humans (Fig. S1, VPS6 and VPS43). The insect orthologs of these two proteins share about 36–44% (Syntaxin-7) and 56–62% (SNAP-25) identities with the human proteins. They also share relatively low amino acid identities, about 17–21% and 14–18%, with the yeast VPS6 and VPS43 proteins, respectively. The cysteine-rich domain of SNAP-25 is required for the palmitoylation of SNAP-25 and the anchoring of SNAP-25 to membrane (Greaves et al., 2010), is also very highly conserved in the insect orthologs of VPS43/SNAP-25 (Fig. S1, VPS43).

VPS10 is a sorting receptor for vacuolar hydrolases. In the trans-Golgi network, VPS10 interacts with hydrolases (such as

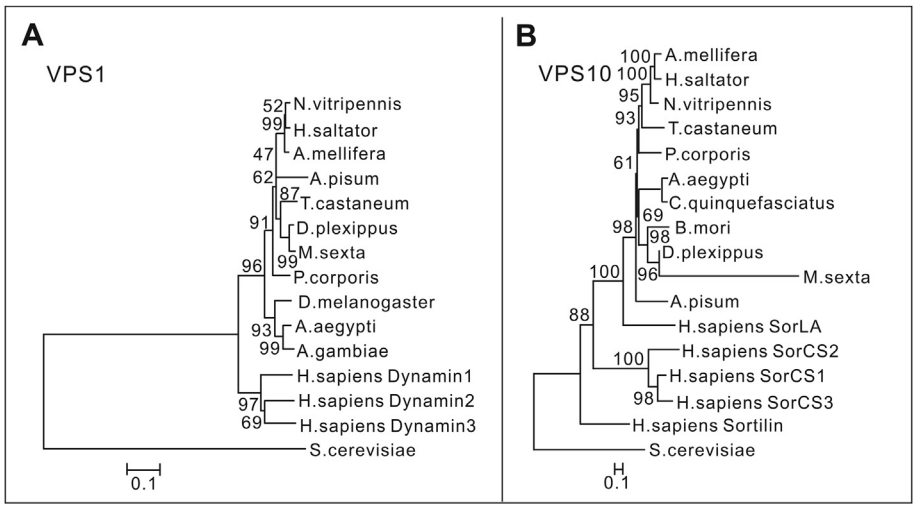

Fig. 11. The neighbor-joining phylogenetic trees of VPS1 (A) and VPS10 (B). Bootstrap confidence values greater than 30% are indicated on the branches. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

carboxypeptidase Y) and the VPS10-hydrolase complex is transported to prevacuolar compartments. After releasing the hydrolase, VPS10 is recycled back to the TGN (Hermey, 2009). In mammals, five proteins (Sortilin, SorCS1, SorCS2, SorCS3, and SorLA) were identified as VPS10 orthologs, and these mammalian VPS10 orthologs also function as sorting receptors for delivery of ligands from the TGN to the late endosome and/or lysosome (Hermey, 2009). Our analysis showed that only the ortholog of SorLA is present in the analyzed insect genomes (Figs. 11B and S1, VPS10). The amino acid identities between SorLA orthologs of humans and insects range from 30 to 39%. Identities between VPS10/SorLA orthologs in insects and yeast are relatively low, ranging from 8 to 14%. Phylogenetic analysis further supports the closely evolutionary relationship of insect orthologs of VPS10 and SorLA of humans (Fig. 11B). Compared with the VPS10/SorLA orthologs of other insects, *M. sexta* SorLA contains an additional approximately 240 amino acids at the N-terminus, plus several large insertions which combined predict a protein >2400 amino acids larger than that of the other insect species (Fig. S1, VPS10).

VPS13 is a class A protein that is required for trafficking of membrane proteins to the vacuole and morphogenesis of prospore membrane in yeast (Park and Neiman, 2012). In humans, four VPS13 orthologs (VPS13A, VPS13B, VPS13C, and VPS13D) were identified (Velayos-Baeza et al., 2004), but the functional roles of these orthologs in humans are not clear. Comparative analysis revealed that in insects, there are three VPS13 orthologs corresponding to human VPS13A, VPS13B, and VPS13D (Fig. S1; VPS13A, B, and D). Orthologs of VPS13A and VPS13D are highly conserved between insects and humans with identities of approximately 25–36%. In contrast, the conservation of VPS13B is relatively low, with identities ranging from 18 to 25%. In addition, we did not identify orthologs of VPS13A and VPS13D in *D. plexippus* and *A. pisum* genomes, and the ortholog of VPS13B also appears to be absent in the *A. pisum* and *P. corporis* genomes.

VPS21 and its mammalian ortholog Rab5 are small Ras-like GTPase proteins and are mainly associated with early endosomes (Fig. 1). Activation of VPS21/Rab5 is mediated by the GDP–GTP exchange factors (GEFs) VPS9 and its mammalian ortholog Rabex5, respectively (Carney et al., 2006). In the active state, Rab5-GTP recruits the tethering factors EEA1, Rabenosyn-5, and the CORVET complex to ensure the proper association of cargo vesicles with target membranes (Balderhaar et al., 2013; Kummel and Ungermann, 2014; Peplowska et al., 2007). Rab5 also interacts with endosomal SNAREs to promote fusion (Kummel and Ungermann, 2014). Comparative analysis shows that VPS21/Rab5 is extremely conserved in yeast, insects, and humans (Fig. S1, VPS21/Rab5). However, even though VPS9/Rabex5 from different species are also highly conserved (Fig. S1, VPS9/Rabex5) and they share the VPS9 core domain (Fig. 12), Rabex5 of insects and humans contain an additional N-terminal zinc finger motif and a C-terminal coiled-coil domain that are not present in yeast (Fig. 12, VPS9/Rabex5). Instead VPS9 of *S. cerevisiae* contains a specific CUE domain at its C-terminus, which is necessary for ubiquitin binding (Carney et al., 2006). In addition, amino acid sequence alignment showed that Rabex5 of humans contains an additional approximately 120 aa at the N-terminus, as compared with Rabex5 of yeast and insects. The C-termini of insect Rabex5 proteins are more variable and larger than that of the yeast and human proteins (Fig. S1, VPS9/Rabex5). Uncovering the significance of these variations will require future experimental analyses.

VPS44/Nhx1 is an endosomal cation/proton exchanger that is involved in evaluating the endosomal and vacuole pH. Nhx1 is essential for vesicle trafficking during the stage of post multivesicular body formation (Kallay et al., 2011). In mammalian cells, NHE9 functions as the ortholog of Nhx1 (Kondapalli et al., 2013). The orthologs of Nhx1/NHE9 are highly conserved in yeast, insects, and humans (Fig. S1, VPS44/NHE9). Amino acid identities between insect and human orthologs range from 49 to 54%, and that between insect and yeast orthologs range from 26 to 31%. Within insect genomes, the Nhx1/NHE9 orthologs show 62–92% identity. Compared with the other insect orthologs of Nhx1/NHE9, *M. sexta* Nhx1/NHE9 lacks the C-terminal region of approximately 210–320 amino acids (Fig. S1, VPS44/NHE9).

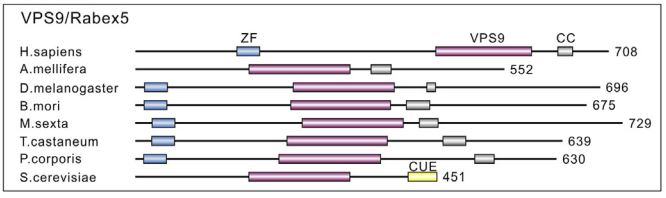

Fig. 12. Domain organization of VPS9 and its ortholog Rabex5. The illustration shows the approximate sizes and the predicted domains/motifs of VPS9/Rabex5 orthologs. Abbreviations: CC, coiled-coil; CUE, ubiquitin system component cue; ZF, zinc finger. Accession numbers for amino acid sequences are listed in Supplemental Table S1.

VPS45 is a class D protein involved in protein trafficking and in humans, splice variants of VPS45 may serve several functions. Similar to VPS33 (the core subunit of CORVET/HOPS complexes), VPS45 is also a Sec1/Muc18 (SM) family protein and is required for activating the assembly of endosomal SNARE complexes through interaction with syntaxin Tlg2 and v-SNARE protein Snc2 (Hong and Lev, 2014; Shanks et al., 2012). Orthologs of VPS45 are well conserved across yeast, insects, and humans (Fig. S1, VPS45). They share 33–38% amino acid sequence identity between yeast and insects, 46–84% identity within insects, and 54–62% identity between insects and humans.

In yeast, VPS55 is a small protein of about 140 aa with four predicted transmembrane domains (Belgareh-Touze et al., 2002). It is mostly localized in late endosomes and forms a complex with VPS68, which has no known ortholog in insects and humans. The VPS55–VPS68 complex is required for endosomal trafficking in yeast (Schluter et al., 2008). Depletion of either VPS55 or VPS68 disrupts recycling to the TGN (Belgareh-Touze et al., 2002; Schluter et al., 2008). Interestingly, the human OB-RGRP protein (obesity receptor gene-related protein), which is distantly related to VPS55, was shown to complement the phenotypic defects of a VPS55 mutant (Belgareh-Touze et al., 2002). Our analysis showed that VPS55 is also moderately well conserved between insects and humans (Fig. S1, VPS55) and we did not identify an ortholog of VPS68 in the *M. sexta* or other insect genomes.

VPS74 was originally characterized as a class A VPS protein (Hedman et al., 2007). Recent data demonstrated that VPS74 and its ortholog GOLPH3 in humans are associated with the cis-Golgi, where it binds the cytosolic domains of mannosyltransferases and promotes the targeting of these glycosyltransferases to Golgi, and their incorporation into COPI-coated vesicles (Schmitz et al., 2008; Tu et al., 2008). Binding of VPS74 with PtdIns(4)P is an essential step for Golgi-localization of VPS74/GOLPH3 (Wood et al., 2009). Alternatively, VPS74/GOLPH3 may also be involved in dephosphorylation of PtdIns(4)P (Wood et al., 2012). Comparative analysis revealed that the orthologs of VPS74/GOLPH3 are extremely well-conserved in yeast, insects, and humans (Fig. S1, VPS74). Insect VPS74 proteins share 40–42% and 70–75% amino acid identity with those of yeast and humans, respectively.

3.7. Conclusions and perspectives

In eukaryotic cells, vesicular trafficking is essential for sorting membrane cargo to different destinations, for recycling, and for degradation. During trafficking processes, numerous protein complexes participate in vesicle formation, transport, and fusion, in order to accurately control each step and maintain the integrity and dynamics of the membrane system (Brighouse et al., 2010; Campelo and Malhotra, 2012). Among these trafficking-related complexes are ESCRT, CORVET, HOPS, retromer, GARP, and PI3K-III, which are mainly composed of VPS proteins. Among the myriad roles of these complexes are biogenesis of MVBs, regulating endolysosomal fusion, recycling receptors to TGN, and targeting functional complexes on specific target membranes (Bonifacino and Hierro, 2011; Henne et al., 2011; Nickerson et al., 2009; Schuh and Audhya, 2014; Seaman, 2012; Vanhaesebroeck et al., 2010). We compared a large variety of VPS proteins across yeast, insect, and human genomes, and across insect genomes representative of six insect orders. Comparative genome analysis shows that these VPS protein complexes are evolutionally conserved in yeast, insects, and humans. In comparison with the yeast genome, VPS genes that encode components of ESCRT, VPS-C complexes CORVET and HOPS, and retromer are expanded into various isoforms in the human genome. However, in the genomes of the six insect Orders examined, a similar expansion was only observed for a few VPS genes, which included VPS2 and VPS37 (subunits of ESCRT), and VPS16 and VPS33 (subunits of VPS-C core). It is of special interest to note that the VPS gene expansions and specific isoforms present within the Insecta appear to be highly uniform across the insect Orders and species examined, suggesting that this expansion predated or occurred with the divergence of the Insecta.

It is well established that ESCRT, VPS-C complexes, and retromer are crucial for endosomal sorting and trafficking. Considering the tight regulation of endocytic and retrieval vesicle trafficking, complexes such as the coat/adapter complexes, ESCRT, retromer, and tethering complexes (CORVET, HOPS, and GARP) must coordinate their functions through cross-talk in order to provide normal cellular functions and maintain homeostasis. Compared with studies of VPS proteins in yeast and mammals, little specific information is known regarding the functions of insect VPS orthologs. Of particular interest are VPS proteins that are associated with gene expansions in mammals, but not in insects. How these VPS proteins accomplish their appropriate functions in insects, without the diversity provided by the gene expansions observed in mammals will be of great interest and should be addressed experimentally. The current study has provided a basis for addressing these questions. Future detailed comparative studies of each VPS gene/protein and their associated complexes will help to illuminate the network of regulatory mechanisms controlling vesicular transport pathways in eukaryotes, and specifically how invertebrates differ in their regulation and function of these essential processes. These and other studies will be important for understanding the molecular mechanisms underpinning invertebrate cellular physiology, as well as insect development, immunity, and pathogen–insect interactions.

Acknowledgments

This work was supported by a grant from the National Natural Science Foundation of China (NSFC, No. 31272088) and a start up program from Northwest A&F University (No. Z111021104) to ZL, and NSF grant (IOS-1354421) to GWB. The *Manduca genome* sequence and RNA-seq dataset were provided by the *Manduca* sequence consortium through grants from the NIH (GM041247) (to Mike Kanost, Kansas State University) and DARPA (to GWB) and was made available at www.agripestbase.org/manduca/ by Sue Brown (Kansas State University).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ibmb.2014.11.007.

References

Abe, M., Setoguchi, Y., Tanaka, T., Awano, W., Takahashi, K., Ueda, R., Nakamura, A., Goto, S., 2009. Membrane protein location-dependent regulation by PI3K (III) and rabenosyn-5 in Drosophila wing cells. PloS One 4, e7306.
Akbar, M.A., Ray, S., Kramer, H., 2009. The SM protein Car/Vps33A regulates SNARE-mediated trafficking to lysosomes and lysosome-related organelles. Mol. Biol. Cell 20, 1705–1714.
Angers, C.G., Merz, A.J., 2009. HOPS interacts with Apl5 at the vacuole membrane and is required for consumption of AP-3 transport vesicles. Mol. Biol. Cell 20, 4563–4574.
Arlt, H., Perz, A., Ungermann, C., 2011. An overexpression screen in *Saccharomyces cerevisiae* identifies novel genes that affect endocytic protein trafficking. Traffic 12, 1592–1603.
Attar, N., Cullen, P.J., 2010. The retromer complex. Adv. Enzyme Regul. 50, 216–236.
Azmi, I., Davies, B., Dimaano, C., Payne, J., Eckert, D., Babst, M., Katzmann, D.J., 2006. Recycling of ESCRTs by the AAA-ATPase Vps4 is regulated by a conserved VSL region in Vta1. J. Cell Biol. 172, 705–717.
Azmi, I.F., Davies, B.A., Xiao, J., Babst, M., Xu, Z., Katzmann, D.J., 2008. ESCRT-III family members stimulate Vps4 ATPase activity directly or via Vta1. Dev. Cell 14, 50–61.

Babst, M., Katzmann, D.J., Estepa-Sabal, E.J., Meerloo, T., Emr, S.D., 2002. Escrt-III: an endosome-associated heterooligomeric protein complex required for mvb sorting. Dev. Cell 3, 271–282.
Babst, M., Sato, T.K., Banta, L.M., Emr, S.D., 1997. Endosomal transport function in yeast requires a novel AAA-type ATPase, Vps4p. EMBO J. 16, 1820–1831.
Bajorek, M., Schubert, H.L., McCullough, J., Langelier, C., Eckert, D.M., Stubblefield, W.M., Uter, N.T., Myszka, D.G., Hill, C.P., Sundquist, W.I., 2009. Structural basis for ESCRT-III protein autoinhibition. Nat. Struct. Mol. Biol. 16, 754–762.
Balderhaar, H.J., Lachmann, J., Yavavli, E., Brocker, C., Lurick, A., Ungermann, C., 2013. The CORVET complex promotes tethering and fusion of Rab5/Vps21-positive membranes. Proc. Natl. Acad. Sci. USA 110, 3823–3828.
Balderhaar, H.J., Ungermann, C., 2013. CORVET and HOPS tethering complexes – coordinators of endosome and lysosome fusion. J. Cell Sci. 126, 1307–1316.
Banta, L.M., Robinson, J.S., Klionsky, D.J., Emr, S.D., 1988. Organelle assembly in yeast: characterization of yeast mutants defective in vacuolar biogenesis and protein sorting. J. Cell Biol. 107, 1369–1383.
Becherer, K.A., Rieder, S.E., Emr, S.D., Jones, E.W., 1996. Novel syntaxin homologue, Pep12p, required for the sorting of lumenal hydrolases to the lysosome-like vacuole in yeast. Mol. Biol. Cell 7, 579–594.
Belgareh-Touze, N., Avaro, S., Rouille, Y., Hoflack, B., Haguenauer-Tsapis, R., 2002. Yeast Vps55p, a functional homolog of human obesity receptor gene-related protein, is involved in late endosome to vacuole trafficking. Mol. Biol. Cell 13, 1694–1708.
Bissig, C., Gruenberg, J., 2014. ALIX and the multivesicular endosome: ALIX in Wonderland. Trends Cell Biol. 24, 19–25.
Bonangelino, C.J., Chavez, E.M., Bonifacino, J.S., 2002. Genomic screen for vacuolar protein sorting genes in *Saccharomyces cerevisiae*. Mol. Biol. Cell 13, 2486–2501.
Bonifacino, J.S., Hierro, A., 2011. Transport according to GARP: receiving retrograde cargo at the trans-Golgi network. Trends Cell Biol. 21, 159–167.
Bonifacino, J.S., Hurley, J.H., 2008. Retromer. Curr. Opin. Cell Biol. 20, 427–436.
Boura, E., Hurley, J.H., 2012. Structural basis for membrane targeting by the MVB12-associated beta-prism domain of the human ESCRT-I MVB12 subunit. Proc. Natl. Acad. Sci. USA 109, 1901–1906.
Brighouse, A., Dacks, J.B., Field, M.C., 2010. Rab protein evolution and the history of the eukaryotic endomembrane system. Cell. Mol. Life Sci. 67, 3449–3465.
Brocker, C., Engelbrecht-Vandre, S., Ungermann, C., 2010. Multisubunit tethering complexes and their role in membrane fusion. Curr. Biol. 20, R943–R952.
Brocker, C., Kuhlee, A., Gatsogiannis, C., Balderhaar, H.J., Honscher, C., Engelbrecht-Vandre, S., Ungermann, C., Raunser, S., 2012. Molecular architecture of the multisubunit homotypic fusion and vacuole protein sorting (HOPS) tethering complex. Proc. Natl. Acad. Sci. USA 109, 1991–1996.
Bugarcic, A., Zhe, Y., Kerr, M.C., Griffin, J., Collins, B.M., Teasdale, R.D., 2011. Vps26A and Vps26B subunits define distinct retromer complexes. Traffic 12, 1759–1773.
Caballe, A., Martin-Serrano, J., 2011. ESCRT machinery and cytokinesis: the road to daughter cell separation. Traffic 12, 1318–1326.
Campelo, F., Malhotra, V., 2012. Membrane fission: the biogenesis of transport carriers. Annu. Rev. Biochem. 81, 407–427.
Carney, D.S., Davies, B.A., Horazdovsky, B.F., 2006. Vps9 domain-containing proteins: activators of Rab5 GTPases from yeast to neurons. Trends Cell Biol. 16, 27–35.
Faelber, K., Held, M., Gao, S., Posor, Y., Haucke, V., Noe, F., Daumke, O., 2012. Structural insights into dynamin-mediated membrane fission. Structure 20, 1621–1628.
Falguieres, T., Luyet, P.P., Bissig, C., Scott, C.C., Velluz, M.C., Gruenberg, J., 2008. In vitro budding of intralumenal vesicles into late endosomes is regulated by Alix and Tsg101. Mol. Biol. Cell 19, 4942–4955.
Felsenstein, J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 783–791.
Ferguson, S.M., De Camilli, P., 2012. Dynamin, a membrane-remodelling GTPase. Nature reviews. Mol. Cell Biol. 13, 75–88.
Fjorback, A.W., Seaman, M., Gustafsen, C., Mehmedbasic, A., Gokool, S., Wu, C., Militz, D., Schmidt, V., Madsen, P., Nyengaard, J.R., Willnow, T.E., Christensen, E.I., Mobley, W.B., Nykjaer, A., Andersen, O.M., 2012. Retromer binds the FANSHY sorting motif in SorLA to regulate amyloid precursor protein sorting and processing. J. Neurosci. 32, 1467–1480.
Fujii, K., Hurley, J.H., Freed, E.O., 2007. Beyond Tsg101: the role of Alix in 'ESCRTing' HIV-1. Nature reviews. Microbiology 5, 912–916.
Ghoujal, B., Milev, M.P., Ajamian, L., Abel, K., Mouland, A.J., 2012. ESCRT-II's involvement in HIV-1 genomic RNA trafficking and assembly. Biol. Cell 104, 706–721.
Gill, D.J., Teo, H., Sun, J., Perisic, O., Veprintsev, D.B., Emr, S.D., Williams, R.L., 2007. Structural insight into the ESCRT-I/-II link and its role in MVB trafficking. EMBO J. 26, 600–612.
Greaves, J., Prescott, G.R., Gorleku, O.A., Chamberlain, L.H., 2010. Regulation of SNAP-25 trafficking and function by palmitoylation. Biochem. Soc. Trans. 38, 163–166.
Hedman, J.M., Eggleston, M.D., Attryde, A.L., Marshall, P.A., 2007. Prevacuolar compartment morphology in vps mutants of *Saccharomyces cerevisiae*. Cell. Biol. Int. 31, 1237–1244.
Henne, W.M., Buchkovich, N.J., Emr, S.D., 2011. The ESCRT pathway. Dev. Cell 21, 77–91.
Hermey, G., 2009. The Vps10p-domain receptor family. Cell. Mol. Life Sci. 66, 2677–2689.
Hierro, A., Sun, J., Rusnak, A.S., Kim, J., Prag, G., Emr, S.D., Hurley, J.H., 2004. Structure of the ESCRT-II endosomal trafficking complex. Nature 431, 221–225.
Hong, W., Lev, S., 2014. Tethering the assembly of SNARE complexes. Trends Cell Biol. 24, 35–43.
Hurley, J.H., 2010. The ESCRT complexes. Crit. Rev. Biochem. Mol. Biol. 45, 463–487.
Hurley, J.H., Hanson, P.I., 2010. Membrane budding and scission by the ESCRT machinery: it's all in the neck. Nat. Rev. Mol. Cell Biol. 11, 556–566.
Jimenez, A.J., Maiuri, P., Lafaurie-Janvore, J., Divoux, S., Piel, M., Perez, F., 2014. ESCRT machinery is required for plasma membrane repair. Science 343, 1247136.
Jones, D.T., Taylor, W.R., Thornton, J.M., 1992. The rapid generation of mutation data matrices from protein sequences. Comput. Appl. Biosci. 8, 275–282.
Jones, P., Binns, D., Chang, H.Y., Fraser, M., Li, W., McAnulla, C., McWilliam, H., Maslen, J., Mitchell, A., Nuka, G., Pesseat, S., Quinn, A.F., Sangrador-Vegas, A., Scheremetjew, M., Yong, S.Y., Lopez, R., Hunter, S., 2014. InterProScan 5: genome-scale protein function classification. Bioinformatics 30, 1236–1240.
Kallay, L.M., Brett, C.L., Tukaye, D.N., Wemmer, M.A., Chyou, A., Odorizzi, G., Rao, R., 2011. Endosomal Na^+ (K^+)/H^+ exchanger Nhx1/Vps44 functions independently and downstream of multivesicular body formation. J. Biol. Chem. 286, 44067–44077.
Katzmann, D.J., Babst, M., Emr, S.D., 2001. Ubiquitin-dependent sorting into the multivesicular body pathway requires the function of a conserved endosomal protein sorting complex, ESCRT-I. Cell 106, 145–155.
Katzmann, D.J., Stefan, C.J., Babst, M., Emr, S.D., 2003. Vps27 recruits ESCRT machinery to endosomes during MVB sorting. J. Cell Biol. 162, 413–423.
Kerr, M.C., Bennetts, J.S., Simpson, F., Thomas, E.C., Flegg, C., Gleeson, P.A., Wicking, C., Teasdale, R.D., 2005. A novel mammalian retromer component, Vps26B. Traffic 6, 991–1001.
Kondapalli, K.C., Hack, A., Schushan, M., Landau, M., Ben-Tal, N., Rao, R., 2013. Functional evaluation of autism-associated mutations in NHE9. Nat. Commun. 4, 2510.
Kostelansky, M.S., Schluter, C., Tam, Y.Y., Lee, S., Ghirlando, R., Beach, B., Conibear, E., Hurley, J.H., 2007. Molecular architecture and functional model of the complete yeast ESCRT-I heterotetramer. Cell 129, 485–498.
Koumandou, V.L., Klute, M.J., Herman, E.K., Nunez-Miguel, R., Dacks, J.B., Field, M.C., 2011. Evolutionary reconstruction of the retromer complex and its function in *Trypanosoma brucei*. J. Cell Sci. 124, 1496–1509.
Kramer, L., Ungermann, C., 2011. HOPS drives vacuole fusion by binding the vacuolar SNARE complex and the Vam7 PX domain via two distinct sites. Mol. Biol. Cell 22, 2601–2611.
Kummel, D., Ungermann, C., 2014. Principles of membrane tethering and fusion in endosome and lysosome biogenesis. Curr. Opin. Cell Biol. 29C, 61–66.
Lata, S., Schoehn, G., Jain, A., Pires, R., Piehler, J., Gottlinger, H.G., Weissenhorn, W., 2008. Helical structures of ESCRT-III are disassembled by VPS4. Science 321, 1354–1357.
Lee, H.H., Elia, N., Ghirlando, R., Lippincott-Schwartz, J., Hurley, J.H., 2008. Midbody targeting of the ESCRT machinery by a noncanonical coiled coil in CEP55. Science 322, 576–580.
Li, Z., Blissard, G.W., 2012. Cellular VPS4 is required for efficient entry and egress of budded virions of *Autographa californica* multiple nucleopolyhedrovirus. J. Virol. 86, 459–472.
Lobingier, B.T., Merz, A.J., 2012. Sec1/Munc18 protein Vps33 binds to SNARE domains and the quaternary SNARE complex. Mol. Biol. Cell 23, 4611–4622.
Lobingier, B.T., Nickerson, D.P., Lo, S.Y., Merz, A.J., 2014. SM proteins Sly1 and Vps33 co-assemble with Sec17 and SNARE complexes to oppose SNARE disassembly by Sec18. eLife 3, e02272.
Lottridge, J.M., Flannery, A.R., Vincelli, J.L., Stevens, T.H., 2006. Vta1p and Vps46p regulate the membrane association and ATPase activity of Vps4p at the yeast multivesicular body. Proc. Natl. Acad. Sci. USA 103, 6202–6207.
Lupas, A., Van Dyke, M., Stock, J., 1991. Predicting coiled coils from protein sequences. Science 252, 1162–1164.
McCullough, J., Colf, L.A., Sundquist, W.I., 2013. Membrane fission reactions of the mammalian ESCRT pathway. Annu. Rev. Biochem. 82, 663–692.
McCullough, J., Fisher, R.D., Whitby, F.G., Sundquist, W.I., Hill, C.P., 2008. ALIX-CHMP4 interactions in the human ESCRT pathway. Proc. Natl. Acad. Sci. USA 105, 7687–7691.
Missotten, M., Nichols, A., Rieger, K., Sadoul, R., 1999. Alix, a novel mouse protein undergoing calcium-dependent interaction with the apoptosis-linked-gene 2 (ALG-2) protein. Cell Death Differ. 6, 124–129.
Moberg, K.H., Schelble, S., Burdick, S.K., Hariharan, I.K., 2005. Mutations in erupted, the Drosophila ortholog of mammalian tumor susceptibility gene 101, elicit non-cell-autonomous overgrowth. Dev. Cell 9, 699–710.
Monroe, N., Han, H., Gonciarz, M.D., Eckert, D.M., Karren, M.A., Whitby, F.G., Sundquist, W.I., Hill, C.P., 2014. The oligomeric state of the active Vps4 AAA ATPase. J. Mol. Biol. 426, 510–525.
Morita, E., Sandrin, V., Alam, S.L., Eckert, D.M., Gygi, S.P., Sundquist, W.I., 2007. Identification of human MVB12 proteins as ESCRT-I subunits that function in HIV budding. Cell Host Microbe 2, 41–53.
Morlot, S., Roux, A., 2013. Mechanics of dynamin-mediated membrane fission. Annu. Rev. Biophys. 42, 629–649.
Nakamura, N., Yamamoto, A., Wada, Y., Futai, M., 2000. Syntaxin 7 mediates endocytic trafficking to late endosomes. J. Biol. Chem. 275, 6523–6529.
Nickerson, D.P., Brett, C.L., Merz, A.J., 2009. Vps-C complexes: gatekeepers of endolysosomal traffic. Curr. Opin. Cell Biol. 21, 543–551.
Park, J.S., Neiman, A.M., 2012. VPS13 regulates membrane morphogenesis during sporulation in *Saccharomyces cerevisiae*. J. Cell Sci. 125, 3004–3011.

Peel, S., Macheboeuf, P., Martinelli, N., Weissenhorn, W., 2011. Divergent pathways lead to ESCRT-III-catalyzed membrane fission. Trends Biochem. Sci. 36, 199–210.
Peplowska, K., Markgraf, D.F., Ostrowicz, C.W., Bange, G., Ungermann, C., 2007. The CORVET tethering complex interacts with the yeast Rab5 homolog Vps21 and is involved in endo-lysosomal biogenesis. Dev. Cell 12, 739–750.
Perez-Victoria, F.J., Bonifacino, J.S., 2009. Dual roles of the mammalian GARP complex in tethering and SNARE complex assembly at the trans-golgi network. Mol. Cell Biol. 29, 5251–5263.
Pirooz, S., He, S., O'Connell, D., Khalilzadeh, P., Yang, Y., Liang, C., 2014. Viruses customize autophagy protein for efficient viral entry. Autophagy 10.
Plemel, R.L., Lobingier, B.T., Brett, C.L., Angers, C.G., Nickerson, D.P., Paulsel, A., Sprague, D., Merz, A.J., 2011. Subunit organization and Rab interactions of Vps-C protein complexes that control endolysosomal membrane traffic. Mol. Biol. Cell 22, 1353–1363.
Raiborg, C., Stenmark, H., 2009. The ESCRT machinery in endosomal sorting of ubiquitylated membrane proteins. Nature 458, 445–452.
Raymond, C.K., Howald-Stevenson, I., Vater, C.A., Stevens, T.H., 1992. Morphological classification of the yeast vacuolar protein sorting mutants: evidence for a prevacuolar compartment in class E vps mutants. Mol. Biol. Cell 3, 1389–1402.
Ren, X., Hurley, J.H., 2011. Structural basis for endosomal recruitment of ESCRT-I by ESCRT-0 in yeast. EMBO J. 30, 2130–2139.
Rodahl, L.M., Haglund, K., Sem-Jacobsen, C., Wendler, F., Vincent, J.P., Lindmo, K., Rusten, T.E., Stenmark, H., 2009. Disruption of Vps4 and JNK function in Drosophila causes tumour growth. PloS One 4, e4354.
Rusten, T.E., Vaccari, T., Stenmark, H., 2012. Shaping development with ESCRTs. Nat. Cell Biol. 14, 38–45.
Saitou, N., Nei, M., 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4, 406–425.
Saksena, S., Wahlman, J., Teis, D., Johnson, A.E., Emr, S.D., 2009. Functional reconstitution of ESCRT-III assembly and disassembly. Cell 136, 97–109.
Schluter, C., Lam, K.K., Brumm, J., Wu, B.W., Saunders, M., Stevens, T.H., Bryan, J., Conibear, E., 2008. Global analysis of yeast endosomal transport identifies the vps55/68 sorting complex. Mol. Biol. Cell 19, 1282–1294.
Schmitz, K.R., Liu, J., Li, S., Setty, T.G., Wood, C.S., Burd, C.G., Ferguson, K.M., 2008. Golgi localization of glycosyltransferases requires a Vps74p oligomer. Dev. Cell 14, 523–534.
Schuh, A.L., Audhya, A., 2014. The ESCRT machinery: from the plasma membrane to endosomes and back again. Crit. Rev. Biochem. Mol. Biol. 49, 242–261.
Seaman, M.N., 2012. The retromer complex – endosomal protein recycling and beyond. J. Cell Sci. 125, 4693–4702.
Seaman, M.N., Gautreau, A., Billadeau, D.D., 2013. Retromer-mediated endosomal protein sorting: all WASHed up! Trends Cell Biol. 23, 522–528.
Shanks, S.G., Carpp, L.N., Struthers, M.S., McCann, R.K., Bryant, N.J., 2012. The Sec1/Munc18 protein Vps45 regulates cellular levels of its SNARE binding partners Tlg2 and Snc2 in *Saccharomyces cerevisiae*. PloS One 7, e49628.
Shestakova, A., Hanono, A., Drosner, S., Curtiss, M., Davies, B.A., Katzmann, D.J., Babst, M., 2010. Assembly of the AAA ATPase Vps4 on ESCRT-III. Mol. Biol. Cell 21, 1059–1071.
Shim, S., Merrill, S.A., Hanson, P.I., 2008. Novel interactions of ESCRT-III with LIP5 and VPS4 and their implications for ESCRT-III disassembly. Mol. Biol. Cell 19, 2661–2672.
Skalicky, J.J., Arii, J., Wenzel, D.M., Stubblefield, W.M., Katsuyama, A., Uter, N.T., Bajorek, M., Myszka, D.G., Sundquist, W.I., 2012. Interactions of the human LIP5 regulatory protein with endosomal sorting complexes required for transport. J. Biol. Chem. 287, 43910–43926.
Solinger, J.A., Spang, A., 2013. Tethering complexes in the endocytic pathway: CORVET and HOPS. FEBS J. 280, 2743–2757.
Stefani, F., Zhang, L., Taylor, S., Donovan, J., Rollinson, S., Doyotte, A., Brownhill, K., Bennion, J., Pickering-Brown, S., Woodman, P., 2011. UBAP1 is a component of an endosome-specific ESCRT-I complex that is essential for MVB sorting. Curr. Biol. 21, 1245–1250.
Stuchell-Brereton, M.D., Skalicky, J.J., Kieffer, C., Karren, M.A., Ghaffarian, S., Sundquist, W.I., 2007. ESCRT-III recognition by VPS4 ATPases. Nature 449, 740–744.
Takats, S., Pircs, K., Nagy, P., Varga, A., Karpati, M., Hegedus, K., Kramer, H., Kovacs, A.L., Sass, M., Juhasz, G., 2014. Interaction of the HOPS complex with Syntaxin 17 mediates autophagosome clearance in Drosophila. Mol. Biol. Cell 25, 1338–1354.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Teo, H., Gill, D.J., Sun, J., Perisic, O., Veprintsev, D.B., Vallis, Y., Emr, S.D., Williams, R.L., 2006. ESCRT-I core and ESCRT-II GLUE domain structures reveal role for GLUE in linking to ESCRT-I and membranes. Cell 125, 99–111.
Teo, H., Perisic, O., Gonzalez, B., Williams, R.L., 2004. ESCRT-II, an endosome-associated complex required for protein sorting: crystal structure and interactions with ESCRT-III and membranes. Dev. Cell 7, 559–569.
Thoresen, S.B., Pedersen, N.M., Liestol, K., Stenmark, H., 2010. A phosphatidylinositol 3-kinase class III sub-complex containing VPS15, VPS34, Beclin 1, UVRAG and BIF-1 regulates cytokinesis and degradative endocytic traffic. Exp. Cell Res. 316, 3368–3378.
Tu, L., Tai, W.C., Chen, L., Banfield, D.K., 2008. Signal-mediated dynamic retention of glycosyltransferases in the Golgi. Science 321, 404–407.
Ungermann, C., Wickner, W., 1998. Vam7p, a vacuolar SNAP-25 homolog, is required for SNARE complex integrity and vacuole docking and fusion. EMBO J. 17, 3269–3276.
Vaccari, T., Rusten, T.E., Menut, L., Nezis, I.P., Brech, A., Stenmark, H., Bilder, D., 2009. Comparative analysis of ESCRT-I, ESCRT-II and ESCRT-III function in Drosophila by efficient isolation of ESCRT mutants. J. Cell Sci. 122, 2413–2423.
Valentine, M., Hogan, J., Collier, S., 2014. The drosophila Chmp1 protein determines wing cell fate through regulation of epidermal growth factor receptor signaling. Dev. Dyn. 243 (8), 977–987.
van der Bliek, A.M., Meyerowitz, E.M., 1991. Dynamin-like protein encoded by the Drosophila shibire gene associated with vesicular traffic. Nature 351, 411–414.
Vanhaesebroeck, B., Guillermet-Guibert, J., Graupera, M., Bilanges, B., 2010. The emerging mechanisms of isoform-specific PI3K signalling. Nature reviews. Mol. Cell Biol. 11, 329–341.
Vanhaesebroeck, B., Leevers, S.J., Panayotou, G., Waterfield, M.D., 1997. Phosphoinositide 3-kinases: a conserved family of signal transducers. Trends Biochem. Sci. 22, 267–272.
Vasquez-Rifo, A., Bosse, G.D., Rondeau, E.L., Jannot, G., Dallaire, A., Simard, M.J., 2013. A new role for the GARP complex in microRNA-mediated gene regulation. PLoS Genet. 9, e1003961.
Vater, C.A., Raymond, C.K., Ekena, K., Howald-Stevenson, I., Stevens, T.H., 1992. The VPS1 protein, a homolog of dynamin required for vacuolar protein sorting in *Saccharomyces cerevisiae*, is a GTPase with two functionally separable domains. J. Cell Biol. 119, 773–786.
Velayos-Baeza, A., Vettori, A., Copley, R.R., Dobson-Stone, C., Monaco, A.P., 2004. Analysis of the human VPS13 gene family. Genomics 84, 536–549.
Votteler, J., Sundquist, W.I., 2013. Virus budding and the ESCRT pathway. Cell Host Microbe 14, 232–241.
Wollert, T., Wunder, C., Lippincott-Schwartz, J., Hurley, J.H., 2009. Membrane scission by the ESCRT-III complex. Nature 458, 172–177.
Wood, C.S., Hung, C.S., Huoh, Y.S., Mousley, C.J., Stefan, C.J., Bankaitis, V., Ferguson, K.M., Burd, C.G., 2012. Local control of phosphatidylinositol 4-phosphate signaling in the Golgi apparatus by Vps74 and Sac1 phosphoinositide phosphatase. Mol. Biol. Cell 23, 2527–2536.
Wood, C.S., Schmitz, K.R., Bessman, N.J., Setty, T.G., Ferguson, K.M., Burd, C.G., 2009. PtdIns4P recognition by Vps74/GOLPH3 links PtdIns 4-kinase signaling to retrograde Golgi trafficking. J. Cell Biol. 187, 967–975.
Xu, H., Jun, Y., Thompson, J., Yates, J., Wickner, W., 2010. HOPS prevents the disassembly of trans-SNARE complexes by Sec17p/Sec18p during membrane fusion. EMBO J. 29, 1948–1960.
Yang, D., Hurley, J.H., 2010. Structural role of the Vps4-Vta1 interface in ESCRT-III recycling. Structure 18, 976–984.
Yang, Z., Vild, C., Ju, J., Zhang, X., Liu, J., Shen, J., Zhao, B., Lan, W., Gong, F., Liu, M., Cao, C., Xu, Z., 2012. Structural basis of molecular recognition between ESCRT-III-like protein Vps60 and AAA-ATPase regulator Vta1 in the multivesicular body pathway. J. Biol. Chem. 287, 43899–43908.
Zhu, G.D., Salazar, G., Zlatic, S.A., Fiza, B., Doucette, M.M., Heilman, C.J., Levey, A.I., Faundez, V., L'Hernault, S.W., 2009. SPE-39 family proteins interact with the HOPS complex and function in lysosomal delivery. Mol. Biol. Cell 20, 1223–1240.