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Vps-C Complexes: Gatekeepers of Endolysosomal Traffic

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Abstract

Genetic studies in yeast, plants, insects and mammals have identified four universally conserved proteins, together called Vps Class C, that are essential for late endosome and lysosome assembly and for numerous endolysosomal trafficking pathways, including the terminal stages of autophagy. Two Vps-C complexes, HOPS and CORVET, incorporate diverse biochemical functions: they tether membranes, stimulate Rab nucleotide exchange, guide SNARE assembly to drive membrane fusion, and possibly act as ubiquitin ligases. Recent studies offer new insight into the complex relationships between Vps-C complexes and their cognate Rab small GTP-binding (G-)proteins at endosomes and lysosomes. Accumulating evidence supports the view that Vps-C complexes implement a regulatory logic that governs endomembrane identity and dynamics.

INTRODUCTION

The Vps-C complexes CORVET (class C core vacuole/endosome tethering) and HOPS (homotypic fusion and protein sorting) are found on endosomes and lysosomes, where they control membrane traffic by keeping a close eye on the membrane fusion machinery. HOPS and CORVET are essential for the maturation, integrity, and inheritance of late endosomes and vacuolar lysosomes, and appear to control all traffic passing into and through these organelles (Figure 1). Hence, Vps-C complexes are likely central regulators of a multitude of cellular and physiological processes associated with late endocytic organelles, including downregulation of growth factor receptors and nutrient transporters [1,2], disposal and recycling of cytoplasmic components through autophagy [3-5], cholesterol and lipid metabolism [6,7], and antigen processing and presentation [8,9]. In this review we focus on the yeast Vps-C complexes because they have been subjected to the closest scrutiny. However, conserved complexes appear to carry out similar functions in all eukaryotes[10-13].

VPS-C COMPLEXES: MOLECULAR ORGANIZATION

Classical genetic screens in *Saccharomyces cerevisiae* identified 41 *VPS* (vacuole protein sorting) genes [14], and over a dozen *PEP* (peptidase sorting) genes [15], involved in delivery of cargo to the yeast vacuole. Mutations in four of these caused the severe Class C phenotype, with mutant cells lacking an identifiable vacuolar lysosome. The proteins encoded by these genes, Vps11 (Pep5), Vps16, Vps18 (Pep3), and Vps33, associate to form the stable Vps-C core complex [16,17]. The *VPS* family today includes about 80 genes, but deletion of no others specifically controlling endocytic traffic produce stronger growth, trafficking and membrane

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morphology defects than those seen in Class C *vps* mutants, reflecting the pivotal roles played by these proteins in multiple endolysosomal trafficking events (Figure 1).

HOPS

Two accessory subunits, Vps39 and Vps41, associate with the Vps-C core to form the ~633 kDa HOPS (homotypic fusion and protein sorting) complex (Table I). HOPS controls all membrane fusion at the vacuolar lysosome. Consistent with this role, *vps39* and *vps41* null mutants contain highly fragmented vacuoles characteristic of the class B *VPS* phenotype [14]. Vps39 functions in yeast as a guanosine exchange factor (GEF) that activates the vacuolar Rab Ypt7 [18], while Vps41 binds Ypt7 as a direct effector, physically linking the HOPS holocomplex to the activated, GTP-bound Rab [19]. HOPS also associates with acidic phospholipids and SNAREs [20,21]

Many Rab effectors are big proteins with open, elongated structures hypothesized to facilitate long-distance membrane tethering. Most subunits of the HOPS complex, with the exception of the Sec1/Munc18 (SM) family subunit Vps33, appear to fit this description, while at the same time displaying a domain architecture common to membrane coat proteins (Figure 2). Computational analyses predict that all Vps-C subunits (except Vps33) possess an amino-terminal β-propellor and an α-solenoid that extends to the carboxy-terminus. This architecture is strikingly similar to common building blocks of other membrane-shaping, multi-subunit complexes, including COPI, COPII, clathrin and subunits of the nuclear pore [22]. Thus, it is no surprise that HOPS might possess similar capacities to self-assemble into higher-order structures on membranes [23-26], with the Vps41 subunit perhaps functioning as a coat protein for AP-3 vesicles [25]. The prospect that HOPS senses or generates membrane curvature and functions as a long-distance tethering apparatus finds analogy in the recently demonstrated capacity of a Golgin, GMAP-210, to tether liposomes with selectivity for degrees of membrane curvature [27].

CORVET

Beyond its established role in HOPS, the Vps-C core was recently shown to associate with a pair of alternative accessory subunits, Vps3 [28] and Vps8 [28,29] which share homology with the HOPS subunitsVps39 and Vps41 (Table I) [28]. This second Vps-C complex, CORVET (class C core vacuole/endosome tethering), associates with the early endosome Rab5 ortholog Vps21 [28]. Despite strong sequence homology between the carboxy-terminal domains of Vps3 and Vps39, the more poorly-conserved amino-terminal domain of Vps39 harbors its Rab-binding site [11,12,18], and there is at this writing no evidence that Vps3 catalyzes nucleotide exchange. The only confirmed GEF for Vps21 is Vps9 [30]; *vps9* null mutants phenocopy trafficking defects found in *vps3*, *vps8* and *vps21* mutants, all of which have defects in traffic through the late endosomal prevacuolar compartment and exhibit Class D *vps* swollen vacuoles [14,28,31].

The discovery of CORVET clarifies the role of Vps33, the SM subunit of the Vps-C core, on endosomes [29]: Vps33 localizes to both endosomes and vacuoles due to its participation in both CORVET and HOPS holocomplexes. The presence of the same SM protein at both endosomes and vacuoles, meanwhile, may explain the partial functional redundancy of the endosomal and vacuolar Q_a-SNARE proteins Pep12 and Vam3 [32-35], both reported to interact with Vps33 [17,29]. Pep12, however, also interacts with a second endosomal SM protein, Vps45 [36]. It will be important to understand the division of labor between Vps33 and Vps45.

The discovery of CORVET raises a host of new questions. While structural homologies between HOPS and CORVET (Figure 2) might tempt us to speculate that CORVET functions

in early or late endosome tethering and fusion, many phenotypes resulting from loss of Vps21, Vps3 or Vps8 are consistent with defects in *retrograde* traffic from endolysosomes. Class D *vps* mutants have swollen vacuoles, indicative of a failure in membrane cycling away from the vacuole (we call this the Roach Motel phenotype: 'membrane checks in, but it doesn't check out'). The Class D defect could in principle arise from a failure of retrograde vesicle formation at the vacuole, a failure of retrograde fusion at the endosome, or both [37]. Deletion of *VPS8* in yeast lacking the vacuole Q_a-SNARE Vam3 substantially rescues the *vam3* fragmented vacuole phenotype [28], suggesting defects in membrane consolidation and divestiture are offsetting and compensatory. Overexpression of the CORVET subunit Vps8 causes clustering of Vps21-positive endosomes, suggestive of a direct tethering function [28]. As this phenotype results from superstoichiometric expression of Vps8, it might reflect an autonomous Vps8 function not requiring the Vps-C core. Overexpression of Vps3 also produces clustering of Vps21-positive endosomes, but is accompanied by vacuolar fragmentation, perhaps due to the capacity of Vps3 to compete with the Ypt7 GEF, Vps39, for assembly on the Vps-C core. These findings, and the aberrant colocalization of endosomal and vacuolar Rabs that occurs in a *vps8* mutant [28], together indicate that the balance between different Vps-C complexes is a key parameter that governs traffic between late endosomes and vacuoles/lysosomes. How these complexes function during normal endolysosomal tranport processes, however, is at present understood only superficially.

Vps-C complexes and ubiquitin

Covalent attachment of single or short chains of ubiquitin to both transmembrane cargo proteins and sorting receptors is a widespread regulatory signal in endolysosomal cargo sorting and membrane dynamics [38]. While ubiquitin-mediated regulation of Vps-C complexes is not firmly established, the conspicuous recurrence of Zn^{2+} finger RING motifs (common to many E3 ubiquitin ligases) within Vps-C core and accessory subunits (Figure 2) is suggestive. The RING domains of Vps18 and Vps8, at least, are required for efficient Vps-C function [31, 39], as is that of Vps11 (our unpublished results). Moreover, the RING domain of mammalian Vps18 was reported to exhibit E3 ligase activity toward the GGA3 adaptor protein, at least *in vitro* [40,41]. However, neither ubiquitination mediated by Vps-C RING domains nor ubiquitination of Vps-C subunits has been demonstrated *in vivo*, and despite evidence that the HOPS-associated Rab Ypt7 is ubiquitinated [42], no study has clearly linked a specific ubiquitination reaction to *in vivo* functions of Vps-C complexes.

CELLULAR FUNCTIONS AND REGULATION

Endosome maturation and Rab/Vps-C complex conversion

Nearly 20 years ago it was recognized that mammalian early endosomes are decorated with Rab5, while late endosomes and lysosomes bear Rab7 [43]. The discovery that CORVET and HOPS complexes specifically interact with endosomal and lysosomal Rabs, and the identification of intermediate complexes containing both CORVET and HOPS accessory subunits [10,28,31], suggests a range of plausible models for compartmental maturation during endolysosomal transport. Through exchange of Vps3 for Vps39, the Ypt7 GEF, endosomes might encourage the recruitment and activation of the downstream lysosomal Rab. An exchange of Rab5 for Rab7 has been observed on mammalian endosomes [10], and while there is yet no study characterizing possible CORVET-HOPS dynamics in this system, Rab5 (ortholog of Vps21) captures a protein complex containing Vps-C core components and the putative HOPS ortholog hVps39, suggesting that Rab5 might similarly promote recruitment of Rab7 through an interaction with the Rab7 GEF [10]. While this model is both appealing and plausible it is far from established, and key issues are unresolved. In yeast, the physiological relevance of intermediate complexes containing both HOPS and CORVET accessory subunits (i.e., Vps8 and 39, or Vps3 and 41; Figure 2D) has yet to be established. The mammalian Vps8

ortholog is unstudied. The major mammalian Vps3/39 ortholog when overexpressed caused clustering and fusion of late endosomes and lysosomes [11,12], but it is unclear whether this protein is a functional homolog of yeast Vps3 or Vps39, or whether it subsumes functions of both. Mammalian cells also contain several largely uncharacterized proteins with homology to the carboxy-terminal domain of Vps39. No mammalian protein has yet been demonstrated to harbor any GEF activity toward Rab7.

The available data from yeast are consistent both with dynamic subunit exchange models in which CORVET and HOPS accessory subunits sequentially dissociate from and associate with the Vps-C core, and with models in which CORVET and HOPS exist predominantly as stable, discrete complexes. In the first case, endolysosomal transport would be accompanied by dynamic remodeling of complex composition. In the second case, association and dissociation of entire complexes would accompany transport. Because HOPS and CORVET are biochemically stable complexes, dynamic subunit remodeling would imply the existence of an as-yet unidentified chaperone or degradation system; the requirement for such a system could conceivably provide the missing link between Vps-C biochemistry and ubiquitin signaling.

There is also the open question of whether the acquisition of Rab7 by Rab5 endosomes results in maturation of the entire organelle [10] or whether discrete Rab5-and Rab7-enriched microdomains emerge to form separate endosome- and lysosome-targeted transport vesicles [44]. The rapid tempo of improvements to live-cell imaging systems and the development of improved fluorescent probes should provide more definitive answers.

SNARE selectivity and chaperone activity by HOPS

HOPS, and perhaps CORVET as well, promote membrane fusion by interacting directly with the core fusion machinery. SNARE-mediated fusion invariably requires four complementary SNARE domains, identified as R, Q_a, Q_b, and Q_c, depending on the presence of Arg or Gln within the central ‘0-layer’ of the SNARE domain. SNARE domains span a docking junction and ‘zipper’ together *in trans*, driving the apposed membranes together to trigger lipid mixing and fusion. Post-fusion *cis*-SNARE complexes are then disassembled by the ATP-dependent chaperone NSF and its α–SNAP cochaperone (Sec18 and Sec17 in yeast), freeing the SNAREs to catalyze subsequent rounds of fusion [45].

How does HOPS interact with SNAREs? Perhaps surprisingly, no study to date has addressed which HOPS subunits directly mediate specific SNARE contacts. While the SM protein Vps33 is an obvious candidate, studies reporting interactions between the HOPS holocomplex and the vacuole Q_a-SNARE, Vam3 [17,46], or the Q_c-SNARE, Vam7 [20], did not exclude participation by other members of the holocomplex. While Vam3 includes an amino-terminal regulatory motif that mediates interactions of other Q_a-SNAREs with their cognate SM proteins, this domain was not required for Vam3-HOPS binding in pulldowns employing cell lysates [46]. HOPS apparently binds not the Vam7 SNARE domain, but the amino-terminal lipid-binding PX domain, a domain found in no other SNARE, including those found on mammalian endosomes and lysosomes [20]. Thus, while the HOPS-Vam7(PX) interaction may be functionally significant in fungi, it is not evolutionarily conserved. In any case, these somewhat unconventional SNARE-binding profiles raise the possibility that Vps33 is an unconventional SM protein, and may indicate that additional HOPS components participate in SNARE recognition. No study to date has specifically examined CORVET-SNARE interactions.

Recent studies affirm the importance of HOPS in *trans*-SNARE complex assembly using both purified organelles [47] and a fully reconstituted system [48]. But does this reflect a HOPS-mediated catalytic SNARE assembly activity or, instead, enhanced membrane tethering and increased local SNARE concentration? Altering the 0-layer amino acids of vacuolar SNAREs

has provided important insights. Mutating the Q_c-SNARE Vam7 to resemble an R-SNARE reduces its ability to mediate fusion, despite an apparent ability to form kinetically stable 2Q•2R SNARE complexes in a HOPS-dependent manner [49]. When provided with both wild-type and mutant Vam7, however, vacuoles overwhelmingly favor incorporation of wild-type Vam7 into *trans*-complexes [49], demonstrating a SNARE filtering function that likely resides in HOPS. As further evidence that HOPS enforces SNARE selectivity, 'rotation' of the 0-layer by compensatory mutation of the R-SNARE, Nyv1, restores the 3Q•1R ratio when mixed with R-mutant Vam7 and partially restores fusion capacity [49]. Addition of exogenous HOPS to fusion reactions inhibits fusion mediated by mismatched and rotated 0-layer SNAREs, but not wild-type SNAREs [50]. Sec17 can outcompete HOPS for binding to *cis*- [47] and *trans*-SNARE complexes [51], so HOPS is readily displaced to allow SNARE disassembly by Sec17-Sec18 [48]. Apparently a multilayered quality control system monitors each stage of SNARE-mediated fusion lest accidental lysis should occur [52].

The primary engine of SNARE disassembly is the ATPase Sec18/NSF, so how could SNARE proofreading and mismatch correction occur in the absence of ATP, a condition under which many of the above results were obtained? Insight has emerged from recent work using carboxy-terminal truncations of SNARE domains to limit the degree of SNARE zippering. While fully-zippered SNAREs are remarkably stable, with spontaneous off-rates much longer than biologically relevant time scales [53], formation of kinetically stable *trans*-SNARE complexes on vacuoles requires zippering three α-helical turns *beyond* the SNARE domain 0-layer [51], a zippering pivot point recently shown to correspond to an energetic barrier to further folding [54]. Taken together, these recent data raise the possibility that HOPS ensures SNARE-pairing fidelity by linking inspection of the *trans*-complex backbone conformation at the 0-layer to the completion of zippering and initiation of membrane fusion.

Rab-dependent activation and phosphoinhibition of HOPS

Yeast vacuoles undergo fusion and fission in response to hypo- and hypertonic stresses [55, 56]. These rapid activations and deactivations of membrane tethering and fusion depend on the nucleotide-binding status of the master regulator Ypt7 and on the phosphorylation of the HOPS subunit Vps41 [56,57]. HOPS consolidates Rab GEF (Vps39) [18,19] and effector (Vps41) [19] functions into a stable complex. This relationship suggests a positive feedback loop similar to a loop described for Rab5 and its Rabex-5/Rabaptin GEF/effector complex [58]. The Ypt7 GAP (GTPase activating protein), Gyp7, inhibits HOPS activity by returning Ypt7 to its GDP-bound, inactive state, but even when heavily overexpressed, Gyp7 is insufficient to terminate vacuole fusion without the aid of a casein kinase I, Yck3 [19]. Yck3 is palmitoylated and traffics via the AP-3 pathway from Golgi to vacuole [59] where it directly phosphorylates the HOPS subunit Vps41 [21,57] and the Q_a-SNARE Vam3 [19; our unpublished results] to inhibit vacuole fusion. Deletion of Yck3, or expression of constitutively 'active' Vps41 that evades phosphoregulation by Yck3, results in hyperaccumulation of Vps41 at the vacuole [60] and missorting of AP-3 cargoes [60,61]. The molecular basis of this sorting defect is unclear.

Yck3 activity toward Vps41 and Vam3 is opposed by active Ypt7 on the vacuole [19], demonstrating that Ypt7 positively regulates HOPS both directly through effector binding and indirectly by counteracting inhibitory phosphorylation. HOPS is anchored to the vacuole through interactions with Ypt7, SNARE proteins, and acidic phospholipids. Phosphorylation of Vps41 by Yck3 does not change the apparent affinity of Vps41 for Ypt7 [21,60], but it dramatically reduces the affinity of HOPS for acidic phospholipids and SNARES [21]. Consequently, in the presence of Yck3 HOPS association with the vacuole, and fusion, are dependent on the activation status of Ypt7. In experiments with proteoliposomes and purified components, HOPS –mediated fusion was Ypt7-dependent when Yck3 was present and active,

but Ypt7-independent when Yck3 was absent [21]. However, experiments with native membranes suggest that this situation is somewhat nonphysiological. Both *in vivo* genetic experiments [19], and experiments with native vacuoles and antibodies raised against Ypt7 [57] indicate that in the absence of Yck3, residual Ypt7 is still essential for fusion. How can this discrepancy be explained? Wickner's group found that native vacuoles bearing surplus SNARES undergo fusion despite inhibition of Ypt7 [52] or, when supplemented with HOPS, in the absence of Ypt7 [48]. Thus, at superphysiological levels of SNAREs and HOPS, Ypt7 is not required for fusion at all. At physiological levels of SNAREs and HOPS, but in the absence of the Yck3 kinase, only residual levels of Ypt7 are needed, and the system is nearly or completely insensitive to the activation status of Ypt7; when Yck3 is present, fusion requires relatively high levels of active Ypt7. Thus, under *in vivo* conditions, Yck3 enforces a tight coupling between the activation status of Ypt7 and HOPS-mediated docking and fusion.

CONCLUDING REMARKS

Vps-C complexes control the locations, timing, specificity and integrity of fusion in the terminal branches of the endocytic system. Besides the fundamental concern of maintaining organelle identity, inappropriate delivery of cargo to the lysosome can result in improper proteolytic activation, as illustrated by mistaken lysosomal maturation of trypsinogen to trypsin, which leads to intracellular proteolysis and pancreatitis [62]. Fusion is an inherently dangerous business, as demonstrated by the lysis that accompanies fusion upon overexpression of vacuolar SNAREs [50,52]. Remarkably, HOPS not only stimulates fusion, but simultaneously reduces lysis. Lipid mixing as membrane bilayers progress from hemifusion to full fusion might produce transient holes adjacent to the fusion pore that require repair mechanisms coordinated with fusion machinery, a topic explored in an excellent recent review [63]. Moreover, HOPS and Ypt7 signaling respond to osmotic stresses [56] that, if uncontrolled, cause lysosomal rupture [64]. Understanding the coordinated mechanisms through which HOPS, SNAREs and specific membrane lipids effect fusion while restricting lysis presents a central challenge.

Recent work suggests possible connections between Vps-C complexes and other membrane trafficking processes. Microautophagy, or the internalization of cytoplasm through invagination of the vacuole limiting membrane, is thought to regulate the balance of membrane in the endolysosomal system through regulation by the EGO (exit from rapamycin-induced growth arrest) and TOR target of rapamycin) complexes at the vacuole [65]. Thus it is intriguing that mutations in the TOR and HOPS complexes are synthetically lethal [66], but the nature of the genetic interaction remains largely unexplored. The mechanism by which microautophagic tubules are released from the vacuole limiting membrane is also unknown, but it is not unreasonable to suppose HOPS and its associated fusion machinery could participate. Beyond microautophagy, the discovery in mammals that UVRAG recruits a Vps-C complex to autophagosomes to permit delivery of autophagosomes to endolysosomes provides a missing link between Vps-C function and autophagosome maturation [67].

The discovery of CORVET at endosomes introduces numerous possibilities for interactions with endosomal cargo sorting machineries. A recent report that human VpsC component hVps18 interacts with Hrs and TSG101 [68] hints at the possibility of cross-talk between Vps-C and ESCRTs (endosomal sorting complexes required for transport) that regulate cargo sorting into multivesicular bodies [69] — a clue that requires further exploration. Also, the apparent defects in retrograde endolysosomal traffic upon disruption of CORVET require study. The recently observed complementary roles of Rab5 and Rab7 in recruiting retromer components [70] brings us back to the idea that CORVET and HOPS might influence retrograde traffic through activation and exchange of Rabs, but this hypothesis is so far untested.

Vps-C complex components have proven resistant to biochemical and structural characterization in large part due to the difficulty of expressing them recombinantly. Even ten years after the discovery of Vps-C holocomplexes, our knowledge remains limited to computational structural predictions (Figure 1). A daunting but essential challenge going forward is to determine which biochemical functions reside within specific Vps-C subunits, and which are emergent properties of the larger complexes. Such information is vital not only to understand Vps-C functions in more than a cartoon outline, but also to extend the findings gleaned from studies of yeast (in which cells lacking entire Vps-C genes are viable) to multicellular organisms in which full-length gene deletions are almost always lethal. While key insights have been gleaned from studies of melanocytes [71], tissue-specific disruptions [72] and partial loss-of-function mutants such as the fly *VPS18* allele *deep orange* [73] have generally proven most tractable to study in whole organisms. Furthermore, dissection of specific Vps-C complex functionalities will enable untangling of the pleiotropic defects seen upon disruption or loss of entire subunits. Given their rapid morphological responses to stress and highly volatile contents, endolysosomal organelles must be simultaneously plastic and indestructable. We are only beginning to appreciate the myriad Vps-C interactions that impart this dual character.

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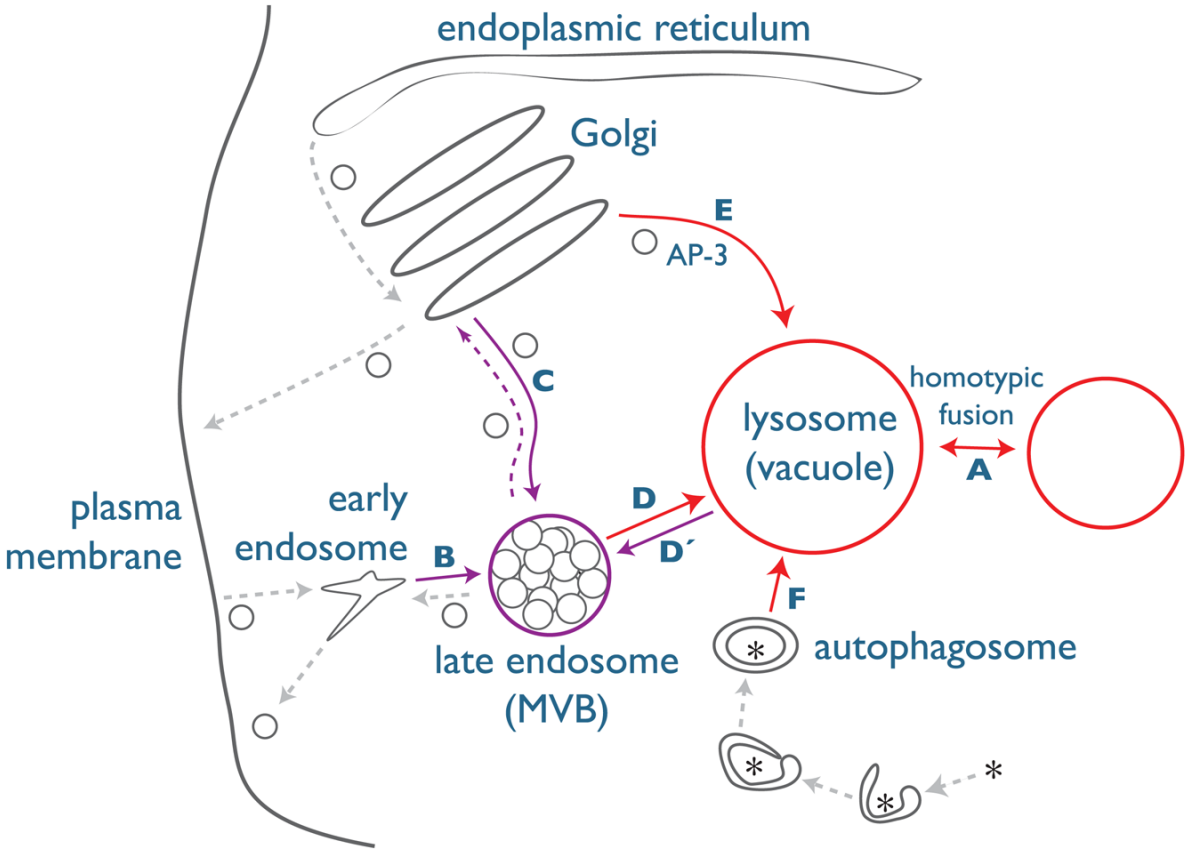

Figure 1. Cellular trafficking pathways governed by Vps-C complexes

The vacuole is the terminal organelle in the yeast endocytic pathway, equivalent to the mammalian lysosome. Vps-C complexes regulate several transport routes to and from the vacuole: (A) vacuoles fuse homotypically in response to environmental signals and during cell division and cell fusion; (B) endocytic vesicles and early endosomes fuse homotypically as they mature; (C) transport vesicles derived from the TGN fuse with endosomes to deliver biosynthetic cargos; (D) late endosome cargo is delivered to the vacuole via heterotypic fusion; (D′) Retrograde traffic from the vacuole passes through the endosome; (E) some vacuole-resident proteins are transported from TGN to vacuole in an AP-3-dependent pathway that bypasses endosomes; and (F) soluble cargos and defective organelles are transported from the cytosol into the vacuole lumen via the CVT and autophagy pathways. Solid arrows depict transport steps thought to require Vps-C complexes. Endosomes and endosomal trafficking events requiring CORVET are depicted in purple, while vacuoles and vacuolar trafficking events requiring HOPS are depicted in red. While this model depicts traffic in yeast, endolysosomal traffic in plants, invertebrates and mammals is broadly similar. AP-3, adaptor protein-3 complex; TGN, trans-Golgi network; CVT, cytoplasm to vacuole targeting; HOPS, fusion and vacuole protein sorting; CORVET, class C core vacuole/endosome tethering complex.

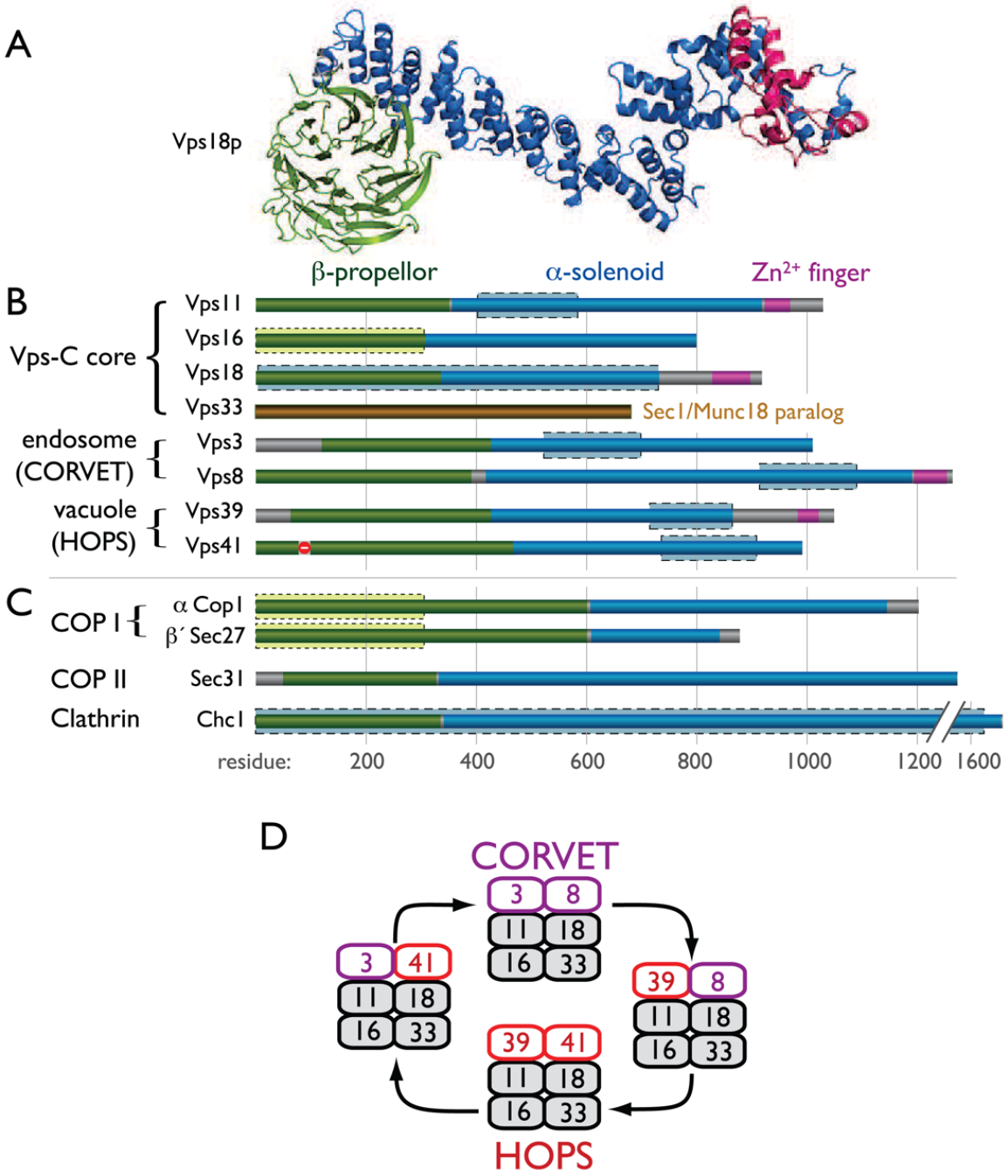

Figure 2. Composition and domain architecture of Vps-C complexes

Predicted folding structures for the Vps-C core and accessory subunits were generated using the tertiary-fold prediction algorithm *Rosetta* [74]. (A) Ribbon diagram depicting folding model for Vps18. (B and C) Summary of tertiary structure predictions indicated by color coding for Vps-C components (B) and vesicle coat outer shell subunits (C). Primary sequence homologies (PSI-BLAST) between Vps-C subunits and clathrin heavy chain are indicated by blue boxes with dashed boundaries. Homology between the Vps16 amino-terminus and COPI subunits is denoted by green boxes with dotted boundaries. Structural predictions for the yeast Vps-C components are shown; nearly identical predictions were obtained for their mammalian orthologs. (D) Working schema for Vps-C complex function, in which interactions of

CORVET and HOPS with endosomal and lysosomal Rabs regulate membrane fusion events at late endosomes and lysosomes, respectively. Hybrid complexes containing both HOPS and CORVET subunits have been detected [28], but their associations with anterograde or retrograde traffic are speculative. We also note that hVps39 interacts with both GDP- and GTP-bound Rab5 [10], but has not been shown to interact with Rab7, indicating the model requires further testing to reconcile differences between the yeast and metazoan systems. COP/Cop, coat protein; Sec, secretory; Chc, clathrin heavy chain.

Table I

Rab GTPase and Vps-C complex components governing endolysosomal membrane fusion

Factor	Yeast	Human	Function(s)
Rab GTPase	Vps21	Rab5	Early endosome membrane identity marker and fusion regulation
	Ypt7	Rab7	Late endosome/lysosome membrane identity marker and fusion regulator
Vps-C core	Vps11/Pep5	hVps11	Elongated, coiled-coil proteins that function in membrane tethering and SNARE chaperone/ proofreading
	Vps16	hVps16	
	Vps18/Pep3	hVps18	Human subunit possesses ubiquitin-ligase activity *in vitro*
	Vps33	hVps33A, B	Sec1/Munc18 (SM) family, putative SNARE interactor
HOPS	Vps39/Vam6	hVps39/hVam6*	Putative GEF for Ypt7*
	Vps41/Vam2	hVps41	Ypt7 effector protein, possibly involved in AP-3 vesicle biogenesis
CORVET	Vps3	hVps3	Putative GEF for Vps21
	Vps8	hVps8	Vps21 effector protein
Other	Yck3	Casein kinase I	Protein kinase regulated by Ypt7, negatively regulates fusion by phosphorylating Vps41 and Vam3
	Gyp7		GAP for Ypt7
	Gdi1/Sec19	GDI	GDI, universal Rab chaperone
	Sec18	NSF	AAA ATPase for SNARE complex disassembly
	Sec17	α-SNAP	SNARE-complex adaptor for Sec18/NSF

Rab, monomeric G protein of the Ras GTPase superfamily; GTPase, guanosine triphosphate phosphatase; Vps, vacuole protein sorting; Ypt, yeast protein targeting; Pep, carboxypeptidase Y-deficient; Vam, vacuolar morphogenesis; SNARE, soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor; SM, Sec1/Munc18 family; HOPS, homotypic fusion and vacuole protein sorting; GEF, guanine nucleotide exchange factor; CORVET, class C core vacuole/endosome tethering complex; Yck, yeast casein kinase; GAP, GTPase activating protein; Gyp, GTPase-activating protein for Ypt; GDI, guanosine nucleotide dissociation inhibitor; Sec, secretory; AAA, ATPase associated with various cellular activities; NSF, *N*-ethylmaleimide-sensitive factor; SNAP, *N*-ethylmaleimide-sensitive factor attachment protein.

* Metazoan Vps39 shares sequence homology with yeast Vps3 and Vps39.