# CORVET and HOPS tethering complexes – coordinators of endosome and lysosome fusion

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## Summary
Protein and lipid transport along the endolysosomal system of eukaryotic cells depends on multiple fusion and fission events. Over the past few years, the molecular constituents of both fission and fusion machineries have been identified. Here, we focus on the mechanism of membrane fusion at endosomes, vacuoles and lysosomes, and in particular on the role of the two homologous tethering complexes called CORVET and HOPS. Both complexes are heterohexamers; they share four subunits, interact with Rab GTPases and soluble NSF attachment protein receptors (SNAREs) and can tether membranes. Owing to the presence of specific subunits, CORVET is a Rab5 effector complex, whereas HOPS can bind efficiently to late endosomes and lysosomes through Rab7. Based on the recently described overall structure of the HOPS complex and a number of *in vivo* and *in vitro* analyses, important insights into their function have been obtained. Here, we discuss the general function of both complexes in yeast and in metazoan cells in the context of endosomal biogenesis and maturation.

**Key words:** HOPS, CORVET, Endosome, Lysosome, Rab7, Rab5, Tethering, Membrane fusion

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## Introduction
Eukaryotic cells rely on a complex interconnected membrane system to transport cargo proteins such as hormones to the extracellular space and use similar principles to clear the surface of certain membrane proteins (Bonifacino and Glick, 2004). The primary carriers are vesicles that form in a cargo-dependent manner at a donor membrane and fuse with the acceptor membrane. Such dynamic fission and fusion processes are a major challenge for organelle identity and are thus tightly controlled. Within the endocytic branch of the endomembrane system, endosomes are the general sorting station, where endocytic vesicles that carry cell surface receptors fuse (Huotari and Helenius, 2011) (Fig. 1). At endosomes, decisions are made regarding whether a receptor is degraded or recycled, which depends on its interaction with substrates as well as posttranslational modifications. For degradation, receptors are marked by ubiquitylation and are sorted into intraluminal vesicles (ILVs) with the help of the ESCRT machinery, thus converting or maturing the early endosome into the multivesicular late endosome or multivesicular body (MVB) (Henne et al., 2011; Huotari and Helenius, 2011) (Fig. 1). In addition to endocytic vesicles, Golgi-derived vesicles that carry lysosomal hydrolases and membrane proteins fuse with endosomes. As several hydrolases require transmembrane receptors for their targeting to endosomes and lysosomes, these receptors need to be sorted back to the Golgi. It can thus be hypothesized that MVBs only fuse efficiently with lysosomes if, first, recycling of sorting receptors has been completed and, second, all ubiquitylated receptors have been cleared off from the endosomal surface by ESCRTs (Epp et al., 2011; Holthuis and Ungermann, 2013). In addition to endosomal fusion with lysosomes, at least two other fusion events take place at the yeast vacuole. Some proteins are targeted directly from the Golgi to the lysosome and arrive via the AP-3 pathway on vesicular carriers (Bowers and Stevens, 2005), whereas autophagosomes that are generated mainly during starvation will deliver organelles and cytosolic compounds to the lysosome to replenish the cell with biosynthetic precursors (Chen and Klionsky, 2011; Mizushima et al., 2011) (Fig. 1). Thus, the biogenesis of endosomes and lysosomes is likely to be tightly linked to the function of the fusion machinery that operates on these organelles.

In general, fusion of membranes within the endomembrane system requires a conserved machinery that consists of Rab GTPases and their interacting effectors, which mediate the first contact, as well as membrane-embedded SNAREs that are found on both membranes (Bonifacino and Glick, 2004). Rab proteins can exist in the inactive GDP- and active GTP-bound form. They are kept soluble in the cytoplasm by binding to the GDP-dissociation inhibitor (GDI). Upon recruitment to membranes, Rabs are converted into the active GTP-bound state by their specific guanine nucleotide exchange factor (GEF), and this reaction might also employ a GDI-dissociation factor. Once activated, the Rab–GTP can bind multiple effectors, which can also interact simultaneously with organelle-specific phosphoinositides (Barr and Lambright, 2010; Bos et al., 2007; Hutagalung and Novick, 2011; Itzen and Goody, 2011). The interaction of GTP-bound Rab proteins with tethering factors appears to be a key event in endosomal fusion as it brings membranes into contact (Bröcker et al., 2010; Yu and Hughson, 2010). The contact between membranes is further enhanced by SNAREs that are present on both membranes (Jahn and Scheller, 2006; Südhof and Rothman, 2009). SNAREs are tail-anchored membrane proteins with, generally, C-terminal transmembrane domains. During the final stage of fusion, SNAREs zip from their N- to C-termini into a tight four-helical

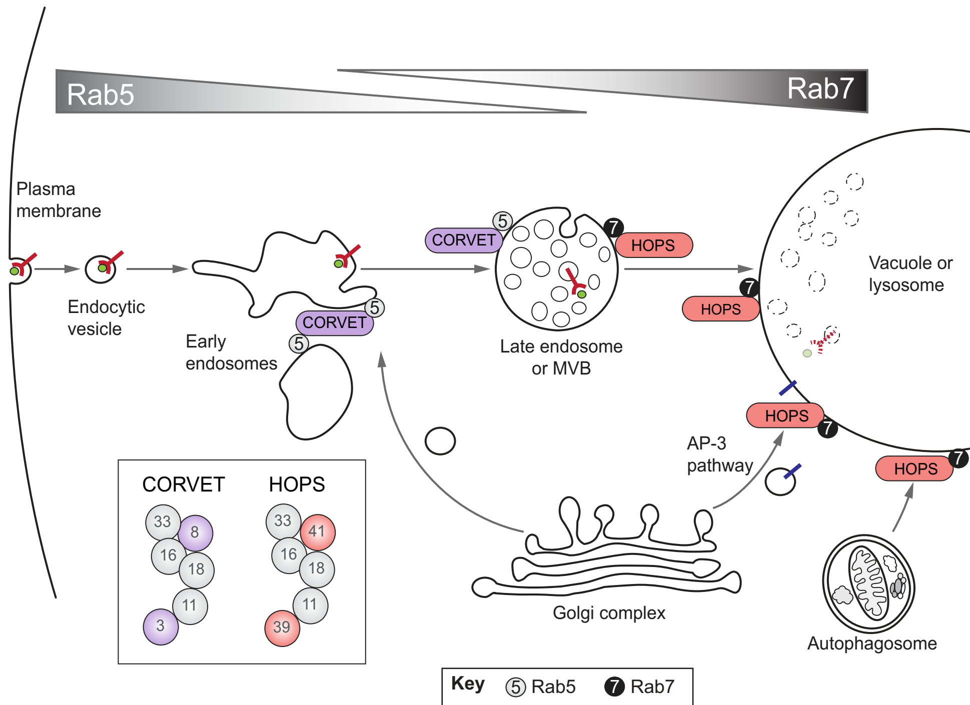


**Fig. 1. Schematic representation of CORVET and HOPS function within the endolysosomal pathway.** Shown here is the fate of a receptor-bound ligand that enters the endocytic pathway and eventually is degraded in the vacuole lumen. Endocytic vesicles are tethered to early endosomes through binding to coiled-coil tethering protein such as Vac1, EEA1 or rabenosyn5 (Christoforidis et al., 1999; Nielsen et al., 2000; Peterson et al., 1999; Tall et al., 1999). CORVET functions in endosome–endosome fusion by binding to the small GTPase Rab5. At the late endosome, Rab5 is replaced by Rab7, which then interacts with HOPS to promote fusion. The arrangement of HOPS and CORVET subunits, based on a recent EM structure, is shown below (boxed; see Fig. 2 for details). HOPS is also required for homotypic vacuole–vacuole (or lysosome) fusion and for fusion of autophagosomes with the vacuole. Golgi-derived AP-3 vesicles (here shown with a cargo in blue) fuse directly in a HOPS-dependent manner with the yeast vacuole.

complex and thus mediate the mixing of the lipid bilayers and hence fusion of the lumina. Once fusion is completed, SNAREs are unzipped and recycled by the ATPase NSF/Sec18 and its cofactor α-SNAP/Sec17 (Jahn and Scheller, 2006; Südhof and Rothman, 2009).

Endosomal biogenesis relies on two Rab proteins – Rab5 and Rab7, which act in a consecutive manner (Huotari and Helenius, 2011) (Box 1). The endosomal Rab5-like proteins promote the fusion of endocytic and Golgi-derived vesicles with early endosomes. Tethering of endocytic vesicles utilizes the EEA1 protein or its functional equivalents such as yeast Vac1 (Huotari and Helenius, 2011) (Fig. 1). The Rab5 GEF protein Rabex5 (yeast Vps9) might be present already on endocytic vesicles before their fusion with endosomes (Carney et al., 2006). In yeast, Vps21 is the main Rab5 homolog, and it is partially complemented by its other yeast homologs Ypt52 and Ypt53 (Cabrera et al., 2013; Horazdovsky et al., 1994; Nickerson et al., 2012; Singer-Krüger et al., 1994). During endosomal maturation, Rab5 is consecutively replaced by Rab7 (Poteryaev et al., 2010; Rink et al., 2005; Vonderheit and Helenius, 2005) (Box 1). In metazoan cells, this turnover appears to include a displacement of Rabex5 by Mon1 (SAND-1) (Poteryaev et al., 2010), which is part of the Mon1–Ccz1 complex (Kinchen and Ravichandran, 2010; Wang et al., 2002). It is now known that the yeast Mon1–Ccz1 complex is the GEF of Rab7-like Ypt7 protein (Nordmann et al., 2010), which was confirmed recently with the mammalian complex (Gerondopoulos et al., 2012). On late endosomes, activated Rab7–GTP can bind the membrane-remodeling retromer complex and might thus support the recycling of receptors from late endosomes (Balderhaar et al., 2010; Liu et al., 2012; Rojas et al., 2008; Seaman et al., 2009). In addition, and probably later on, Rab7 also promotes fusion with the lysosome, although the exact order has not been clarified (Balderhaar et al., 2010; Huotari and Helenius, 2011; Liu et al., 2012). Intriguingly, Rab5 and Rab7 bind to two hexameric tethering complexes, the endosomal CORVET ('class C core vacuole/endosome tethering') and the late endosomal/lysosomal HOPS ('homotypic fusion and protein sorting') complex (Abenza et al., 2010; Abenza et al., 2012; Bröcker et al., 2012; Ostrowicz et al., 2010; Peplowska et al., 2007; Seals et al., 2000; Wurmser et al., 2000) (Fig. 1; Fig. 2A). Here, we will review molecular insights into the role of both complexes, which have been mainly, but not exclusively, characterized in yeast, and will discuss their function in and regulation of membrane fusion

### Box 1. The functions of Rab5 and Rab7 within the endolysosomal system

Two small Rab GTPases are of key importance for the endolysosomal system. Rab5 (in yeast, Vps21) functions at the early endosome, whereas Rab7 (in yeast, Ypt7) is required on late endosomes and lysosomes. As discussed in the main text, Rab proteins interact with their effectors when bound to GTP as this stabilizes the effector-binding site within the Rab (Barr and Lambright, 2011). On early endosomes, Rab5–GTP interacts with multiple effectors, including the tethering factor EEA1 and the phosphoinositide 3-kinase Vps34 (Christoforidis et al., 1999) and thus generates endosomal domains involved in fusion and maturation. Early endosomes fuse in a Rab5-dependent manner to generate larger structures and form intraluminal vesicles with the help of the ESCRT complexes. These processes lead to a maturation of the early to the late endosome (Huotari and Helenius, 2011). The subsequent fusion of the mature late endosome with the lysosome depends on the homologous Rab7, which needs to be recruited and activated to its GTP-loaded form. In parallel, Rab5 is inactivated (Poteryaev et al., 2010; Lachmann et al., 2012). Once activated, Rab7–GTP interacts with HOPS to mediate fusion with lysosomes. Although the role of Rab5 and Rab7 as crucial factors in endolysosomal transport is widely accepted, the coordination of their transition and their multiple interactions on endosomes and lysosomes are far from being understood.

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at endosomes and lysosomes. Unless mentioned otherwise, we will focus initially on the function of HOPS and CORVET in yeast and will later extend our discussion into metazoan cells.

## Composition and subunit function of CORVET and HOPS

Upon classification of yeast mutants, it was apparent that the deletion of four proteins, Vps11, Vps16, Vps18 and Vps33, resulted in the most severe defect in endosomal biogenesis and vacuole morphology. Unlike wild-type cells, which have one to three vacuoles (termed class A), these mutants had highly fragmented vacuoles, and the proteins were thus coined class C proteins (Banta et al., 1988; Raymond et al., 1992). It later turned out that the four proteins function together along the endocytic pathway also in metazoan cells (Peterson and Emr, 2001; Richardson et al., 2004; Sriram et al., 2003; Rieder and Emr, 1997; Srivastava et al., 2000), although they are part of two complexes in yeast – CORVET (Peplowska et al., 2007) and HOPS (Seals et al., 2000; Wurmser et al., 2000) (Fig. 2A) – which was nicely confirmed in the fungus *Aspergillus nidulans* (Abenza et al., 2010; Abenza et al., 2012). In agreement with a shared function of the four class C proteins, deletion of the remaining two subunits of either complex is less dramatic (Horazdovsky et al., 1996; Markgraf et al., 2009; Nakamura et al., 1997; Raymond et al., 1992; Raymond et al., 1990; Wada et al., 1992), suggesting that there is some functional redundancy of HOPS and CORVET within the endocytic pathway. In addition to the four shared class C proteins, CORVET contains two Rab5-binding subunits, Vps3 and Vps8, whereas HOPS has instead the Rab7-binding subunits Vps39 and Vps41 (also known as Vam6 and Vam2) (Abenza et al., 2010; Abenza et al., 2012; Markgraf et al., 2009; Peplowska et al., 2007; Price et al., 2000; Seals et al., 2000; Wurmser et al., 2000). The similarity between HOPS and CORVET suggests that intermediate complexes also exist, and indeed a complex of low abundance can be isolated that consists of the class C subunits, plus HOPS Vps41 and CORVET Vps3 (Ostrowicz et al., 2010; Peplowska et al., 2007) (Fig. 2A). Structurally, most HOPS and CORVET subunits are likely to have a very similar secondary structure, with a predicted β-propeller at their N-terminal part and a possible α-solenoid at the C-terminal half (Nickerson et al., 2009) (Fig. 2B). Furthermore, Vps8, Vps11 and Vps18, as well as mammalian Vps41, have C-terminal RING domains (McVey Ward et al., 2001; Rieder and Emr, 1997). RING domains can function as E3 ubiquitin ligases

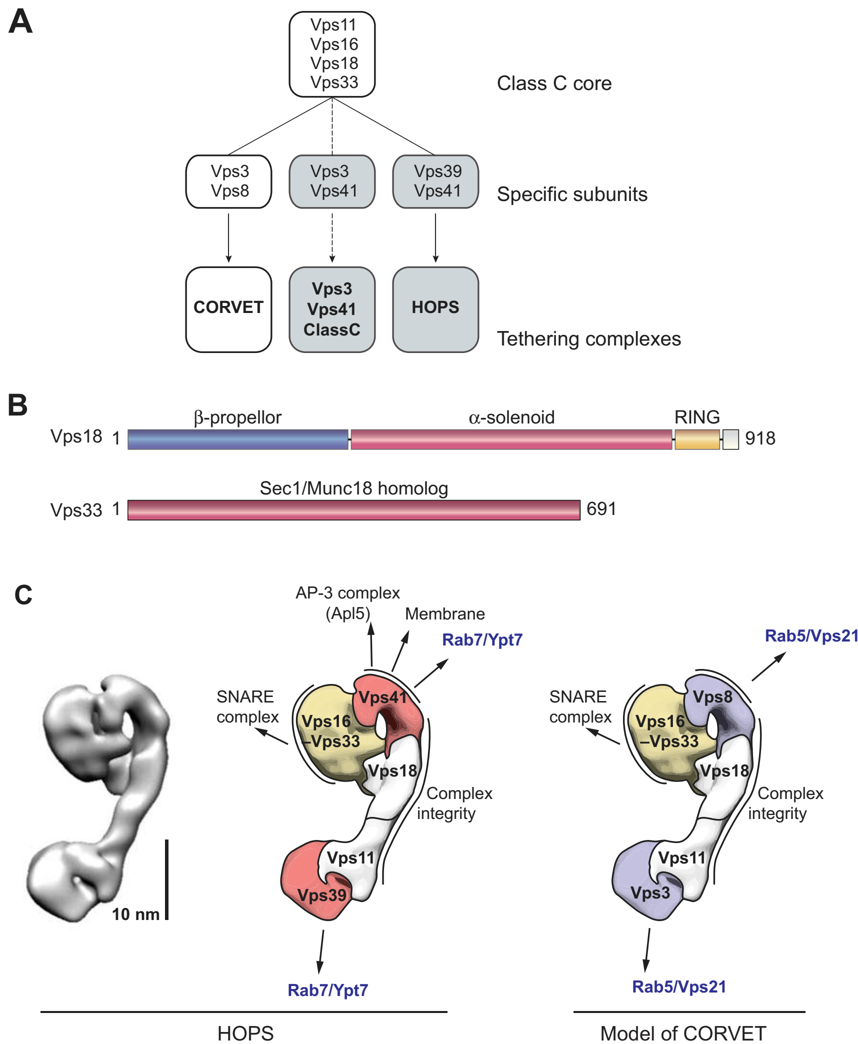


**Fig. 2. The structure of CORVET and HOPS.** (**A**) Module-like assembly of CORVET and HOPS, which consist of four shared class C subunits (Vps11, Vps16, Vps18, Vps33) and two Rab-specific subunits. The low-abundance intermediate complex is also shown (Vps3–Vps41–class-C). (**B**) Secondary-structure arrangement of subunits. A more detailed summary can be found elsewhere (Nickerson et al., 2009). (**C**) The three-dimensional overall structure of HOPS is shown on the left. The structure was determined by negative-stain electron microscopy (Bröcker et al., 2012). In the middle, the position and interactions of subunits within HOPS are illustrated. HOPS has two Rab-binding sites at opposite ends and binds to SNAREs through the Vps33 subunit and others. The Vps41 β-propeller domain contains an ALPS motif that binds membranes and can interact with the AP-3 subunit Apl5 (see text for details). The subunit arrangement in CORVET is shown on the right and is based on the HOPS structure. The position of the equivalent Rab-binding subunits Vps8 and Vps3 is shown in pale blue.

(Budhidarmo et al., 2012), and mammalian Vps18 is indeed able to promote ubiquitylation of the Gga3 adaptor protein that functions between Golgi and endosome (Yogosawa et al., 2006; Yogosawa et al., 2005). Furthermore, deletions of the RING domains of Vps11, Vps18 and Vps8 affect protein sorting to various degrees, although it is not yet clear whether this is related to assembly of the complex or additional functions in fusion that might relate to ubiquitylation (Plemel et al., 2011). The only subunit with a different structure is the mostly α-helical Vps33, which belongs to the SNARE-interacting Sec1/Munc18 (SM) family (Seals et al., 2000; Subramanian et al., 2004) (Fig. 2B). These proteins were initially thought to function alone, although several studies showed that Sec1-like proteins cooperate and interact with other fusion factors (Laufman et al., 2009; Peterson et al., 1999; Tall et al., 1999; Wiederkehr, 2004; Südhof and Rothman, 2009). However, Vps33 seems to be the only SM protein that is an integral part of a large multiprotein complex.

Recent structural data (Bröcker et al., 2012) combined with previous subunit-interaction analyses (Ostrowicz et al., 2010; Plemel et al., 2011; Pulipparacharuvil et al., 2005; Rieder and Emr, 1997; Wurmser et al., 2000) now provide a first glimpse into the organization of HOPS, which is likely to extend to CORVET (Fig. 2C). As both complexes have Rab-binding and SNARE-binding subunits, one attractive hypothesis is that HOPS and CORVET interact with Rab proteins on one membrane and SNAREs on the opposite membrane (Ostrowicz et al., 2010). However, tethering of membranes is already observed if HOPS is added to membranes that just carry GTP-loaded Ypt7 (yeast Rab7) (Hickey and Wickner, 2010). Recently, the yeast HOPS structure has been solved by negative-stain electron microscopy (EM) (Bröcker et al., 2012) (Fig. 2C). To assign the approximate position of the individual subunits, the EM structures of previously identified subunit dimers (Vps11–Vps39 and Vps16–Vps33) and trimers (Vps11–Vps39–Vps18) were solved (Ostrowicz et al., 2010; Plemel et al., 2011; Pulipparacharuvil et al., 2005) and modeled into the overall structure (Bröcker et al., 2012). By combining antibody labeling with EM, the positions of selected subunits were assigned, thus identifying their relative position in the complex (Fig. 2C). HOPS is a 30 nm long seahorse-shaped particle, with a large head, a flexible linker and a smaller tail. Surprisingly, the Rab7/Ypt7-binding subunits Vps39 and Vps41 are located at opposite ends of HOPS. At the head, Vps41 is located next to Vps16 and the SNARE-binding Vps33 subunit, whereas Vps18 and Vps11 connect to the Vps39 subunit (Bröcker et al., 2012) (Fig. 2C, middle). Earlier mapping analysis had revealed that Vps11 and Vps39 interact through their C-terminal segments (Ostrowicz et al., 2010; Plemel et al., 2011; Wurmser et al., 2000), thus suggesting that the N-terminal β-propeller of Vps39 occupies much of the C-terminal tail. Furthermore, Ypt7–GTP could be localized to the two opposite ends of the HOPS complex by EM, thus confirming the yeast Rab7/Ypt7-binding ability of Vps39 and Vps41 and their positions within the complex (Bröcker et al., 2012).

The overall picture emerging from the HOPS structure now provides a general outline of its function as a tethering complex, which probably also extends to CORVET, in which Vps3 replaces Vps39, and Vps8 replaces Vps41 at the opposite end (Fig. 2C, right), whereas the shared subunits are located at the center of each complex. It is, however, clear that these four subunits need to co-assemble with the Rab-specific subunits, as they cannot be isolated as a tetrameric core complex (Ostrowicz et al., 2010). Tethering should therefore include Rab-binding at opposite ends of the complex, and thus opposite membranes. This probably precedes SNARE binding through the common Vps33 and other subunits. As such, both HOPS and CORVET would be tethers that preferentially cluster Rab7- and Rab5-decorated membranes. In the following section, we will discuss this model in the context of the available literature.

## The role of CORVET at the endosome

The yeast class C proteins were initially only implicated in the HOPS complex (Seals et al., 2000; Wurmser et al., 2000), although it was known that mutants in Vps8 could suppress Vps11 mutants (Woolford et al., 1998; Plemel et al., 2011). Vps8 has been isolated from yeast and shown to form a complex with Vps3 and the four class C proteins (Peplowska et al., 2007). The identification of the yeast CORVET complex as a heterohexamer could then explain both the severe phenotype of the class C mutants and their link to Vps8 and Vps3 (Peplowska et al., 2007). CORVET is an effector of Rab5 both in yeast and *Aspergillus* (Peplowska et al., 2007; Abenza et al., 2010; Balderhaar et al., 2013). Both Vps8 and Vps3 bind to the yeast Rab5-like Vps21 protein (Horazdovsky et al., 1996; Markgraf et al., 2009; Pawelec et al., 2010; Plemel et al., 2011), and their deletion results in a large vacuole in yeast (Banta et al., 1988; Raymond et al., 1992). Among the gene products that result in a similarly enlarged vacuole (class D) are also the Rab5-like Vps21, its GEF Vps9, the early endosomal tethering protein Vac1, the SM-protein Vps45 and the endosomal SNARE Pep12. Interestingly, all class D genes encode proteins involved in membrane fusion at endosomes (Bowers and Stevens, 2005). Recently, it was shown that the CORVET subunits have a function that is distinct and possibly downstream from Vps45 and Vac1 (Cabrera et al., 2013), which also binds Vps21–GTP (Peterson et al., 1999; Tall et al., 1999). The CORVET subunits Vps3 and Vps8 localize independently of Vac1, Vps45 and Pep12 but still require activated Rab5 proteins for their localization and function (Cabrera et al., 2013). The most plausible interpretation of these results is that CORVET is a tether for the fusion of endosomes with each other, so-called homotypic fusion, whereas Vac1 might be required primarily for the fusion of endocytic vesicles with endosomes, although the possibility of cooperation occurring between the two tethering machineries cannot be excluded. In agreement with this, it has been found that purified CORVET is able to tether efficiently vacuole-associated endosomes in a Rab5/Vps21-dependent manner both *in vitro* and *in vivo* (Balderhaar et al., 2013). Endosomal fusion and fission appear to be crucial for the generation of MVBs and protein sorting as Rab5 mutants are defective in both processes (Cabrera et al., 2013; Nickerson et al., 2012; Russell et al., 2012). Indeed, the generation of an average MVB of a radius of 100 nm that carries up to 50 intraluminal vesicles (Luhtala and Odorizzi, 2004) would require several fusion events to provide sufficient membrane for the vesicle generation. Consistently, the acute removal of all Rab5 homologs in mouse liver results in a loss of Rab7-positive MVBs (Zeigerer et al., 2012). Finally, overexpression of the Vps8 subunit of CORVET in yeast leads to an accumulation of MVBs, suggesting a delay in fusion while maintaining the CORVET-driven tethering function (Markgraf et al., 2009). Taking these data together, we believe that CORVET is the general tether that promotes endosomal fusion by binding Rab5–GTP (Balderhaar et al., 2013; Cabrera et al.,

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2013) and, consequently, provides the membrane material for efficient generation of MVBs.

## The role of HOPS in endolysosomal biogenesis and fusion

The existence of the HOPS complex has not only been known for a longer period of time, but this complex is also by far the better studied and understood in terms of its function compared with its 'sibling' CORVET. HOPS is an effector of yeast Rab7/Ypt7 (Abenza et al., 2012; Bröcker et al., 2012; Seals et al., 2000), which is, as mentioned above, activated by the Rab7 GEF complex Mon1–Ccz1 (Nordmann et al., 2010). As Mon1–Ccz1 resides on early and late endosomes, activated Rab7–GTP is probably targeted to the vacuole or lysosome during the course of endosomal maturation (Nordmann et al., 2012; Poteryaev et al., 2010). Indeed, yeast HOPS binds to the GTP-form of the Rab7-homlog Ypt7 through its Vps41 and Vps39 subunits (Bröcker et al., 2012; Ostrowicz et al., 2010; Plemel et al., 2011; Seals et al., 2000) and is able to tether membranes through the yeast Rab7 Ypt7 (Hickey and Wickner, 2010; Zick and Wickner, 2012) (Fig. 3A). Of note, some binding of HOPS to Ypt7–GDP has also been observed, which suggests that HOPS had a weaker sensitivity for the activated Rab or had a strong preference for the small pool of Ypt7–GTP that was still present in the binding assay (Wurmser et al., 2000; Zick and Wickner, 2012). Furthermore, it was proposed that Vps39 is not only an effector of Ypt7, but also a Ypt7 GEF (Wurmser et al., 2000), which could not be confirmed (Nordmann et al., 2010; Peralta et al., 2010). However, it has been shown both for yeast and for metazoan cells that the Mon1 subunit of the Mon1–Ccz1 GEF complex interacts with HOPS subunits, including Vps39 (Wang et al., 2003; Nordmann et al., 2010; Poteryaev et al., 2010).

In addition to the initial characterization of HOPS as a Rab7-effector complex, its ability to restore *in vitro* vacuole fusion greatly extended insights into its function (Stroupe et al., 2006). Wickner and colleagues further established a novel proteoliposome fusion assay that depends both on isolated components such as vacuolar SNAREs, their activating Sec17 (α-SNAP) and Sec18 (NSF) proteins, the HOPS complex and Ypt7 (Stroupe, 2012; Stroupe et al., 2009; Zucchi and Zick,

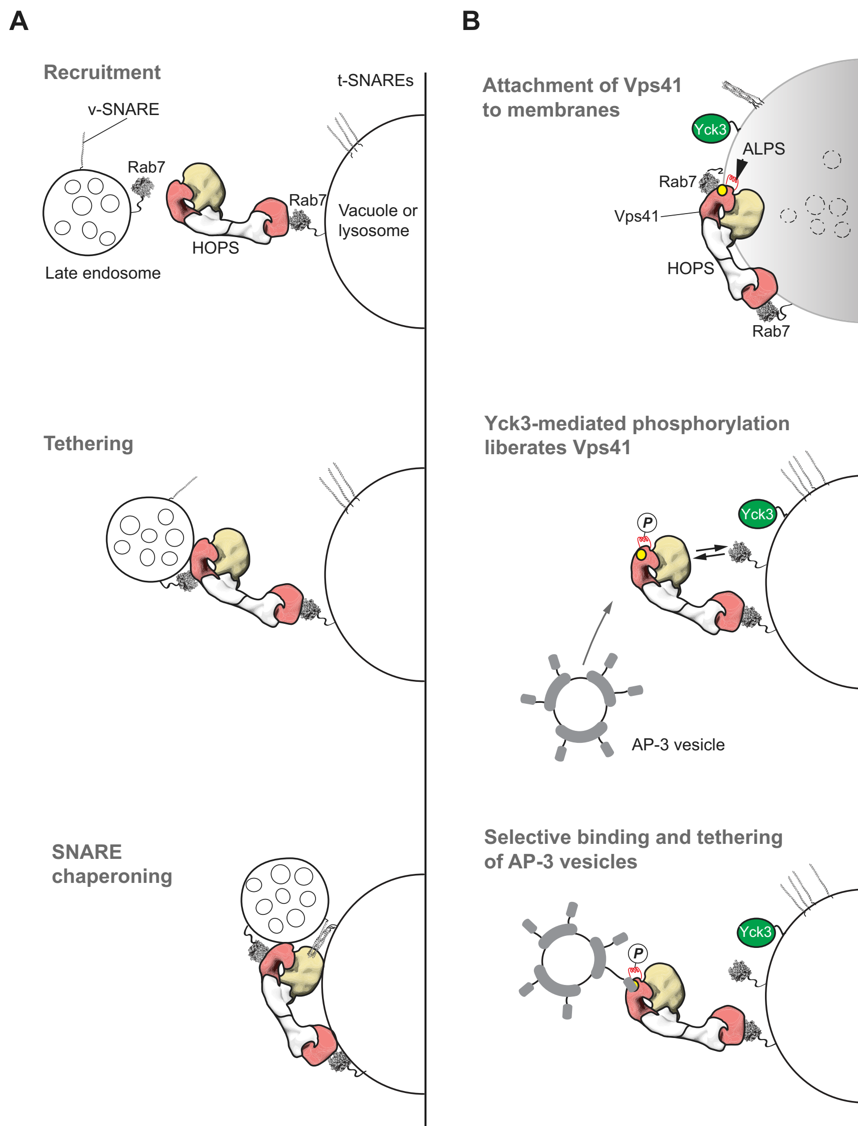


**Fig. 3. The function of HOPS in fusion at the vacuole or lysosome.** (**A**) HOPS-mediated tethering of late endosomes with vacuoles. HOPS (top) binds to Ypt7 (Rab7) located on the vacuole and late endosomes for its tethering (centre) and utilizes its SNARE-chaperoning activity to capture SNAREs and assemble them into the fusogenic four-helix bundle (bottom). (**B**) Regulation of the role of HOPS in AP-3 vesicle fusion by phosphorylation. At vacuoles (top), HOPS binds to the membrane through Ypt7–GTP and its ALPS membrane-interacting motif (in red; the vacuole surface is shown in gray). The casein kinase Yck3, which arrives via the AP-3 pathway on the vacuole surface, then phosphorylates the ALPS motif and loosens the interaction of Vps41 with the membrane (centre). Vps41 phosphorylation fully exposes an AP-3-binding site (yellow) in Vps41 (bottom) that is required for the fusion of AP-3 vesicles with vacuoles. For further details, see main text.

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2011). Thus, not only the general function but also specific properties of HOPS in fusion could be elucidated with this assay. Interestingly, the requirement of yeast Rab7 protein Ypt7 for efficient fusion can be bypassed *in vitro* if the lipid composition is altered (Mima et al., 2008; Stroupe et al., 2009). HOPS probably binds these membranes directly and thus can promote fusion even in the absence of Ypt7 (Cabrera et al., 2010; Stroupe et al., 2006). Such a membrane binding would then replace the *in vivo* recruitment of HOPS by means of Ypt7–GTP, although at least one membrane-binding site is also crucial for HOPS function *in vivo* (Cabrera et al., 2010) (see below). Once it is targeted to membranes, HOPS then catalyzes membrane fusion, presumably by capturing, chaperoning and proofreading SNAREs at the fusion site through its Vps33 subunit (Akbar et al., 2009; Krämer and Ungermann, 2011; Lobingier and Merz, 2012; Pieren et al., 2010; Starai et al., 2008) (Fig. 3A). These assembled SNAREs at the fusion site can also be a target of Sec17 and Sec18, which would result in SNARE disassembly (Xu et al., 2010). Thus, HOPS not only supports SNARE assembly but can also protect SNAREs at fusion sites from Sec17 and Sec18. Whether the ability of HOPS to bind SNAREs differs considerably from that of CORVET is uncertain. Vps33, which is present in both complexes, is probably the main SNARE interactor, and binding of Vps33 to endosomal SNAREs has been reported (Subramanian et al., 2004) (Fig. 2C), even though additional SNARE binding sites have been identified within other subunits (Krämer and Ungermann, 2011; Lobingier and Merz, 2012). It is, however, possible that Vps33 is differentially posttranslationally modified or that its conformation varies depending on whether it is located close to Vps41 (in HOPS) or Vps8 (in CORVET). Moreover, the overall architecture of CORVET and HOPS could influence the SNARE affinity of Vps33. An alternative model is that Vps33 binds SNAREs in both complexes, and specificity is brought about by the Rab-specific subunits in either complex.

## Regulation of HOPS during vacuole biogenesis

As mentioned above, HOPS mediates multiple fusion events at the yeast vacuole and probably at mammalian endosomes (Fig. 1). It is likely that the function of HOPS is regulated, possibly beyond Rab binding, but thus far only one regulatory mechanism has been dissected to any degree. By following the posttranslational modification of the Vps41 subunit, the Yck3 casein kinase has been identified as a specific modulator of fusion (LaGrassa and Ungermann, 2005). Yck3 is a substrate of the AP-3 pathway, which bypasses the late endosome on its way to the vacuole (Fig. 1; Fig. 3B) (Sun et al., 2004). Thus, the AP-3 pathway can ensure that proteins that could act on endosomal proteins or mark the lysosome as an autonomous organelle, such as the syntaxin-like SNARE Vam3, are delivered directly to the lysosomal surface (Darsow et al., 2001; Bowers and Stevens, 2005). In addition, this pathway bypasses the sorting of proteins into intraluminal vesicles at the late endosome. Interestingly, the HOPS subunit Vps41 interacts with components of the AP-3 coat and could thus facilitate fusion of AP-3 vesicles with vacuoles (Rehling et al., 1999; Darsow et al., 2001; Angers and Merz, 2009). Two striking effects have been observed in the absence of the Yck3 casein kinase: first, Vps41 strongly accumulates at late endosomal sites (Cabrera et al., 2009; LaGrassa and Ungermann, 2005) and, second, vacuoles isolated from the yeast deletion strain are strongly resistant to the yeast Rab7 inhibitors Gdi1 or Gyp7 (Brett et al., 2008; LaGrassa and Ungermann, 2005). These data are consistent with the idea that non-phosphorylated Vps41 is strongly membrane associated, thus shielding the bound Ypt7–GTP from Rab inhibitors (Cabrera et al., 2009; Hickey et al., 2009) (Fig. 3B). Furthermore, the phosphorylation sites reside within an amphipathic lipid packaging sensor (ALPS) helix of yeast Vps41, which is part of the β-propeller domain (Cabrera et al., 2010) (see Fig. 3B). What then is the function of this helix? We believe that Vps41 initially binds both activated Ypt7–GTP and the late endosomal membrane through segments of its β-propeller – an example of coincidence detection. Upon fusion with vacuoles, the membrane-binding ALPS helix is phosphorylated by Yck3 and thus weakens the membrane interaction of Vps41 (Cabrera et al., 2009; Cabrera et al., 2010) (Fig. 3B). Consequently, in the presence of phosphorylated Vps41, Ypt7 is not shielded as strongly, and thus fusion is more sensitive to Rab inhibitors (Cabrera et al., 2009; LaGrassa and Ungermann, 2005). In addition, the weakened interaction exposes a binding site in HOPS for the AP-3 coat (Angers and Merz, 2009; Rehling et al., 1999) and thus allows the fusion between AP-3 vesicles and the Yck3-containing vacuole (Cabrera et al., 2010) (Fig. 3B). In agreement with this, *in vitro* phosphorylation of HOPS with recombinant Yck3 makes fusion strongly dependent on Ypt7–GTP, whereas HOPS could also bind the GDP-form of Ypt7 and even support fusion in the absence of Yck3-driven phosphorylation (Hickey et al., 2009; Zick and Wickner, 2012). This suggests that the kinase is necessary to sharpen also the nucleotide specificity of HOPS for Ypt7–GTP – a model that requires further investigation. Considering the complexity of this reaction, it is actually surprising that this motif does not seem to be conserved in metazoan Vps41, as it allows the exclusive and specific targeting of SNAREs such as the syntaxin Vam3 to the lysosomal vacuole (Cabrera et al., 2010; Darsow et al., 1998). Of note, a separate function for human Vps41 has recently been proposed for the delivery of the LAMP1 lysosomal membrane protein (see below) (Pols et al., 2013).

An additional level of regulation of CORVET and HOPS could occur by posttranslational modifications or by subunit exchange, as intermediate complexes that contain HOPS Vps41 and CORVET Vps3 also exist in yeast, albeit at low abundance, as mentioned above (Ostrowicz et al., 2010; Peplowska et al., 2007) (Fig. 2A). Even though an excess of Vps3 will promote the formation of CORVET at the expense of HOPS – presumably by competing with Vps39 for interaction with Vps11 (Ostrowicz et al., 2010; Plemel et al., 2011) – it is not yet clear whether such a transition also accompanies endosomal maturation. At present, it also cannot be excluded that the function of CORVET or HOPS is regulated by the dynamics of single subunits.

## Functions of HOPS and CORVET beyond yeast

Metazoan HOPS has been extensively characterized in mammalian cells, *Drosophila* and *Caenorhabditis elegans*, whereas specific information on metazoan CORVET is still fragmentary (reviewed by Fairn and Grinstein, 2012; Pols et al., 2012; Zlatic et al., 2011b; Solinger et al., 2013). As metazoan cells have evolved additional isoforms of subunits, we consider it a major challenge to decipher from the literature which analyzed reactions indeed require metazoan HOPS and which might depend instead on the admittedly poorly described metazoan CORVET. In the current state of affairs, HOPS or even single subunits are assigned to a specific trafficking reaction during

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endocytosis or phagocytosis, even though only single, possibly even shared, subunits have been analyzed.

Regarding the subunit repertoire, metazoan cells seem to contain all the necessary components for at least one CORVET and one HOPS complex. They encode the shared subunits Vps11, Vps18 and two A and B isoforms of Vps16 and Vps33. Furthermore, metazoan cells encode Vps8, Vps41 and two isoforms of Vps39 (hVps39-1/TLP and hVps39-2/TRAP-1) (Warner et al., 1998; Charng et al., 1998; Akbar et al., 2011; Caplan et al., 2001; Huizinger et al., 2001; Kim et al., 2001; Wurthner et al., 2001; Felici et al., 2003; Pevsner et al., 1996; Pulipparacharuvil et al., 2005; Richardson et al., 2004; Kinchen et al., 2008), of which one (hVps39-1/TLP) is similar to yeast Vps3 (Peplowska et al., 2007). Interestingly, like their yeast homologs, the Vps16 and Vps33 isoforms can form two distinct subcomplexes (Ostrowicz et al., 2010; Sato et al., 2000). Vps33A interacts with Vps16A, and both function in endolysosomal fusion and are probably HOPS specific (Akbar et al., 2009; Pulipparacharuvil et al., 2005), whereas the interacting Vps33B and Vps16B (VIPAR or SPE-39) are required for phagocytosis and earlier endosomal fusion reactions (Akbar et al., 2011; Cullinane et al., 2010; Gissen et al., 2004; Zhu and L'Hernault, 2003; Zhu et al., 2009). Thus, metazoan CORVET might contain, in addition to Vps11 and Vps18, Vps8, hVps39-1, Vps16B and Vps33B, whereas metazoan HOPS probably has hVps39-2, Vps41, Vps16A and Vps33A as specific subunits. It can, of course, not be excluded that additional variants of HOPS or CORVET complexes exist, similar to the low-abundance hybrid complex in yeast (Peplowska et al., 2007). Below, we will summarize some of the findings that have been linked to subunits of HOPS and CORVET, even though the literature refers mostly to HOPS or even single subunits.

Multiple interactions between HOPS or CORVET subunits and other proteins have been identified, including binding to actin and tubulin, and the cytoskeletal binding protein HOOK (Richardson et al., 2004; Xu et al., 2008), the interaction with components of the autophagy machinery (Liang et al., 2008), the Arl8 GTPase (Garg et al., 2011), which is involved in lysosomal mobility (Hofmann and Munro, 2006; Mrakovic et al., 2012), Merkel virus (Liu et al., 2011) and the AP-3 complex (Zlatic et al., 2011a). Furthermore, HOPS/CORVET subunits have been identified on clathrin-coated vesicles (Zlatic et al., 2011a), as well as on late endosomes and lysosomes (Poupon et al., 2003; Pols et al., 2012; Sriram et al., 2003; Swetha et al., 2011), which suggests a role for HOPS/CORVET in endosomal fusion and even maturation. Defects in HOPS or CORVET subunits in mammalian tissues result in strong deficiencies. For instance, loss of hVps39-1 (TLP), the homologous hVps39-2 (TRAP-1), hVps41 (hVam2) or hRab7 results in embryonic lethality as early as gastrulation (Aoyama et al., 2012; Kawamura et al., 2012; Messler et al., 2011) and also causes extensive developmental defects in zebrafish (Schonthaler et al., 2008). Likewise, mutants in HOPS impair infection by Ebola virus (Carette et al., 2011) and export of HIV virions (Tomita et al., 2011), affect endosomal, phagosomal and lysosomal biogenesis (Caplan et al., 2001; Poupon et al., 2003; Pols et al., 2012; Sriram et al., 2003; Swetha et al., 2011; Kinchen et al., 2008) and, subsequently, development (Wilkin et al., 2008). Mutations in Vps33B and Vps16B, which could be part of the metazoan CORVET, are further linked to diseases such as arthrogryposis–renal-dysfunction–cholestasis (ARC) syndrome, an autosomal recessive disorder, and cancer (Gissen et al., 2004; Roy et al., 2011). Several of the observed defects are likely to be associated with defective signaling through the endosome, which impairs morphogen gradients, receptor degradation and subsequently affects embryonic development or causes strong developmental defects within the entire organism (Charng et al., 1998; Wurthner et al., 2001; Felici et al., 2001; Aoyama et al., 2012; Kawamura et al., 2012; Messler et al., 2011; Wilkin et al., 2008). Data from localizing hVps39-1 and hVps41 as well as other class C proteins in mammalian cells are consistent with the observations in yeast that HOPS is involved in endosome–lysosome fusion (Caplan et al., 2001; Pols et al., 2012). Recent results also suggest that hVps41 has an additional specific function in the fusion of carriers that deliver the LAMP1 membrane protein to the lysosome (Pols et al., 2013), which might be related to the function of yeast Vps41 in the fusion of AP-3 vesicles with vacuoles (Darsow et al., 2001; Angers and Merz, 2009; Cabrera et al., 2010). In addition, Rab5-mediated homotypic fusion is required for endosomal maturation (Zeigerer et al., 2012), and one hVps39 isoform has been isolated previously with Rab5–GTP (Rink et al., 2005). As isolated fungal CORVET and HOPS are clearly Rab specific (Abenza et al., 2010; Abenza et al., 2012; Balderhaar et al., 2013; Bröcker et al., 2012; Ostrowicz et al., 2010; Peplowska et al., 2007; Zick and Wickner, 2012; Plemel et al., 2011), Rab5-dependent fusion events in metazoan cells probably require the CORVET instead of Rab7-dependent HOPS, as suggested previously (Peplowska et al., 2007). Overall, the functions of metazoan HOPS and CORVET appear to be equivalent to their functions in membrane fusion as characterized in fungi. It will be important to investigate whether additional binding partners are involved in altered functions or more complex regulations and whether the proposed composition of each complex is indeed observed *in vivo* or whether additional complexes exist. Furthermore, it will be crucial to understand the functions of the reported interactions of endosomal proteins with individual CORVET and HOPS subunits in the context of their role as part of the tethering complex. Future studies will be necessary to reveal the precise function of metazoan HOPS at late endosomes, as well as a much more detailed characterization of metazoan CORVET.

## Concluding remarks

Rab-driven membrane-tethering that is mediated by CORVET and HOPS is of central importance for the biogenesis of endosomes and lysosomes in eukaryotic cells. Both complexes probably function similarly in catalyzing the assembly of SNARE complexes and thus facilitate the mixing of lipid bilayers in confined zones on membranes. It is plausible that the function of fusion complexes is coordinated with that of other complexes that act at endosomal and lysosomal membranes. Separate domains or micro-compartments exist on endosomes (Derivery et al., 2012; Sönnichsen et al., 2000) that not only allow a separation of fission and fusion events by Rab GTPases and membrane-remodeling complexes but also the coordination of these events (Holthuis and Ungermann, 2012). Moreover, one can predict that additional regulatory circuits beyond the currently identified casein-kinase-I-mediated phosphorylation of yeast Vps41 will control the functions of specific subunits. Considering the importance of both complexes for the biogenesis of endosomes and lysosomes, it will be important to elucidate the molecular details of their function and regulation. This should also include a

much more detailed molecular understanding of HOPS and CORVET function in metazoan cells in the context of endosomal and lysosomal signaling, endosomal maturation and the associated fusion events, which could eventually facilitate interventions during pathogen or viral infections.

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