Characterization of the Mammalian CORVET and HOPS Complexes and Their Modular Restructuring for Endosome Specificity*

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Background: The CORVET and HOPS complexes regulate endosomal cargo trafficking but have not been well characterized in mammals.

Results: A detailed analysis of subunit interactions within the mammalian CORVET, HOPS, and VIPAS39/VPS33B complexes. A detailed analysis of interactions within the mammalian CORVET and HOPS as well as an additional endosomal-targeting complex (VIPAS39-VPS33B) that does not exist in yeast. We show that core interactions within CORVET and HOPS are largely conserved but that the membrane-targeting module in HOPS has significantly changed to accommodate binding to mammalian-specific RAB7 interacting lysosomal protein (RILP). Arthrogryposis-renal dysfunction-cholestasis (ARC) syndrome-associated mutations in VPS33B selectively disrupt recruitment to late endosomes by RILP or binding to its partner VIPAS39. Within the shared core of CORVET/HOPS, we find that VPS11 acts as a molecular switch that binds either CORVET-specific TGFBRAP1 or HOPS-specific VPS39/RILP thereby allowing selective targeting of these tethering complexes to early or late endosomes to time fusion events in the endo/lysosomal pathway.

The endocytic pathway is a dynamic system in which vesicles are continuously fusing, moving, and budding in order to deliver their cargo to the correct compartment. After internalization, endocytic cargo is delivered to the early endosome (EE)3 from which it can be recycled or degraded (1). Cargo destined for degradation such as internalized nutrients, activated growth receptors, and endocytosed pathogens are targeted to the late endosomes (LEs) and lysosomes where the acidic environment and resident proteases allow for degradation. Rab5 (EE) and Rab7 (LE) are master regulators of endosomal transport and fusion and regulate cargo flux through the endocytic system in conjunction with multisubunit motor and tethering complexes. In yeast, EE and LE tethering is regulated by the CORVET (class C core vacuole/endosome tethering) and HOPS (homotypic fusion and vacuole protein sorting) complexes, respectively, which have been characterized in high detail (2–8). Both complexes consist of a shared core (vps16, vps18, vps11, vps33) that associates with CORVET-specific (vps3 and vps8) or HOPS-specific (vps39 and vps41) subunits. These different subunits target the complexes to membranes by interaction with respectively vps21 (yeast Rab5) and Ypt7 (yeast Rab7) (6, 7). In addition, vps41 can also bind lipids (9). Apparent homologues of CORVET and HOPS subunits in mammals have been implicated in endosomal maturation (10), EE fusion (11), and fusion of lysosomes with late endosomes, phagosomes or autophagosomes (12–15). Based on sequence alignment, homologs of all eight yeast HOPS and CORVET subunits are present in mammalian cells with the addition of two novel homologues; VIPAS39 (also known as SPE-39 or VIPAR) and VPS33B. Recently it was shown that mammalian VPS8 and TGFBRAP1 are the vps8 and vps3 homologues (11), and the mammalian CORVET, therefore, consists of VPS8, TGFBRAP1, VPS18, VPS16, VPS33A, and VPS11, whereas HOPS consists of VPS41, VPS39, VPS18, VPS16, VPS33A, and VPS11. Although VPS33B was initially shown to interact with HOPS subunits (16), more

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3 The abbreviations used are: EE, early endosome; LE, late endosome; RILP, Rab7 interacting lysosomal protein; ARC, arthrogryposis-renal dysfunction-cholestasis; IP, immunoprecipitation; WB, Western blot; CLSM, confocal laser-scanning microscope.
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Experimental Procedures

Reagents—Rabbit anti-GFP and rabbit anti-mRFP antibodies were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been excluded by Western blot analyses with various mRFP- or GFP-labeled fusion proteins. Other antibodies used were: mouse anti-CD63 (28), mouse anti-EEA1 (ab2900; Abcam), mouse anti-VPS8 was purchased from Origene and inserted into pcDNA3.2-HA/DEST vector (Invitrogen) using Gateway recombination cloning. Truncation constructs were generated in-house using purified His-mRFP or His-GFP recombinant proteins, respectively. Cross-reactivity has been exclude
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5% CO₂ humidified culture hood at 37 °C. All specimens were analyzed by confocal laser-scanning microscopes (TCS-SP1, TCS-SP2, or AOBs; Leica) equipped with HCX Plan-Apochromat 63× NA 1.32 and HCX Plan-Apochromat ldb.bl 63× NA 1.4 oil-corrected objective lenses (Leica) using LCS (Leica) acquisition software or Deltavision wide field microscope (Applied Precision) with a 100/1.4A immersion objective. Widefield images were deconvolved using SoftWorx software (Applied Precision).

**Transfection**—Expression constructs were transfected using Effectene reagents (Qiagen) according to the manufacturer’s instructions. For silencing, cells were transfected with Dharmacon siRNA (Thermo Fisher Scientific).

**Microscopy Sample Preparation**—Transfected cells were fixed 24 h post-transfection with 4% formaldehyde in PBS for 15 min and permeabilized for 10 min with 0.05% Triton X-100 in PBS at room temperature. Non-specific binding of antibodies was blocked by 0.5% BSA in PBS for 40 min, after which cells were incubated with primary antibodies in 0.5% BSA in PBS for 1 h at room temperature. After washing 3 times with PBS, primary antibodies were visualized with Alexa-Fluor secondary antibody conjugates and detected in affinity purified rabbit anti-Alexa-Fluor-594 (Invitrogen). After three washes with PBS samples were mounted in Vectashield mounting medium (Vector Laboratories).

**Protein Immunoprecipitation**—MelJuSo cells were washed with ice-cold PBS and scraped into cell lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM MgCl₂, 1% Nonidet P-40, 10% glycerol, pH 7.4) supplemented with complete EDTA-free protease inhibitor mixture. Cell lysates were obtained by incubation on ice for 10 min followed by centrifugation for clearing. The supernatants were incubated for 2 h with respective antibodies followed by capture with protein G-Sepharose 4 FF resin and washed extensively with wash-buffer (50 mM Tris, 150 mM NaCl, 5 mM MgCl₂, 10% glycerol, pH 7.4). For GFP-tagged pull-down, GFP-TRAP beads were used (Chromotek). All experiments were repeated multiple times.

**Statistical Analysis**—For calculation of correlation coefficients the signal intensity over a vector was plotted using the plot profile tool in ImageJ (http://imagej.nih.gov). Correlation coefficient for colocalization assays were calculated from plot profiles measuring intensity for the indicated fluorescence channels over a vector through the cells (as in van der Kant et al. (30)). Correlation coefficients for the two plots were calculated in Excel using the function,

\[
\text{Correl}(X,Y) = \frac{\sum(x - \bar{x})(y - \bar{y})}{\sqrt{\sum(x - \bar{x})^2\sum(y - \bar{y})^2}} \quad (\text{Eq. 1})
\]

Statistical testing was performed in Graphpad Prism 6 using a one-way analysis of variance with Tukey’s multiple comparison test for significance.

**Results**

**Characterization and Definition of the Mammalian CORVET, HOPS, and VPS33B-VIPAS39 Complex**—The yeast CORVET and HOPS complexes are assembled from eight different proteins (a Vps16/Vps33/Vps18/Vps11 core with Vps3/Vps8 or Vps39/Vps41). Mammals do not possess 8, but 10 homologues to the yeast CORVET/HOPS subunits. Two mammalian homologues for yeast Vps33 exist (VPS33A and VPS33B) as well as an additional protein with weak homology to Vps16 (VIPAS39). To probe the interactions between the 10 putative CORVET/HOPS subunits and determine the topology of the different subunits in the CORVET-HOPS complexes we performed an extensive array of co-immunoprecipitations (IP) in human MelJuSo cells with antibodies against endogenous VIPAS39, VPS33B, VPS33A, VPS11, VPS16, VPS8, and VPS33A (Fig. 1A) (antibodies specific for other components were either not available or did not work for IP). We observed extensive interactions between VPS11, VPS16, VPS8, TGFBRAP1, and VPS33A. In contrast, VIPAS39 and VPS33B interacted with each other but not with any of the other canonical CORVET/HOPS subunits in line with recent data (12).

To further map the HOPS complex and define possible interactions between VIPAS39 and VPS33B with HOPS components that we could not map in endogenous pulldown experiments, we ectopically co-expressed all six putative HOPS complex subunits as well as VPS33B and VIPAS39 as tagged proteins. We previously showed that expression levels of these tagged proteins in our systems are low (comparable to endogenous) and that the tagged subunits incorporate into functional complexes (30).

We pulled down GFP-VPS16, GFP-VPS11, or GFP-VPS33B and detected the associated proteins by Western blotting with antibodies against the different epitope tags (Fig. 1B). GFP-VPS16 specifically interacted with VPS41, VPS18, and VPS33A. In addition, GFP-VPS11 interacted with VPS39. In contrast to the endogenous co-IP (Fig. 1A), no interactions between the two groups (VPS16/41/18/33a and VPS11/39) were observed. A pulldown experiment with GFP-VPS11 also failed to detect interactions between GFP-VPS11 and endogenous VPS16/VPS33A (data not shown). This may reflect a limitation of the overexpression system or an interference with normal interactions by the associated tags. In line with the endogenous IP (Fig. 1A), we again observed strong interactions between VPS33B and VIPAS39 but not with any other subunit of the HOPS complex. This suggests that mammalian HOPS consist of VPS11/VPS16/VPS18/VPS33A/VPS39/VPS41, whereas VPS33B-VIPAS39 assembles in a distinct complex (Fig. 1C). Furthermore, the observation that tagged subunits recapitulate in large part endogenous interactions indicates we can use these constructs for further detailed mapping of the respective complexes.

**A High Detail Interaction Map of the Mammalian HOPS Complex**—The yeast HOPS complex resembles a seahorse-shaped complex with a head (Vps16/Vps33/Vps41/Vps18) and a tail (Vps11/Vps39), and interactions within the complex have been extensively mapped (6, 7) (Fig. 2A). No structure for the mammalian HOPS complex is available, yet our experiments...
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Figure 1. Biochemical definition of mammalian CORVET, HOPS, and VIPAS39-VIPAS39. A, MelJuSo lysates were immunoprecipitated (IP) by the indicated antibodies and analyzed by WB using indicated antibodies. Each panel represents an independent experiment. Upper left panel, MelJuSo cell lysates were generated (at which point a total lysate (TL) fraction was taken) and divided over five fractions, and an immunoprecipitation was performed on each lysate using one of the indicated antibodies (generating five experimental conditions, horizontal axis). The five experimental conditions were run on the same blot with the same exposures for each detection antibody. A separate gel was run for each detection antibody (on the same blot with the same exposures for each detection antibody). A check mark indicates interaction detected. ND = not detected; empty boxes, not tested. B, lysates of MelJuSo cells expressing eight tagged (experimental condition (exp)) or seven tagged subunits (control condition (ctrl)) not-expressing the GFP-tagged subunit were immunoprecipitated (IP) with anti-GFP (to pull down VPS16, VPS11, or VIPAS39) and analyzed by WB. Shown are total lysates for experimental and control lanes and the immunoprecipitated fraction for experimental and control lanes. C, Summary of experimental data A-E, VIPAS39 and VIPAS39 do not interact with HOPS complex subunits.
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VET-HOPS complex would be VPS11, as we already identified interactions between VPS11 and VPS39 (Fig. 1). In line with our endogenous IP with VPS11 antibodies, an interaction between TGFBRAP1 and GFP-VPS11 was observed (Fig. 3C), and co-expression of VPS11 and TGFBRAP1 resulted in the recruitment of cytosolic GFP-VPS11 to EE (Fig. 3D). To map the interactions between TGFBRAP1 and VPS39 with VPS11, we expressed tagged truncation mutants

FIGURE 2. Interactions within the mammalian HOPS complex. A, structure of the yeast HOPS complex. Yeast HOPS interacts with Ypt-7 via Vps41 and the N terminus of Vps39. B, domain organization of the mammalian HOPS complex subunit orthologs. CLH, clathrin heavy chain repeat; CC, coiled coil; R, ring finger; V11C, PFAM VPS11 C terminus; V16N, PFAM VPS11 N terminus; V16C, PFAM VPS11 C terminus; V39_1, VPS39 domain 1; V39_2, VPS39 domain 2; CNH, citron homology. C, lysates of MelJuSo cells co-expressing tagged VPS constructs as indicated were immunoprecipitated (IP) with anti-GFP antibodies and analyzed by WB using anti-GFP and anti-TGFBRAP1 antibodies. Experimental conditions were run on the same blot with the same exposures for each detection antibody, and cutouts were taken and grouped for presentation purposes. TL, total lysate. D, MelJuSo cells expressing mRFP-VPS11 (blue) or co-expressing mRFP-VPS11 (blue) and GFP-TGFBRAP1 (green) were fixed, stained with antibodies against EEA1 (red), and imaged by CLSM. Scale bars: 10 μm. Graphs show average correlation coefficient (CC) ± S.E n > 25, with EEA1 and VPS11 ± ectopically expressed TGFBRAP1 ***, p ≤ 0.0001).
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of VPS11 and assessed putative interactions (Fig. 4, A and B). TGFBRAP1 and VPS39 bound to the same domain in VPS11 (Fig. 4, A and B), implying that these two proteins might compete for VPS11 binding (Fig. 4C). As TGFBRAP1 binds VPS11 and VPS11 binds RILP, we argued that a RILP-VPS11-TGFBRAP1 interaction could exist that could induce (erroneous) recruitment of TGFBRAP1 to LE. However, when VPS11 was co-expressed with TGFBRAP1 and RILP, VPS11 was exclusively recruited to EE and no longer recruited to LE (even though high levels of RILP were present) (Fig. 4D). This suggests a regulatory mechanism in which binding of TGFBRAP1 to VPS11 in the CORVET complex prevents VPS11 binding to RILP, thereby blocking the targeting of VPS11 to LE and premature assembly of the HOPS complex on LE. Such a mechanism would safeguard a sequential assembly of the CORVET and HOPS and thereby time the fusion events of EE and LE.

ARC Mutations Specifically Disrupt VPS33B-VIPAS39 Interactions or Complex Recruitment to Late Endosomes by RILP—Published data regarding the role of VPS33B and VIPAS39 as members of the HOPS complex are seemingly conflicting (12, 16). Our endogenous IP data (Fig. 1A) as well as our IPs with tagged subunits (Fig. 1B) indicated that VPS33B and VIPAS39 did not interact with other HOPS subunits. To further substantiate the existence of a separate VPS33B-VIPAS39 complex, we depleted CORVET/HOPS subunits by siRNA and assessed the effects on the stability of their endogenous interaction partners by Western blot (WB) (Fig. 5A). Silencing of VPS16 significantly decreased VPS33A and VPS11 protein levels, whereas VPS33B and VIPAS39 levels did not decrease (Fig. 5A). Similarly, silencing of VPS33B compromised VIPAS39 levels without affecting VPS16, VPS33A, or VPS11 (Fig. 5A). These findings reinforce the notion that VPS33B and VIPAS39 assemble into a separate complex.

Interestingly, autosomal recessive mutations in VPS33B or VIPAS39 cause ARC syndrome (23, 33), a fatal multisystem disorder characterized by defects in apical transport in polarized cells. In line with this, VPS33B and VIPAS39 have previously been shown to function at RAB11 positive recycling endosomes (21, 22). Under steady-state conditions VPS33B and VIPAR do not significantly localize to LE (22), but we have shown that these proteins can be recruited to LE by RILP (30). In line with a function of VPS33B and VIPAR at the LE, it was recently shown that VPS33B is important for the maturation of some vesicles (21, 22). Under steady-state conditions VPS33B and VIPAS39 have previously been shown to function at RAB11 positive recycling endosomes (21, 22). Under steady-state conditions VPS33B and VIPAR do not significantly localize to LE (22), but we have shown that these proteins can be recruited to LE by RILP (30).

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The L30P mutation in VPS33B had a different effect; although VPS33B L30P still interacted with VIPAS39 (Fig. 5B), it failed to be recruited to LE by RILP (Fig. 5C; Ref. 30). Thus, our results indicate that two specific mutations in VPS33B, as found in ARC patients, affect assembly with VIPAS39 (VPS33B 1–437) or LE recruitment of the VPS33B-VIPAS39 complex (VPS33B L30P) by RILP (Fig. 5D).

Discussion

We have investigated the interactions between the proposed mammalian subunits of the multisubunit CORVET and HOPS tethering complexes and found, with modifications, that the organization of these complexes is largely conserved from yeast to mammals (Fig. 6A). As in yeast, the mammalian HOPS complex consists of VPS16, VPS11, VPS33A, VPS18, VPS41, and VPS39. We confirm that TGFBRAP1 and VPS8 are the mammalian CORVET-specific subunits (with TGFBRAP1 as the yeast vps3 ortholog) (11, 35). In contrast to previous data (16), however, our data indicate that VIPAS39 and VPS33B, two CORVET/HOPS subunit homologues not present in yeast, are not part of CORVET/HOPS as also suggested by others (12, 21, 36). We cannot formally exclude the occurrence of transient

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interactions of VPS33B and/or VIPAS39 with CORVET/HOPS or the existence of VPS33B-VIPAS39-HOPS interactions in other cellular systems. However, the existence of a VPS33/ VIPAS39 complex independent of other core subunits might explain why only mutations in VPS33B and VIPAS39 but not any of other HOPS subunits are associated to ARC syndrome (21–23, 33) and why all HOPS complex subunits, but not VPS33B and VIPAS39, are required for Ebola-virus infection (19). Conservation of these separate complexes is not apparent in all organisms. For example, in Caenorhabditis elegans the two VPS33 homologues (VPS33.1 and VPS33.2) are present in HOPS (VPS33.1) and CORVET (VPS33.2) (37). It is unclear, however, whether VPS33.1 and VPS33.2 are direct orthologous for mammalian VPS33A and VPS33B or reflect other evolutionary divergence. Similar to mammals, Drosophila VPS33A (Carnation) and VPS33B have distinct functions (38) and Drosophila VPS16 interacts with VPS33A, whereas Drosophila VIPAS39 interacts with VPS33B (39). However, Drosophila VIPAS39 also interacted with VPS18, suggesting that VIPAS39-VPS33B in Drosophila can interact with CORVET/HOPS core subunits (39). In mice, mutations in VPS33A yielded hypopigmentation and mild platelet deficiency but did not cause ARC, indicating that VPS33A and VPS33B functions do not completely overlap in mice (40). This suggests that VPS33B-VIPAS39 interactions might have gradually diverted from that of CORVET/HOPS during mammalian evolution. Yet, functions of VIPAS39 seem conserved between different organisms. For example, the observation that Drosophila lacking VIPAS39 (dVPS16) has a profound defect in phagosomal acidification is in line with our observation that VIPAS39 functions at LE compartments (30). Here we report that ARC-syndrome mutations in VPS33B differentially affect the binding of VPS33B to VIPAS39 or membrane recruitment of VPS33B to RILP. This consolidates the finding that VPS33B-VIPAS39 func-

FIGURE 6. Model of membrane binding specificity of the CORVET and HOPS complex and their conversion during maturation. A, model of the mammalian HOPS complex superimposed on the yeast structure, depicting RILP-binding domains. Asterisks indicate poorly conserved regions in the N terminus of VPS39 and loss of the lipid binding motif of VPS41, which have altered membrane targeting in the mammalian complex. CLH, clathrin heavy chain repeat; CC, coiled coil; R, ring finger; CNH, citron homology. B, the CORVET complex binds to RAB5 on EE and within this complex, TGFBRAP1 binding to VPS11 prevent association of VPS11 to RILP (1). 2, to allow RAB7-RILP binding, TGFBRAP1 is replaced by VPS39, possibly controlled by CCZ1/MON1 and TGFβ signaling. Replacement of VPS8 with VPS41 adds an additional RILP binding motif, completing the conversion of CORVET to HOPS complex from RAB5- to RAB7- during endosomal maturation (3). Both the head and tail of mammalian HOPS can bind the homodimer RAB7-RILP. There may be two conditions; an inactive conformation where HOPS binds back to RILP on the same vesicle failing to contact a fusion partner and an active conformation in which RILP-HOPS contacts other vesicles (LE, phagosomes or autophagosomes containing HOPS interactors such as Arl8b, PLEKHM1, or RILP) for tethering and subsequent fusion.
tion at LEs (34) as well as recycling endosomes (16, 21, 22) and that both membrane binding and VIPAS39 binding are needed for correct VPS33B function.

We show that HOPS subunits have evolved to accommodate binding to the RAB7 effector RILP (for a mammalian model super-imposed on the yeast structure see Fig. 3G). The N terminus of yeast vps39 that binds Ypt7 is poorly conserved in the mammalian VPS39 ortholog, which might explain why the mammalian HOPS complex has shifted to bind RAB7 effectors such as RILP (30, 32) and PLEKH1 (14, 15). The HOPS complex binds RILP on either side of the complex (VPS39/VPS11 in the tail and VPS41/VPS18 in the head) and may bridge RILP molecules on opposing vesicles. Because VPS33A in the head of HOPS also binds to SNAREs (41), a connection between two vesicles will then support tethering and subsequent fusion. It is interesting to note that the HOPS complex can also bind the GTPase Arl8b on lysosomes (20, 42) and PLEKH1 on LE, phagosomes, and autophagosomes (14, 15), possibly tethering these vesicles with RILP-containing organelles (Fig. 6B). RAB7-RILP concomitantly binds the HOPS complex and the dynein-motor complex for retrograde transport (30), whereas the Arl8b effector SKIP also concomitantly regulates HOPS binding and anterograde transport by kinesin (42), indicating that timing of vesicle tethering is likely coupled to their transport.

We found that binding of TGFBRAP1 to VPS11 prevents the interaction of VPS11 with RILP and erroneous recruitment of CORVET complexes to RILP-bearing LE. This might provide an important regulatory step in the conversion of CORVET to HOPS complexes (Fig. 5) where TGFBRAP1 in CORVET would have to release VPS11, thereby allowing VPS11 to bind VPS39 and recruit the entire HOPS complex to RAB7-RILP during RAB5 (EEs) to RAB7 (LE) conversion. Specific targeting of tethering complexes by modulation of targeting subunits was recently also shown for GARP and EARP tethering on Golgi and EEs, respectively, indicating this might be a common mechanism in the regulation of vesicular fusion (43). TGFBRAP1 to VPS39 exchange might further be a common mechanism in the regulation of vesicular GARP and EARP tethering on Golgi and EEs, respectively, indicating that this complex has a function at the LE. Through mapping of the CORVET and HOPS complexes we found that within the shared CORVET/HOPS core, VPS11 is a molecular switch that, depending on its interacting proteins (TGFBRAP1 or VPS39), determines targeting of CORVET and HOPS to EE and LE.

In conclusion, we describe the molecular architecture of the mammalian CORVET, HOPS, and VIPAS39-VPS33B complexes. We find that different ARC syndrome mutations affect VIPAS39-VPS33B or VPS33B-RILP interactions, indicating that this complex has a function at the LE. Through mapping of the CORVET and HOPS complexes we found that within the shared CORVET/HOPS core, VPS11 is a molecular switch that, depending on its interacting proteins (TGFBRAP1 or VPS39), determines targeting of CORVET and HOPS to EE and LE.

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