The CHEVI tethering complex: facilitating special deliveries

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Abstract:

VPS33B and VIPAR comprise the two known components of the recently christened class C Homologues in Endosome-Vesicle Interaction (CHEVI) complex, thought to act as a tethering complex in endosomal trafficking distinct from the HOPS and CORVET complexes in mammalian cells. In this issue Dai et al further explore the role of the CHEVI complex in the biogenesis of α-granules in megakaryocytes; identifying two novel interactors of this complex: α-TUBULIN and SEC22B and demonstrating that VPS33B expression is required for the localisation of SEC22B and the α-granule cargo VWF to proplatelets in megakaryocytes. These findings advance the current knowledge of the function of the CHEVI complex in α-granule biogenesis and together with studies in other systems corroborates its role in the specialised delivery of cargo in different cell types.

Keywords:

VPS33B, VIPAR, vesicle trafficking, tethering complexes, megakaryocyte, platelet, α-granules, CHEVI complex

The vacuolar protein sorting (vps) protein VPS33B forms a complex with VIPAR, also known as VIPAS39 [1]. Based on homology studies these proteins are thought to act in membrane tethering, mediating interactions between intracellular compartments and regulating vesicular fusion. Spang, 2016 [2] proposed that the VPS33B-VIPAR complex should be referred to as the class C Homologues in Endosome-Vesicle Interaction (CHEVI) complex, and the intracellular function of CHEVI is starting to become clearer following the recent insights into the pathology resulting from VPS33B or VIPAR deficiencies.

VPS33B is one of the two (A and B) Vps33p homologues in metazoans. In yeast Vps33p is a class c vps protein required for protein trafficking to the yeast vacuole [3] as part of class c core vacuole/endosome tethering (CORVET) or homotypic fusion and vacuole protein sorting (HOPS) multiprotein complexes [2]. Vps33p and its homologues are members of the Sec1/Munc18 protein family which regulate vesicular membrane targeting and fusion through interactions with soluble NSF attachment protein receptors, SNAREs [4]. VIPAR the 'vps33b-interacting protein involved in polarity and apical protein restriction' is weakly homologous to Vps16p, another component of the yeast CORVET and HOPS complexes [1] and contains a golgin A5 domain. Members of the golgin family are typically coiled-coil membrane proteins and have been implicated in formation of Golgi cisternae and in retrograde intra-Golgi transport through their action as membrane tethers [5].

The presence of two Vps33p homologues in metazoans suggested that each of the homologues; VPS33A and VPS33B, could impart further specificity to the endosomal trafficking system. Work in model organisms implicated VPS33A as part of the

HOPS complex [6,7] and this was borne out by structural work [8]. In mammalian cells VPS33B and VIPAR were lately shown to not be required for endosome fusion with lysosomes or autophagosomes as part of the HOPS complex [9], supporting the conclusion that VPS33A and not VPS33B is part of the HOPS complex.

It was subsequently postulated that VPS33B could be the Vps33p homologue component of the CORVET complex and indeed work in the model organism *C. elegans* implicated the worm homologue of VPS33B, VPS-33.2, as part of the CORVET complex with the VPS33A homologue, VPS-33.1, part of the HOPS complex [10]. However, work in mammalian systems indicate VPS33A and not VPS33B acts with the CORVET complex in early endosome fusion in mammalian cells [11]. Structural analysis also demonstrated that VIPAR and VPS33B interact with each other, but not VPS33A and VPS16 respectively, and mutations in VPS33A that make the sequence resemble VPS33B abrogate the interaction with VPS16, suggesting VPS33B could not also interact with VPS16 as part of the HOPS or CORVET complexes due to structural restrictions [8]. In addition, the most recent paper on the components of the mammalian HOPS complex [9] did not detect any interaction between VPS33B or VIPAR with VPS18. This strongly indicates that the VPS33B-VIPAR complex cannot form part of the mammalian HOPS or CORVET complexes and seem to form a distinct tethering complex, regulating vesicular transport independent of the HOPS and CORVET endosomal trafficking.

VPS33B and VIPAR deficiencies in various model systems have shown cell specific phenotypes and further underlined the divergence from the phenotypes of HOPS and CORVET complex components deficiencies. To date VPS33B and VIPAR have

been implicated in apical protein localization, phagosome-lysosome and HOPS complex independent endosome-lysosome fusion, lysosome-related organelle biogenesis and integrin internalization [1,12–15].

In this issue Dai et al, 2016 describe studies of the role of the CHEVI complex in α-granule biogenesis, a process that is still poorly characterised. Bem et al. 2015 showed that ubiquitous VPS33B deficiency leads to abnormal platelet biogenesis with reduced formation of the megakaryocyte and platelet specific lysosome related organelle, the α-granule [13], mirroring abnormalities seen in VPS33B deficient patients [4]. Subsequently it was shown that the function of VPS33B is extremely complex even within megakaryocytes, as a megakaryocyte specific *PF4-Cre* driven deletion of VPS33B did not lead to the aforementioned α-granule phenotype but did cause defective integrin internalisation [15]. In agreement with this literature, Dai et al, 2016 have shown that while ubiquitous VPS33B deletion in the mouse embryo is lethal, the hematopoietic stem cell specific VPS33B deficiency in mice leads to a platelet α-granule biogenesis defect. They also suggest that presence of α-granules in megakaryocyte specific *PF4-Cre* driven VPS33B deletion, can be attributed to the incomplete removal of VPS33B expression in the megakaryocytes of these mice.

There have been few publications identifying other potential components of the CHEVI complex, which Dai et al, 2016 seek to address. To date VPS33B and VIPAR have been shown to interact with the small GTPases Rab11a [1], Rab10 and Rab25 [16], integrin subunits [15] and a Rab7 effector, RILP [17]. Dai et al, 2016 identify further interactors for the CHEVI complex; α-TUBULIN and SEC22B, and show that they each interact with one of VPS33B's Sec1-like domains. Interactions

with the cytoskeletal protein α-TUBULIN may indicate the method of transport of CHEVI targeted vesicles across the cell, similarly to other tethering complexes which have been shown to interact with cytoskeletal components [18]. They subsequently demonstrate that VPS33B is essential for the trafficking of SEC22B and VWF to megakaryocyte proplatelets, placing CHEVI into the trans-Golgi network (TGN) to α-granule trafficking pathway. SEC22B, in addition to its previously known roles in ER-Golgi transport, has been implicated in the regulation of endosomal fusion in dendritic cells [19] and therefore could be required for endosomal fusion in other cells [12,14, Figure 1]. The SEC22B-CHEVI interaction may impart specificity to vesicles delivered to endosomal compartments that develop into α-granules. Little overlap between the proteins obtained from the pull downs of the CHEVI complex components [15,16,20] may suggest that the interactions of CHEVI complex besides VPS33B-VIPAR are transient or cell type specific.

VPS33B and VIPAR mutations cause the severe multisystem disorder Arthrogryposis Renal dysfunction and Cholestasis (ARC) syndrome with a wide number of symptoms that go beyond the α-granule biogenesis defect [21]. Defects present in the polarised cell types of the kidney, liver, skin and inner ear. However, no specific phenotypes due to VPS33B or VIPAR deficiencies have been described in polarised systems such as intestinal or pulmonary epithelial cells. In ARC patients and murine ARC models VPS33B deficiency can cause incorrect targeting of apical membrane proteins which require transit through Rab11a positive endosomes for their localisation [1,21]. Recently we have also shown that the CHEVI complex is essential for the delivery of a collagen modifying enzyme LH3 from the TGN to intracellular collagen IV containing compartments, CIVCs, in mouse kidney cells [16].

In VPS33B or VIPAR deficient cells LH3 accumulated in the TGN, suggesting a functional block at the exit of LH3 from the TGN (Figure 1) possibly due to a proximal build-up of LH3 in the pathway. Additionally the dry skin phenotype of ARC patients has been shown to be due to abnormally formed lamellar bodies (Figure 1) in the epidermis of patients [22]. Lamellar bodies, which are essential for skin barrier function, are also lipid-filled lysosome related organelles postulated to be formed at the TGN requiring Rab11a for their biogenesis [23]. These studies suggest probable functions for CHEVI in delivery of cargo from the TGN and also for trafficking at Rab11a endosomes. These likely roles ensure protein delivery to diverse organelles, corroborated by the variety of cell specific phenotypes seen in VPS33B and VIPAR deficiencies (Figure 1).

Thus Dai et al, 2016 further illustrate the role of CHEVI in the α-granule biogenesis pathway and indicate its interaction with SEC22B. The phenotype of VPS33B and VIPAR deficient cells indicate that they are likely to act as part of an as yet uncharacterised tethering complex, CHEVI, distinct from the HOPS and CORVET complexes. Further work is required to elucidate the mechanism of CHEVI function in deliveries of cargo to endosomes in diverse cell types.

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Author contributions:

CR and PG both reviewed the literature and wrote this commentary.

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Figure 1: The CHEVI complex in delivery of cargoes in several cell types.

LEFT In megakaryocytes the CHEVI complex is required for correct formation of α-granules and the transport of α-granule cargo to proplatelet extensions. In this issue Dai et al, 2016 also demonstrate interactions with α-TUBULIN and SEC22B. Together with the CHEVI complex these proteins may be important for delivery of α-granule contents to the endosomal compartments acting as precursors of α-granules, from these compartments to the α-granules themselves or for the transport of α-granules into the proplatelet extensions.

CENTRE Granular epidermal skin cells produce lamellar bodies essential for skin barrier function and in these cells the CHEVI complex is required for correct lamellar body biogenesis and/or secretion. The CHEVI complex interactor Rab11a has also been shown to be required for lamellar body biogenesis. It could be suggested that Rab11a together with the CHEVI complex is required for the formation of lamellar bodies from the TGN, transport of cargo from other vesicles to the lamellar bodies, or fusion of lamellar bodies with the plasma membrane at the interface between the granular layer and the cornified layer of the epidermis.

RIGHT In mouse kidney collecting duct cells the CHEVI complex is known to be essential for correct delivery of a cargo, LH3, to CIVCs from the TGN. Initially the activity of Rab10 is required for exit from the Golgi before the activity of Rab25 is subsequently required before LH3 delivery to CIVCs.