Abstract

Vesicular trafficking pathways in a eukaryotic cell are mediated by small GTPases of Rab, Arf and Arf-like (Arl) families and their effectors/interaction partners including tethering factors, motor proteins and SNAREs. HOmotypic fusion and Protein Sorting (HOPS) complex is a multi-subunit tethering complex conserved from yeast to mammals that regulates endocytic trafficking to vacuoles/lysosomes. The six subunits are namely Vacuole Protein Sorting (Vps) 11, Vps16, Vps18 and Vps33 subunits that form the core complex, while Vps39 and Vps41 act as the accessory subunits. Previously, a lysosomal small GTPase of the Arl family, Arl8b was shown to directly bind and recruit the human (h)Vps41 subunit to the lysosomes. Here by using GST-pull down and purified proteins, we have shown that this interaction takes place through the N-terminal WD40 domain of hVps41 and this domain is both essential and sufficient for this interaction. Further, a previously-reported single nucleotide polymorphism (T146P) within this domain disrupts the binding to Arl8b and prevents association of Vps41 with lysosomes. These results also explain how this SNP leads to loss-of-function of Vps41 in mediating delivery of cargo and their degradation in lysosomes. Further as part of another study, I have also explored the interaction of late endosomal and lysosomal protein, Pleckstrin homology domain-containing family M member 1 (PLEKHM1), with Vps39 subunit of the HOPS complex. PLEKHM1 was previously identified as an interaction partner for late endosomal small GTPase Rab7. PLEKHM1 and Vps39 colocalize on lysosomes and this interaction is dependent upon the second Pleckstrin Homology (PH) domain of PLEKHM1. These findings will be crucial in exploring how small GTPases and their effectors collaborate to mediate vesicular trafficking towards lysosomes.