Title:	Characterizing the interaction of a novel autophagy regulatory protein with multisubunit tethering factor HOPS complex
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Abstract:	Eukaryotic cells maintain constant communication among the organelles by uptaking cargos from extracellular and intracellular spaces and sorting them to their correct functional location. External cargos like growth factors and intracellular cargos such as misfolded proteins and damaged organelles are delivered to lysosomes by endocytic and autophagic pathways, respectively. Key molecular players such as the small GTPases and tethering factors regulate these cargo trafficking pathways. HOPS (HOmotypic fusion and vacuole Protein Sorting) complex is a multisubunit tethering factor that mediates the fusion of autophagosomes and late endosomes with lysosomes. This hexameric multisubunit complex consists of subunits including Vps11, Vps16, Vps18, Vps33a, Vps39, and Vps41 that are conserved across evolution from yeast to mammals. A previous report indicates that a protein belonging to the TECPR family of proteins, TECPR2 (Tectonin beta- propeller repeat containing 2), interacts with HOPS complex. We confirmed TECPR2 interaction with multiple HOPS subunits by yeast-two-hybrid assay. Next, we have constructed the domain-deletion mutants of TECPR2 and set up yeast two-hybrid assays with HOPS subunits. We found that the C-terminal TECPR domains of the protein are important for binding with HOPS subunits. Furthermore, confocal microscopy imaging of overexpressed TECPR2 showed mostly cytosolic distribution of the protein with few punctae colocalizing with lysosomal protein LAMP1. Future work is required to decipher the minimal region required for TECPR2 interaction with the HOPS complex.
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