Vps29: a Phosphoesterase Fold that acts as an Interaction Scaffold in the Assembly of Retromer

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Membrane sorting between secretory and endocytic organelles is predominantly controlled by small carrier vesicles or tubules that are layered on their cytoplasmic faces by specific protein coats. Recently we have begun studies of a novel putative membrane coat complex termed retromer. Retromer contains five subunits, Vps35, Vps26, Vps29, Snx1 and Snx2 and is responsible for tubule-based retrieval of proteins from the endosomal system to the Golgi, for example recycling mannose-6-phosphate receptors that traffic lysosomal hydrolases from the TGN to endosomes. We have determined the crystal structure of the mammalian retromer subunit Vps29, showing that it has structural similarity to divalent metal-containing phosphoesterases. However, although Vps29 can coordinate metals in a similar manner it has no detectable phosphatase activity *in vitro*, suggesting a novel specificity or function. We show that Vps29 and Vps26 bind directly to distinct regions of Vps35 and together form a high affinity heterotrimeric sub-complex. Mutagenesis reveals the structural basis for interaction of Vps29 with Vps35 and subsequent membrane association of Vps29 *in vivo*. Furthermore, we demonstrate that a conserved hydrophobic surface distinct from the primary Vps35 binding site can mediate assembly of the Vps29p-Vps26p-Vps35p sub-complex with sorting nexins in yeast, and mutation of either site results in a defect in retromer-dependant membrane trafficking.

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