Tracking N- and C-termini of *C. elegans* polycystin-1 reveals their distinct targeting requirements and functions in cilia and EVs

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Introduction: Cilia are cellular organelles for both receiving signals and broadcasting signals, the latter via ciliary extracellular vesicles (EVs). In *C. elegans* and mammals, the Autosomal Dominant Polycystic Kidney Disease (ADPKD) gene products polycystin-1 (PC1) and polycystin-2 (PC2) localize to cilia and EVs, act in the same pathway, and function in a sensory capacity, suggesting ancient conservation. The functions of the polycystins on cilia and EVs remain enigmatic**.** In EVs, the *C. elegans* polycystins may function non-autonomously to signal between other animals.

Methods: We used our *C. elegans* model and CRISPR/Cas9 endogenously fluorescent-tagged LOV-1/polycystin-1 to study LOV-1 processing, trafficking, transport, EV biogenesis, and function in living animals. Microscopy was used to analyze subcellular localization and interactions of N- and C-terminal tagged LOV-1 reporters with polycystin-2 PKD-2 in living animals. We then conducted functional assays to correlate ciliary presence of LOV-1 with ciliary function.

Results: Super resolution, real time imaging reveals that LOV-1 is processed into N-terminal and C-terminal forms via a conserved GPCR proteolytic site (GPS). The LOV-1 NTM is secreted into the extracellular matrix and not localized to ciliary tip EVs. In contrast, LOV-1 CTM and PKD-2 are co-trafficked, co-transported, and co-localized in cilia and on environmentally released ciliary EVs. LOV-1 CTM requires PKD-2 for ciliary EV localization, while PKD-2 localizes to ciliary EVs independent of LOV-1. We find that LOV-1 but not PKD-2 is required for chemosensation of an ascaroside mating pheromone.

Conclusions: These data indicate that the polycystins LOV-1 and PKD-2 function together and independently and provide insight to how cargo selected and packaged in ciliary EVs. Our findings will lead to better understanding of the *in vivo* functions of the polycystins in cilia and ciliary EVs, which may inform understanding of the human polycystins in normal and ADPKD states.

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