Impact of the RAB-28 GTPase on the Biogenesis and Uptake of Extracellular Vesicles

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Abstract

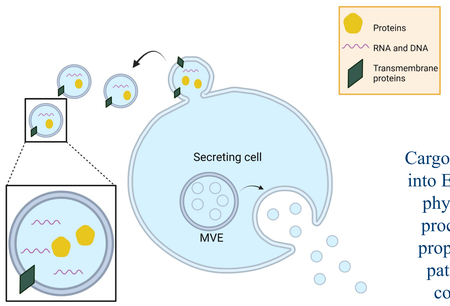
Extracellular vesicles (EVs) are bioactive molecular signaling structures enclosed by a lipid bilayer that mediate intercellular communication by transporting proteins, RNA, lipids, and metabolites between cells. Although EVs are known to play critical roles in both normal physiology and disease, the mechanisms controlling their biogenesis and release remain incompletely understood. In *Caenorhabditis elegans*, sensory neurons within the male tail shed two distinct subpopulations of EVs from separate compartments of the cilia: the distal tip, which releases EVs containing the TRP channel PKD-2, and the ciliary base, which releases EVs containing the ion channel CLHM-1. Interestingly, previous findings suggest that the small GTPase RAB-28 may differentially regulate these EV subpopulations.

A 2019 study by Akella et al. reported that a *rab-28* mutation caused accumulation of EVs between the cilium and surrounding glia, suggesting a potential role in promoting EV shedding. However, subsequent observations in a different *rab-28(gk1040)* allele revealed a reduction in CLHM-1-containing EVs, with no effect on PKD-2 EVs. This unexpected effect raises questions about the precise role of RAB-28 in regulating distinct EV subpopulations.

To address this, I performed a genetic cross between the *rab-28(tm2636)* mutant and strains expressing CLHM-1::GFP and MKS-2::mScarlet, with *him-5* to facilitate male enrichment. My goal was to generate a strain that will enable high-resolution imaging and quantification of CLHM-1 EV release. Over the fall, I was able to complete and verify the successful cross through genotyping via gel electrophoresis and screening of transgenes. This winter, I have begun to gather images to analyze in order to visualize the distribution of our cargoes of interest within the primary cilium of the male tails.

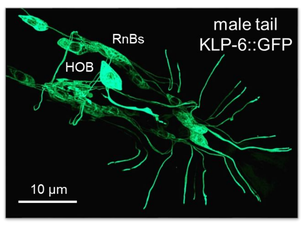
These experiments aim to clarify whether RAB-28 plays a role in promoting or restricting EV release, and whether this function is context-dependent based on the subcellular origin of the vesicles. A better understanding of this regulatory mechanism could shed light on conserved pathways that govern EV-mediated signaling across species.

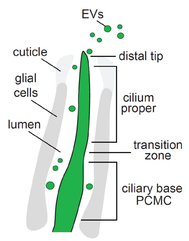
Extracellular vesicles (EVs) are a mode of intercellular communication



Cargoes packaged into EVs regulate physiological processes and propagation of pathological conditions

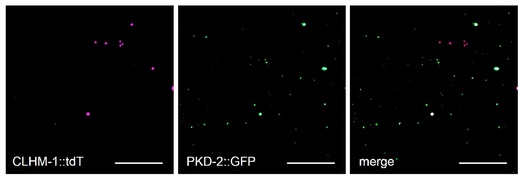
Extracellular vesicles are released from cilia of *C. elegans* sensory neurons





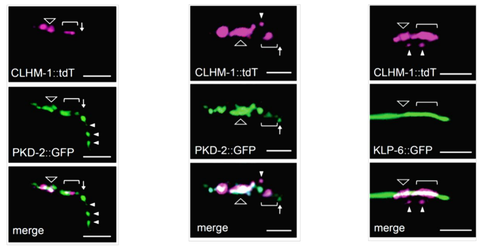
In *C. elegans*, ectosomes are shed from primary cilia of IL2, CEM, RnB, and HOB neurons, then released through a pore in the cuticle and into the environment

CLHM-1 and PKD-2 EV cargoes are enriched in distinct EV subpopulations



The CLHM-1 ion channel and PKD-2, ortholog of TRPP channel PKD2L1, are cargoes in EVs. These two cargoes co-localize in sensory cilia, but are enriched in distinct EV subpopulations

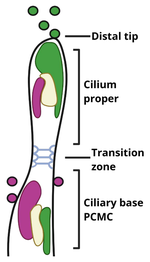
CLHM-1 and PKD-2 EVs are shed from different locations



PKD-2::GFP EVs shed from the distal tip

EV with CLHM-1, not PKD-2

CLHM-1 EVs adjacent to a cilium



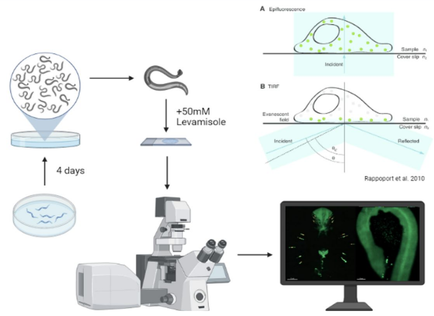
ciliary base — cilium proper — distal tip

PKD-2 EVs are primarily shed from the distal tip, while CLHM-1 EVs are shed from the PCMC of the ciliary base

What regulates EV biogenesis and cargo sorting?

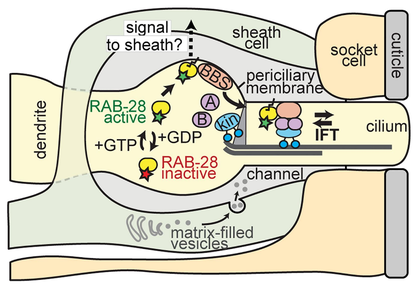
scale, 2 µm

Imaging background



We utilize total internal reflection microscopy (TIRF) to image the EVs released into the environment and spinning disk confocal microscopy to image within the cilia of the male tail.

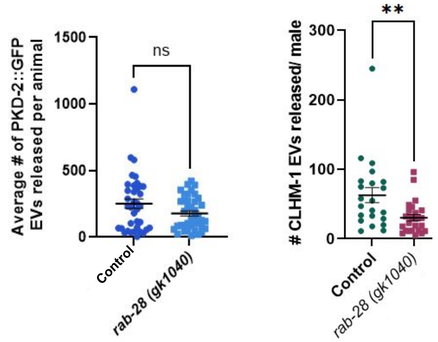
RAB-28 GTPase background



Jensen et al. 2016

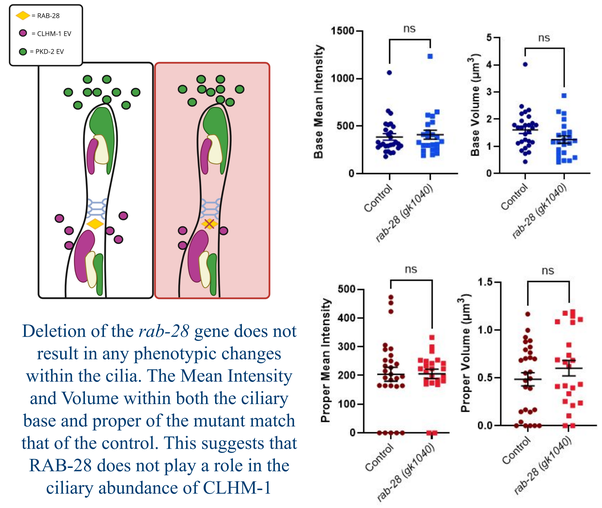
RAB-GTPases function as molecular switches; when Rab-28 is bound to GTP it is activated, and associates with the downstream effector, BBS, which then goes on to associate with intracellular transport machinery.

Δrab-28 differentially regulates EV subpopulations



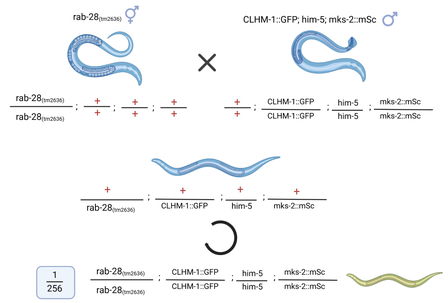
Deletion of the *rab-28* gene results in decreased CLHM-1 release into the environment, while release of PKD-2 remains unaffected, suggesting RAB-28 functions as a differential regulator of distinct EV subpopulations.

CLHM-1 ciliary abundance is not impacted by Δ*rab-28*

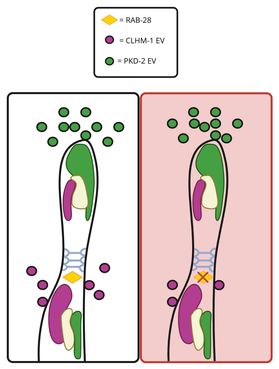


Deletion of the *rab-28* gene does not result in any phenotypic changes within the cilia. The Mean Intensity and Volume within both the ciliary base and proper of the mutant match that of the control. This suggests that RAB-28 does not play a role in the ciliary abundance of CLHM-1

Four point genetic cross



Conclusions/Future Directions

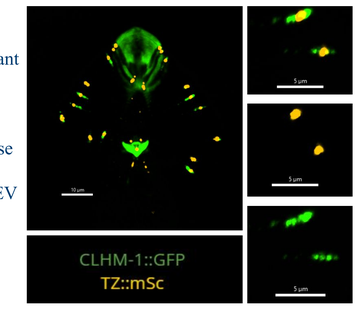


Deletion of the *rab-28* gene:

- Does **not** result in any phenotypic changes within the cilia. The Mean Intensity and Volume within both the ciliary base and proper of the mutant match that of the control.
  - This suggests that RAB-28 does not play a role in the ciliary abundance of CLHM-1
- Results in decreased CLHM-1 release into the environment, while release of PKD-2 remains unaffected
  - Suggesting RAB-28 functions as a differential regulator of distinct EV subpopulations.

Future Directions

- Image the *rab-28* hypomorph cross that I completed
  - Analyze the images for ciliary abundance of the EV subpopulations
  - Analyze the images for EV release of both cargoes of interest
- Create a strain with a different mutant for the same gene.



Acknowledgements

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