MOL231: CRISPR/Cas9 induced TRP knockouts in *Salpingoeca rosetta*

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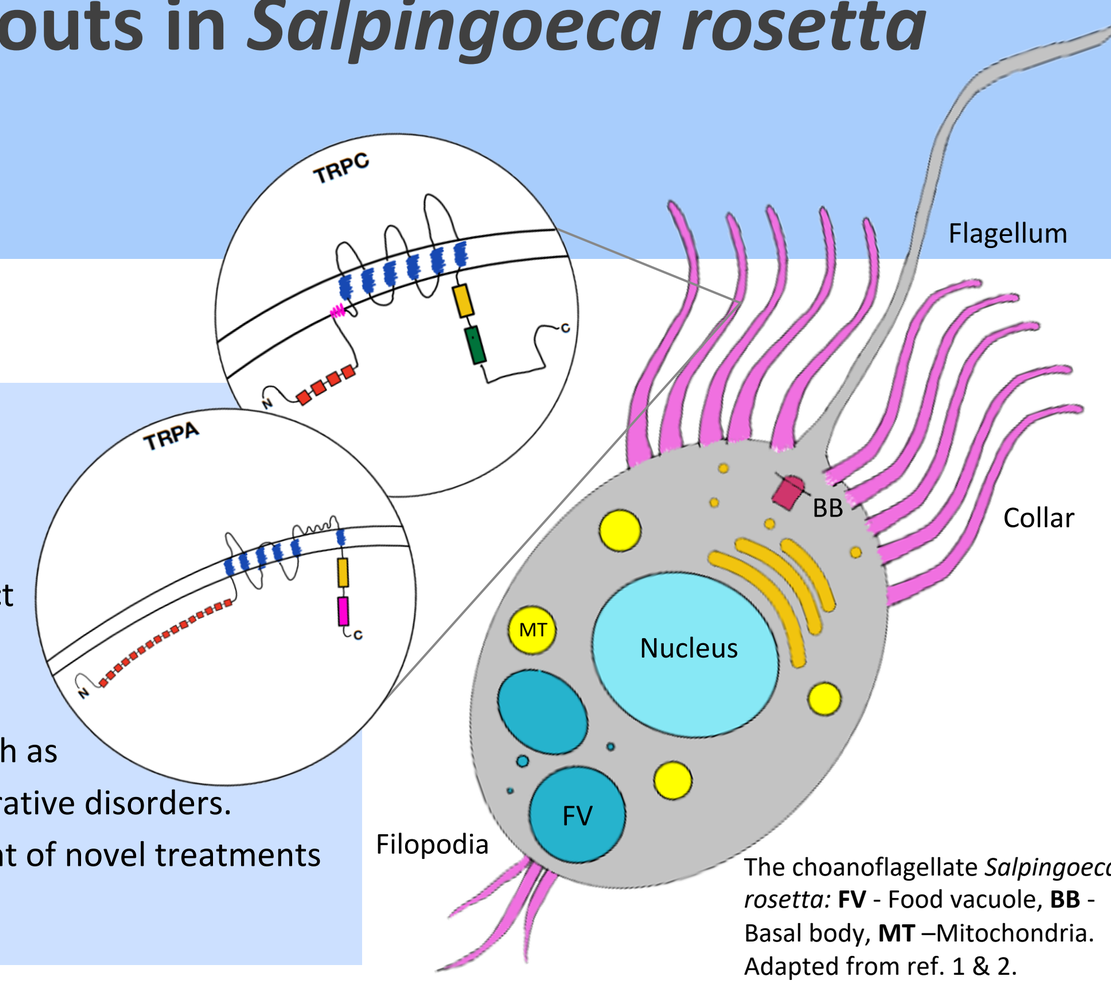
"To understand how animals evolved, we must study choanoflagellates."
Peter Holland

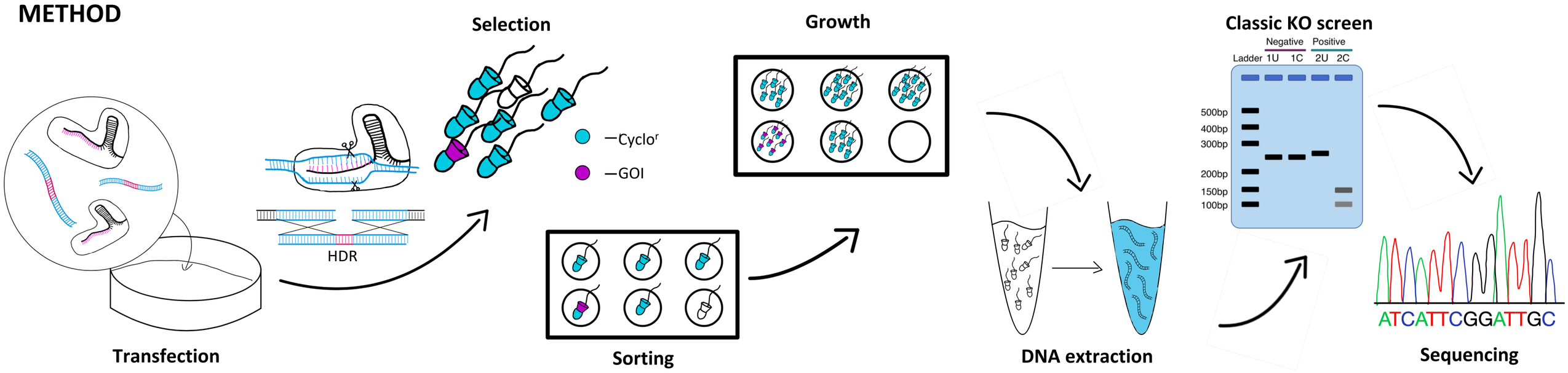
BACKGROUND

Choanoflagellates are the closest living unicellular relative to animals, and possess many genes previously assumed exclusive to metazoans. They may hold information pertaining to **animal multicellularity**. **Sensory neurons** are crucial for animal's ability to navigate and interact efficiently within their environment. In humans, sensory systems are spearheaded by **transient receptor potential** (TRP) channels. Activation of these calcium ion channels triggers transduction of signal to the central nervous system, inducing **somatosensory experiences**, such as pressure, pain, and temperature sensation. TRP channels are linked to cancer and neurodegenerative disorders. Studying their function in the **Choanoflagellate** could provide information aiding in development of novel treatments for disease, and help understand the evolutionary origin of animal sensory systems.

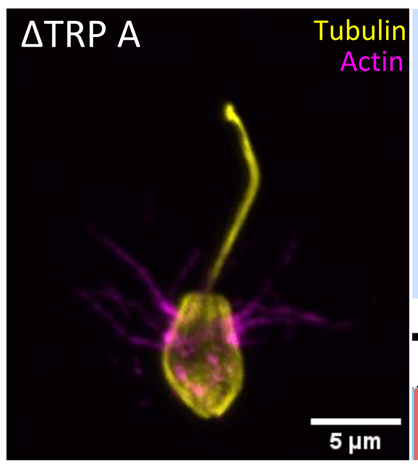


The choanoflagellate *Salpingoeca rosetta:* **FV** - Food vacuole, **BB** - Basal body, **MT** –Mitochondria. Adapted from ref. 1 & 2.

METHOD

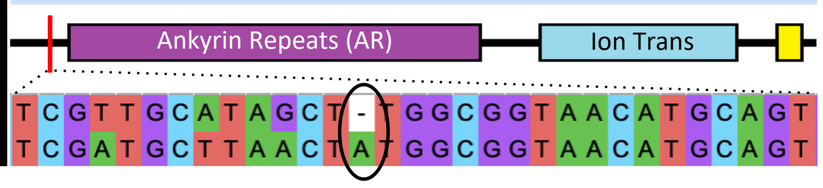


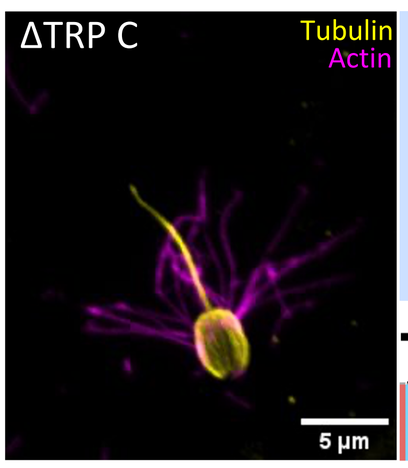
RESULTS



Left - Immunostaining of TRP A mutant. Tubulin - yellow, Actin - magenta. Immunostaining of structural proteins. Morphology is unaltered when compared to wild type.

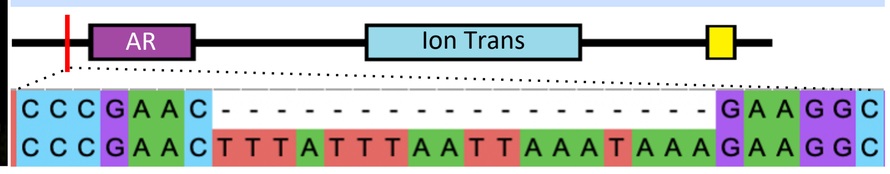
Bottom - Basic protein domain structure, and sequence alignment of TRP A mutant and wild type. Bottom - Mutant sequence; Showing insertion of single nucleotide, causing downstream frameshift.



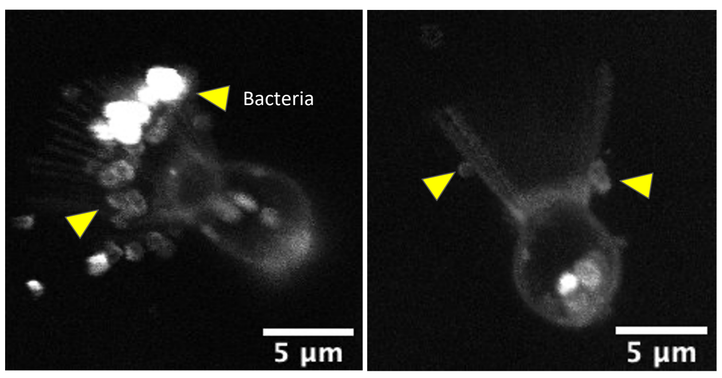


Left - Immunostaining of TRP C mutant Tubulin - yellow, Actin - magenta. Immunostaining of structural proteins. Morphology is unaltered when compared to wild type.

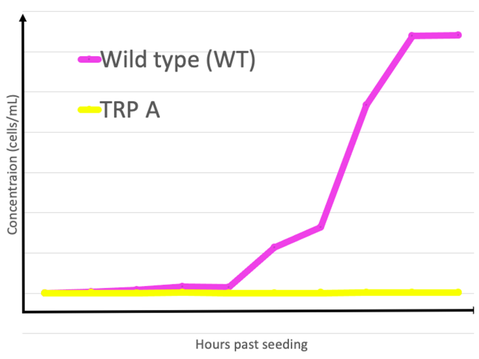
Bottom - Basic protein domain structure and, sequence alignment of TRP C mutant and wild type
Bottom - Mutant sequence; Homology directed repair induced insertion of stop cassette in mutant sequence.



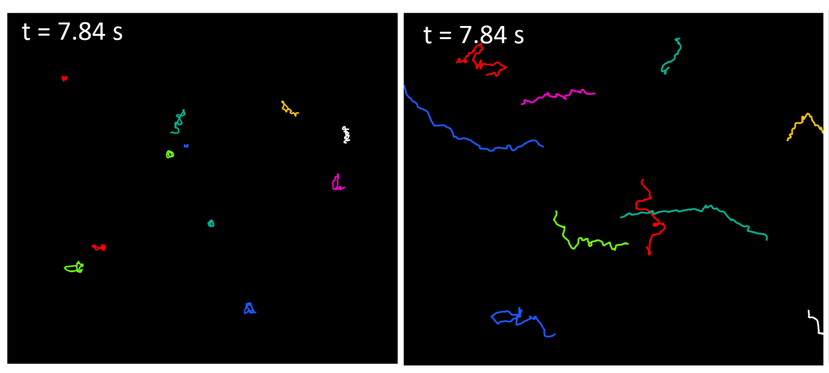
Bacteria in collar



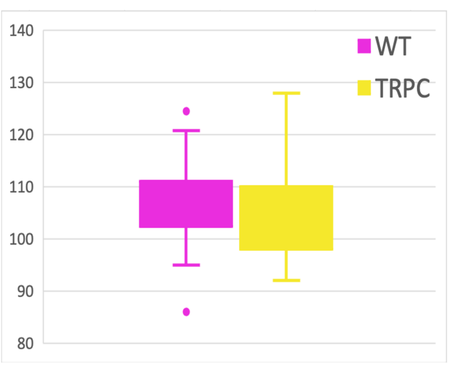
Growth rate



Swim speed



Collar angles



ΔTRP A
Large bacterial clumps litter the collar.

Wild Type
Bright spots in cell body show consumed bacteria.

Growth curve
Culture concentration measured over 6 days, twice a day.

ΔTRP C
Greatly reduced travel distance for mutant.

Wild Type
Tracking of distance travelled during time interval.

Collar morphology
Collar angles were measured for WT and mutant to look for discrepancies.

Discussion

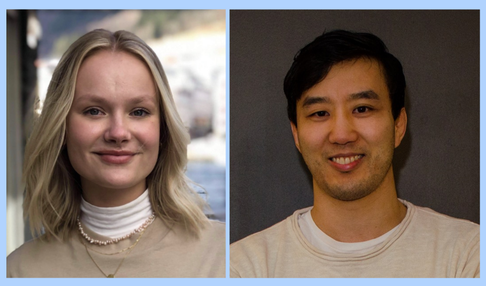
TRP A - Severely impaired growth rate.
- Difficulty feeding?

TRP C - Impaired swim speed.
- Discrepancies in collar morphology?

Choanoflagellates are important models to understand origin of animal sensation.

Future work

- Tagging endogenous TRP A channels to map protein location.
- Calcium imaging to visualize ion flow through the channels.
- Observe feeding during controlled bacterial concentrations.



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References:
1. Himmel, Nathaniel J., Cox, Daniel N.. *Transient receptor potential channels: current perspectives on evolution, structure, function and nomenclature.* 2020;
2. Hoffmeyer, Tarja T., Burkhardt, Pawel. *Choanoflagellate models — Monosiga brevicollis and Salpingoeca rosetta.* 2016;
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