Radial displacement assay distinguishes ALA from RIS mutants during recovery from heat stress in *Caenorhabditis elegans*

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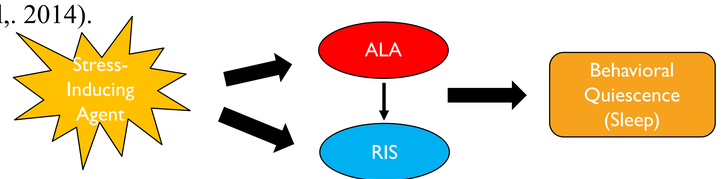
Introduction

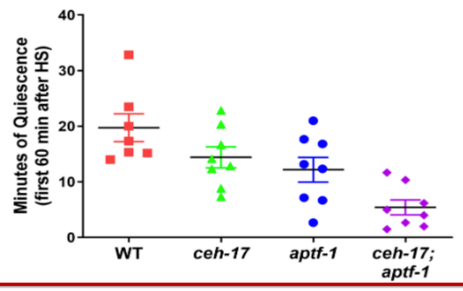
- *C. elegans* offers a powerful genetic system to understand the mechanism of sickness-induced sleep (SIS).
- Sickness-inducing events, such as viral infection or high temperatures, activate the ALA and RIS neurons, which release their respective neuropeptides to cause quiescence/sleep (Hill et al,. 2014).



- Mutants are defective in sleep when sick
  - *ceh-17* mutants are defective in ALA
  - *aptf-1* mutants are defective in RIS
- These two strains exhibit different behavior after being stressed. *ceh-17* have full-body motion while *aptf-1* can only move their noses.

Sleep defects in *aptf-1* and *ceh-17* mutants

- Current methods (WorMotels) cannot readily distinguish between *ceh-17* and *aptf-1* mutants (Raizen, unpublished observations).
- The strains have similar amounts of quiescence when quantified with a WorMotel.

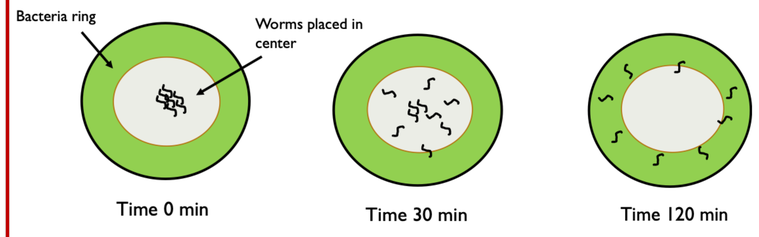


Radial Displacement Assay

- We developed a radial dispersion assay that could potentially distinguish between ALA and RIS-deficient mutants.
- The worms were placed unto the center of a plate with food on its circumference and exposed to a high temperatures (heat shock).
- After heat shock, sleep-less mutants that make full body bends will more effectively move across the agar surface to reach the food.
- Mutants that move only their noses and wild-type worms will lag behind.

Methods

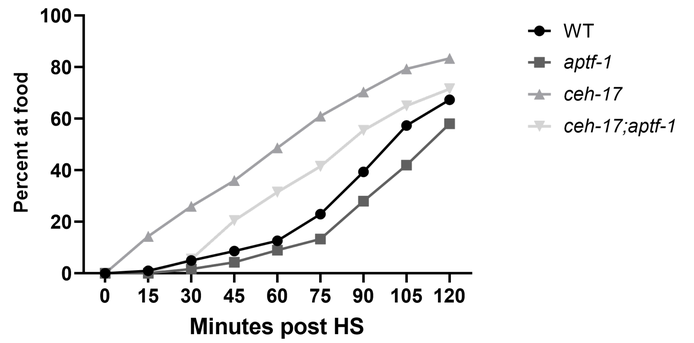
- 10.0cm-diameter agar plates were seeded with a 1.0cm-wide *E.coli* (DA837) ring around the periphery.
- Approximately 100 adult worms of each strain were pipetted unto the center of seeded agar plates.
- The plates were submerged into a circulating water bath at 35° C for 30 minutes to induce a heat shock.
- Once out of the water, the plates were observed every 15 minutes for two hours.
- As a control, approximately 100 adult worms of each strain were also plated without being heat shocked.
- The worms that had reached the food ring were tallied and removed using a Pasteur pipette connected to vacuum suction.



Conclusion

- The radial dispersion assay distinguishes between *ceh-17* and *aptf-1* mutants.
- *ceh-17* mutants' ability to make full-body bends allows them to reach the bacterial ring significantly faster than N2 (WT) and *aptf-1*.

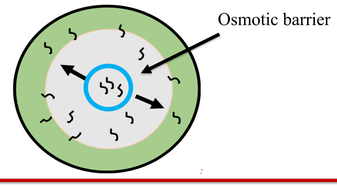
Results



- *aptf-1* lags behind N2 (wild type) and *ceh-17* in reaching the food lawn.
- *ceh-17* is about twice as fast as N2 to reach the food.

Discussion

- The different phenotypes exhibited by *ceh-17* and *aptf-1* could be used to identify other ALA and RIS-defective mutants respectively and classify known sleep mutants.
- This assay could potentially screen for movement quiescent-defective mutants after random mutagenesis and amplify weak phenotypes.
- In addition, we can expand this design to measure motivation of worms to reach the food after heat shock by adding an osmotic barrier between the worms and the food.



Acknowledgments & References

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- Contact Carlos Chavez Perez by cchave03@sas.upenn.edu

Hill AJ, Mansfield R, Lopez JM, Raizen DM, Van Buskirk C. Cellular stress induces a protective sleep-like state in C. elegans. *Curr Biol*. 2014;24(20):2399-2405. doi:10.1016/j.cub.2014.08.040