# Finding genes required for sickness sleep in *C. elegans*

Hina Sako (COL 2026), Kerry M. Lecure, David M. Raizen
Department of Neurology, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, 19104

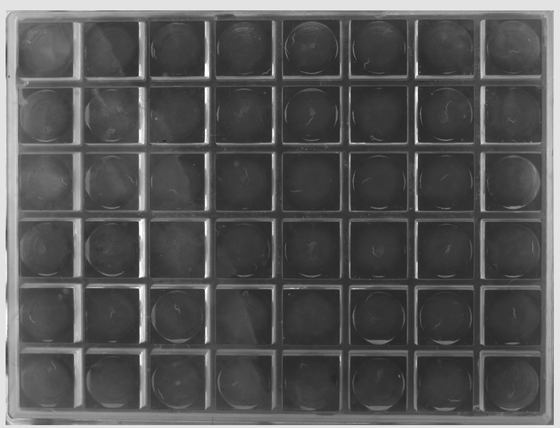
PURM Penn Undergraduate Research Mentoring Program

## Background

- *C . elegans* decrease movement and feeding when exposed to environmental stressor. This is called sickness induced sleep, or SIS. This behavior is seen in all animals is relevant to understanding human fatigue during illness.
- The Mullion Mutation Project (MMP) is a collection of 2007 strains of *C. elegans* mutagenized with UV/TMP, EMS, ENU, or EMS+ENU (Thompson et al. 2013).
- The MMP strain collection contains a mutation in almost every gene of the *C. elegans* genome.
- Given a few strains from the MMP collection, I found that the transcription factor, APTF-1 is required for sickness sleep in *c. elegans.*

## Methods

- A **WorMotel** contains 48 wells, each containing one worm.
- L4 worms are picked the day before and assays are performed on day one adults.
- Stressed worms are shocked with 1500 $J/m^2$ in a UVC crosslinker for 17 seconds.
- Unstressed controls are added to their wells. Images are captured every 10 seconds for 4 hours.



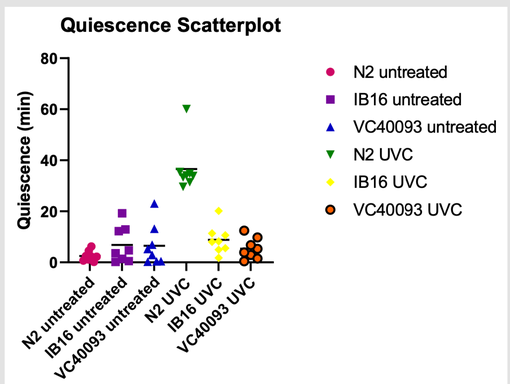
## UV-induced feeding quiescence results

- Wild type worms, N2, exhibit feeding quiescence after UV, which can be seen by the lack of pumping of their pharynx.
- IB16 has a known pumping defect due to a mutation in a gene called *ceh-17.*
- Day 1 adult worms were transferred onto an unseeded plate and stressed with 1500 $J/m^2$ in a UVC crosslinker.
- Assessed worms after 2 hours.

| Strain | Fraction pumping >10 ppm |
|---|---|
| N2 (wild type) | 2/20 |
| IB16 (*ceh-17* mutant) | 10/15 |
| VC40093 (MMP strain) | 4/21 |

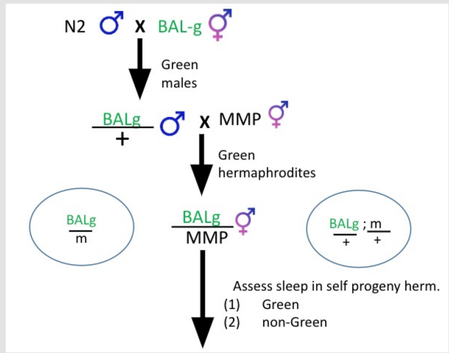
## UV-indued movement quiescence results

- Worms exhibit movement quiescence (SIS) after UV exposure, as seen in the wild type.
- The strain IB16, has a mutation in *ceh-17*, which was already known to have a defect in SIS. This strain was used as a positive control.
- My strain of interest, VC40093 had a defect in SIS, similar to IB16.

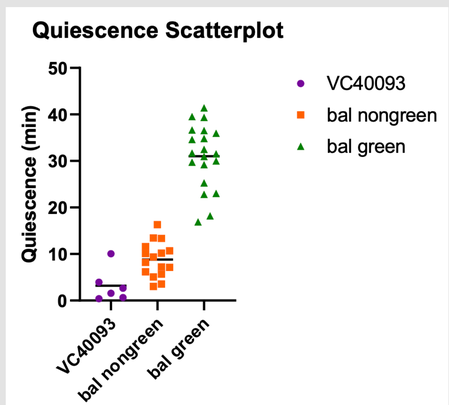



## Balancer mapping to find candidate genes

- Balancer strains are marked with Green Fluorescent Protein and prevent recombination. They are used to determine which chromosome contains a gene of interest.
- My strain of focus, VC40093, has a missense mutation in *aptf-1* where a glutamic acid is changed to a lysine.

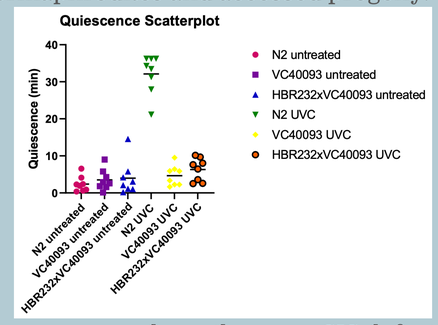



- If the phenotype is linked to the balancer, non-green worms will be 100% SIS defective and greens 0%
- If the phenotype is not linked, 25% of both green and nongreen worms will be SIS defective.
- The results show that the phenotype is linked to the balancer and that the mutation is on chromosome II.




## Complementation testing

- If a phenotype is seen when two recessive mutations that have been mapped to the same area, are combined in trans, it can be concluded that they are alleles of the same gene (Yook, 2005).
- Crossed HBR233 males with VC40093 hermaphrodites and accessed progeny.




- Cross progeny have the same SIS defect as VC40093. It can be concluded that the two mutations fail to complement and are alleles of the same gene.

## APTF-1

- *aptf-1* is a highly conserved AP2 transcription factor that is required for a sleep active neuron, RIS, to induce quiescence in *c. elegans* (Turek , et al. 2013).
- A mutation in *aptf-1* causes a defective in movement quiescence, but not pumping quiescence.

| Species/Abbrv | * | | * | * | | * | | | * | | | * |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. aptf-1 (c. elegans) | E | E | E | A | I | H | M | A | K | E | F | A | L | V |
| 2. TfAP-2-PD (D. melanogaster) | E | G | E | A | T | H | L | A | K | D | F | H | F | V |
| 3. ENSEMBL (H. sapiens) | E | G | E | A | V | H | L | A | R | D | F | A | Y | V |

↑ VC40093 has a mutation in amino acid 237 on protein *aptf-1.*

- VC40093 has a missense mutation on chromosome II: *aptf-1.*
- APTF-1 has highly conserved orthologs including drosophila (TfAP-2) and humans (ENSEMBL)

## Acknowledgements

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