May 2nd, 12:00 AM

# Times of Action and Evolutionary Conservation of Heterochronic Genes

Maria Ivanova
*Rowan University*

Eric G. Moss
*Rowan University*

Rowan University

GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

# Times of action and evolutionary conservation of heterochronic genes.

Maria Ivanova, Eric G. Moss
Department of Cell and Molecular Biology, Rowan University School of Osteopathic Medicine

## Background

Heterochronic genes control the timing and sequence of developmental events during larval stages of *C. elegans*. Mutations in heterochronic genes can cause skipping or reiteration of cell fates associated with certain larval stages (Ls).

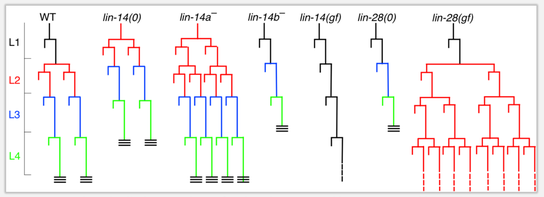



*Figure 1. Hypodermal cells divisions during four larval stages in wild-type worms and heterochronic mutants. WT – wild-type, 0 – null allele, gf – gain-of-function, a', b', – loss-of-function.*

*lin-14* and *lin-28* are two well-studied heterochronic genes.

LIN-14 acts during the first larval stage and controls the events of stages L1 and L2, LIN-28 acts during the second larval stage and controls its events.

First studies of the times of action were carried out using LIN-14 temperature-sensitive mutants. They revealed that it has *two separate* activities not overlapping in time and that the activity of this protein on the L1 stage defines cell fates of the L2 stage.

Nobody studied times of action for other heterochronic genes.

## Aim 1

To implement the degron-tagging for studying the times of action of LIN-14 and LIN-28.

## Results

The worms carrying LIN-14 fused with degron were healthy on plates without auxin and had *lin-14*(0) phenotype on plates with auxin. Closer examination of worms showed that apparently wild-type worms have 19 seam cells as long as true wild-type worms have 16 seam cells.

Observations of seam cell divisions during L1-L2 stages revealed that at least some of the anterior end seam cells undergo divisions characteristic to L2 during L1.

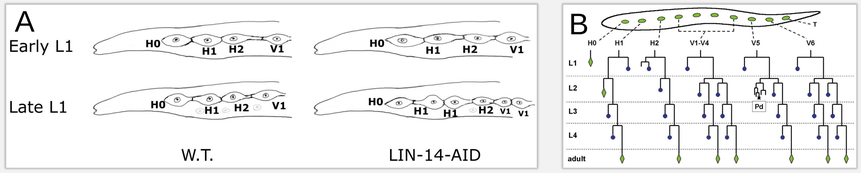



*Figure 5. (A) Anterior end seam cells in wild-type worms and worms carrying LIN-14-AID grown without auxin. V1 undergoes S2 divisions at L1 and then repeats them at L2. Apparently, H1 undergoes S2 divisions at L1 and then repeats them at L2 as well. That results in 19 seam cells in adult worms. (B) The scheme of wild-type seam cell divisions, based on Sulston and Horvitz (1977), interpreted by Šilhánková M., et al. (2004).*

Outcrossed worms that carry LIN-14-AID but do not express TIR1, the ubiquitin ligase required for the degradation, are completely wild-type. That suggests that differences arise because of leaky degradation and reduced levels of LIN-14. Interestingly, it influences only anterior end cells.

The results on deactivation of LIN-14 during L1 are consistent with those of Ambros and Horvitz (1987). The number of seam cells drops when LIN-14 is deactivated in the expected time frame of the *lin-14b* activity that was identified previously.

However, experiments did not work in the opposite direction. Worms that hatched at plates with auxin and started their development on plates without auxin developed *lin-14*(0) phenotype and the time of *lin-14a* activity could not be identified. Thus, experiments on re-activation require another approach.

Further plans include:

- removal of the *lin-14* regulatory region from 3' UTR
- testing of DHFR degron (an alternative to AID)
- application of AID in LIN-28 studies.

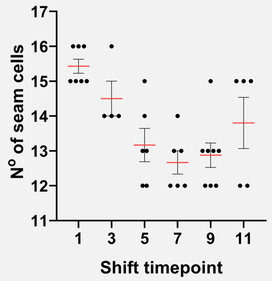



*Figure 6. Shifts from no auxin to auxin plates (LIN-14 deactivation) and the resulting number of seam cells in 50 hours old larvae. Each dot represents an observed worm.*

## Evolutionary conservation

Another important question is the conservation of roles of the heterochronic genes in the evolution. *lin-28* homologs were studied in the fruit fly, clawed frog, zebrafish, mouse, and human. *lin-28* is associated with the early developmental stages and undifferentiated cells and downregulated on the way to the differentiation.

LIN-28 in *C. elegans* and *C. briggsae* has an 89% identity with the accumulation of mutations within the first 80 amino acids.

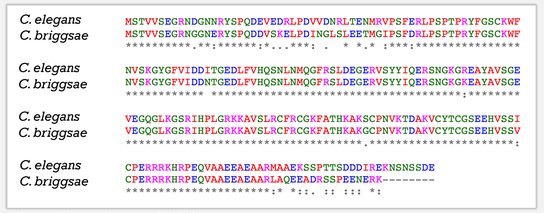



*Figure 7. Protein alignment of LIN-28 sequences.*

Our preliminary data show that *C. briggsae* with the loss of *lin-28* function expresses a distinct phenotype than the same mutant of *C. elegans*. This suggests that *lin-28* may play different roles in the evolutionary close species.

## Aim 2

To characterize the *C. briggsae lin-28* mutant

## Materials and Methods

I use the auxin-inducible degradation (AID) system derived from *Arabidopsis thaliana* that allows for the targeted degradation of proteins.

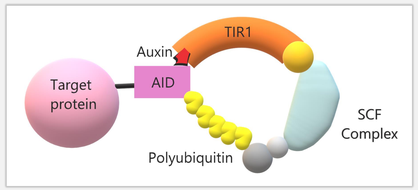



*Figure 2. Auxin-inducible degradation. TIR1 binds to SCF ubiquitin ligase complex. Auxin binds to TIR1, then it starts to recognize degron tag (AID) and ubiquitin ligase attaches multiple ubiquitins to it and thus direct the protein to the proteasomal degradation.*

To attach the degron to proteins of interest I use CRISPR/Cas9 system with a repair template that has single-stranded ends and double-stranded middle.

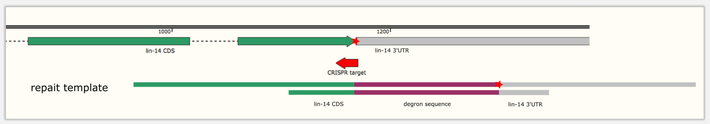



*Figure 3. Strategy for attaching the degron to the LIN-14 protein. I synthetized in vitro the gRNA targeted to a site at lin-14 3' end (indicated by orange arrow). After cutting, some DNA breaks are repaired by recombination with homologous regions (shown in pink) on the repair template that contains degron coding sequence.*

After obtaining the line of worms with the insertion of interest, I carried out experiments on the deactivation of LIN-14 at different time points during the development.

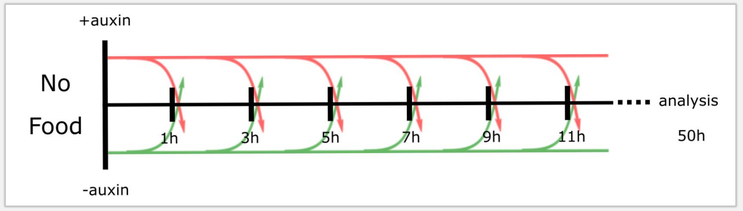



*Figure 4. Experimental design for lin-14 deactivation. Larvae were synchronized by hatching at plates without food. Then transferred at plates with food and with or without auxin and subsets of worms were transferred at the opposite type of plate at certain time points.*

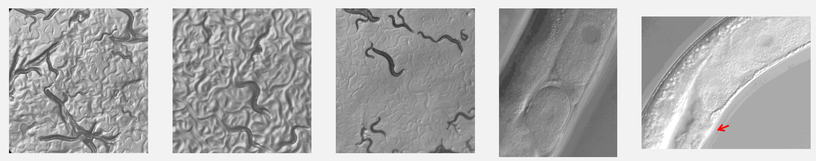
Then I count the number of seam cells 50 hours after the start when worms were at L4 stage to identify if they developed the heterochronic phenotype.

## Preliminary results

*C. briggsae lin-28* loss-of-function mutant displays:

- Slower movements
- Internal vacuolization
- Reduced pumping rate
- Molting defects
- Egg-laying defects

} Not observed in *C. elegans*. Resembles premature aging.



*C. briggsae, lin-28(-)* | *C. briggsae, wild type* | *C. elegans, lin-28(-)* | *C. briggsae, wild type, oocytes* | *C. briggsae, lin-28(-), oocytes, vulva*

Future plans

Analyze for the presence of aging markers:

- Sarcomere deterioration and reduced muscle tone
- Lipofuscin accumulation and lipid peroxidation
- Mitochondrial network disruption

Analyze for the presence of heterochronic phenotype markers:

- Lower number of seam cells
- Precocious adult alae