Caenorhabditis heterochronic genes: conservation and divergence of developmental roles

Maria Ivanova and Eric G. Moss
Department of Molecular Biology, Rowan University, Graduate School of Biomedical Sciences, Stratford, NJ
ivanovam2@rowan.edu

Rowan University
GRADUATE SCHOOL OF BIOMEDICAL SCIENCES

Background

Heterochronic genes control the timing of developmental events during four *C. elegans* larval stages. They encode for proteins and miRNA that control them. Mutations in heterochronic genes can cause skipping or reiteration of cell fates associated with certain larval stages (L1 through L4).

lin-28 and *lin-46* are two well-studied heterochronic genes.

lin-28 acts early in larval development and controls what happens in many tissues during the L2 stage: if it is missing or down-regulated too soon, L2-specific events are skipped. If it is not down-regulated on time, L2-specific events are repeated. This seems to be the sole function of *lin-28* in *C. elegans*.

lin-46 works at the same time and controls L2 events in the opposite way: When *lin-46* is missing, L2-specific events are sometimes repeated. Interestingly, a *lin-28; lin-46* double null mutant, where both genes are inactive, develops nearly perfectly normally.

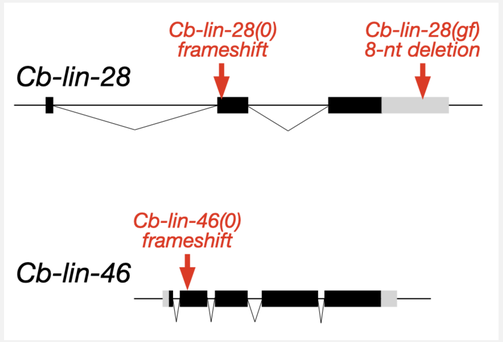
An important question is the conservation of roles of the heterochronic genes in the evolution. *lin-28* homologs were studied in the fruit fly, clawed frog, zebrafish, mouse, and human. *lin-28* is associated with the early developmental stages and undifferentiated cells and downregulated on the way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

Aim

To create mutations in *lin-28* in *lin-46* of *C. briggsae* and determine (1) whether they produce the same phenotypes as in *C. elegans*, and (2) determine whether they genetically suppress each other, as in *C. elegans*

Mutant alleles



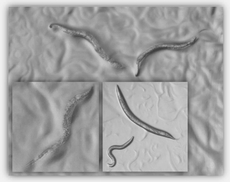
Several mutant alleles were created by injecting worms with Cas9 protein and in-vitro transcribed guide RNAs. Two frameshift alleles of *Cb-lin-28* which are likely nulls. One 3'UTR deletion of *Cb-lin-28* was obtained which likely affects a miRNA binding site, causing a gain-of-function (gf) allele. Two frameshift mutation of *Cb-lin-46* were obtained, which are also likely nulls.

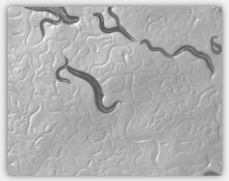
Cb-lin-28(0)

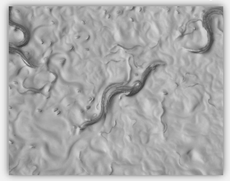
C. briggsae lin-28(0) mutant displays traits that are **not** observed in *C. elegans lin-28(0)*:

- Slower movements, including pumping rate
- "Disorganized" internal structure, vacuoles
- Molting defects
- Swollen excretory canals in the anterior end
- Decreased reproduction
- Dauers appear in the presence of food and sometimes die without developing further

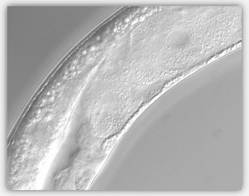
Additionally, dauer stage does not rescue the phenotype as it does in *C. elegans lin-28(0)*.

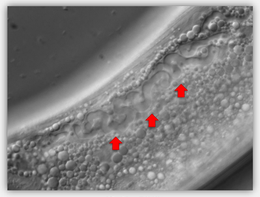

C. briggsae, lin-28(0)


C. elegans, lin-28(0)


C. briggsae, WT


Cb-lin-28(0),
"Disorganized" internal structure


Cb-lin-28(0),
Leaking oocytes and underdeveloped vulva


Cb-lin-28(0),
Swollen excretory canal

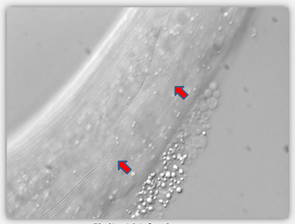
C. briggsae lin-28(0) has a variable expressivity of the phenotype. In an experiment started with 98 *Cb-lin-28(0)* stage 1 larvae, 2 worms developed as wild-type, 61 worms died in the first 3 days after reaching L4 stage and they had viable progeny, other worms survived past day 4 did not have viable embryos and were sick. The maximal lifespan of sick worms was 11 days. WT worms lived longer.

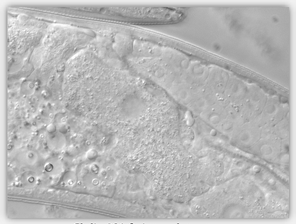
In contrast to *C. elegans lin-28(0), C. briggsae* do not have full precocious alae at L4 stage. Only 1-3 cells produce precocious alae.

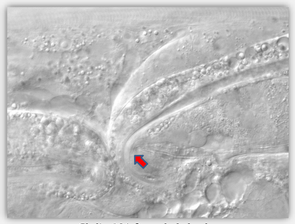
Cb-lin-28(gf)

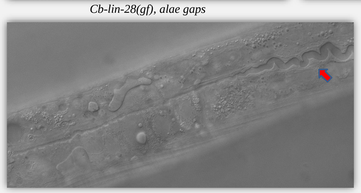
C. briggsae lin-28(gf) mutant has been obtained by targeting 3' UTR control region of the gene with CRISPR/Cas9. I obtained several lineages of worms expressing a peculiar phenotype that I was not able to maintain because of their severe defects.

Mutant worms have disorganized internal structure and 0-2 viable embryos. They also have full or partial alae that proves further that *lin-28* in *C. briggsae* does not play completely similar role in the cell fates control in comparison to *C. elegans*.


Cb-lin-28(gf), alae gaps


Cb-lin-28(gf), internal structure


Cb-lin-28(gf), exploded vulva


Cb-lin-28(gf), swollen excretory canal

Vulval explosion is a characteristic of *C. elegans lin-28(gf)* mutant. In *Cb-lin-28(gf)* this was observed only under the cover glass at microscopy.

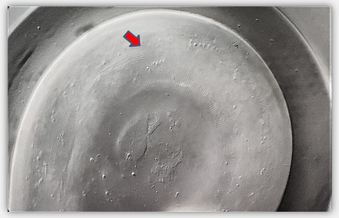
Cb-lin-46(0)

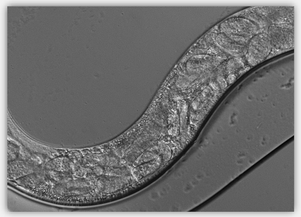
C. briggsae lin-46(0) mutants have:

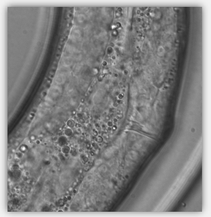
- Egg-laying defects
- Alae gaps in 75-85% of molting L4

Thus, the phenotype is retarded and expressed more than in *C. elegans* where only 10% of molting L4 have alae gaps and worms do not have egg-laying defects.

Cb-lin-46(0) mutants produce more eggs than *lin-28(0)* mutant.


Cb-lin-46(0), alae gap


Cb-lin-46(0), a worm filled with embryos


Cb-lin-46(0), vulval development

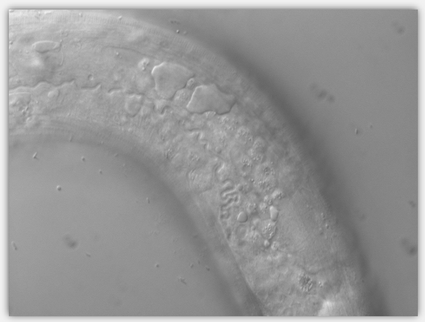
Cb-lin-28(0); Cb-lin-46(0)

An important question is whether the relationship between these two genes is conserved. Unlike *C. elegans*, The *C. briggsae* double mutant is still quite unhealthy. They are wider and shorter. In particular, animals sometimes have the disorganized internal structure like *Cb-lin-28(0)*. They also produce lower number of eggs and embryos in comparison to *Cb-lin-46(0)*.

Notably, double mutants do not have swollen excretory canals.

WT worms appear more often than in *lin-28(0)* mutant.


Cb-lin-28(0); Cb-lin-46(0), one egg


Cb-lin-28(0); Cb-lin-46(0), internal structure

Future aims

Create mutations in other *C. briggsae* heterochronic gene homologs:

- *Cb-lin-14 (loss- and gain-of-function)*
- *Cb-lin-4*
- *Cb-let-7*
- *Cb-hbl-1 (loss- and gain-of-function)*

Test other double-mutant combinations for suppression:

- *lin-4; lin-14(lf)*
- *lin-28(lf); let-7*
- *hbl-1(lf) let-7*