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Evolutionary Conservation of the Heterochronic Pathway in C. elegans and C. briggsae

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Background

Heterochronic genes control the sequence and timing of developmental events during four larval stages of *Caenorhabitis* nematodes. Mutations in these genes may cause skipping or reiteration of developmental events.

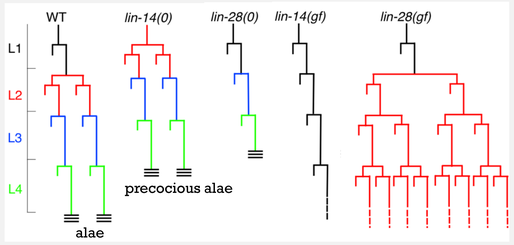

Figure 1. The patterns of seam cell divisions in WT and heterochronic mutants of *C. elegans*. Different colors show stage-specific divisions. Seam cells undergo symmetric divisions at L2 and their number doubles.

C. briggsae is a close relative of *C. elegans*. These species have similar morphology and share the same ecological niche. *C. briggsae* undergoes the same developmental pathway consisting of four larval stages before reaching the adulthood. It also has the same set of heterochronic genes.
lin-28 is one of the heterochronic genes that also exists in other animals from flies to humans. It conservatively blocks the maturation of *let-7* miRNA, the process is generally associated with the stem cell state. *lin-28* is silenced as cells differentiate.

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C. elegans   MSTVVSEGRNDGNNRYSPQDEVEDRLPDVVDNRLTENMRVPSFERLPSPTPRYFGSCKWF
C. briggsae  MSTVVSEGRNGGNERYSPQDDVSKELPDINGLSLEETMGIPSFDRLPSPTPRYFGSCKWF
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C. elegans   NVSKGYGFVIDDITGEDLFVHQSNLNMQGFRSLDEGERVSYYIQERSNGKGREAYAVSGE
C. briggsae  NVSKGYGFVIDDNTGEDLFVHQSNLNMQGFRSLDEGERVSYYIQERSNGKGKEAYAVSGE
             ********** ************************************:********

C. elegans   VEGQGLKGSRIHPLGRKKAVSLRCFRCGKFATHKAKSCPNVKTDAKVCYTCGSEEHVSSI
C. briggsae  VEGQGLKGSRIHPLGRKKAVSLRCFRCGKFATHKAKGCPNVKTDAKVCYTCGSEEHVSSV
             ************************************.**********************:

C. elegans   CPERRRKHRPEQVAAEEAEAARMAAEKSSPTTSDDDIREKNSNSSDE
C. briggsae  CPERRRKHRPEQVAAEEAEAARLAQEEADRSSPEENERK--------
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Figure 2. Protein alignment of LIN-28 sequences of *C. elegans* and *C. briggsae*.

C. elegans mutants of *lin-28* have a reduced number of seam cells and precocious alae. Despite the highly conserved protein sequence, *C. briggsae* develop a distinct phenotype when its *lin-28* is disrupted. Worms did not have a characteristic vulval development defect, they also became lethargic and had a reduced fertility.

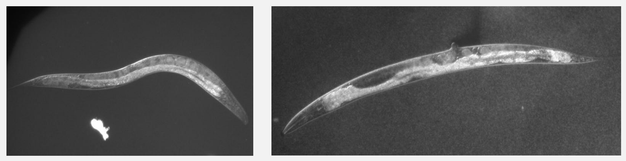

Figure 3. lin-28 loss-of-function mutants of *C. briggsae (left) and C. elegans (right).*

This observation led to a question of how conserved the heterochronic pathway is in close species.

Aims

- To determine conserved and changed parts of the heterochronic pathway of *C. briggsae* in comparison with *C. elegans*.
- To characterize heterochronic gene mutants of *C. briggsae*.

cbr-lin-28

I created *C. briggsae lin-28* mutant by targeting the second exon of the gene with CRISPR/Cas9 and obtained two frameshift alleles of the gene in addition to one that was created in the lab before. All of them had a disrupted cold-shock domain that provides the main function of the protein.

A close examination showed that in contrast to *C. elegans*:

- *cbr-lin-28* mutants have a wild-type number of seam cells.
- Might have fragments of precocious alae at L4 but some individuals lack it completely
- Enter L4 lethargus and arrest their development. Some individuals undergo partial molt, rarely worms undergo a complete molt and continue their development to adult stage.
  - "Arrested L4" have gonads and vulva similar to late L4 stage. They remain in this state indefinitely. Worms start to produce gametes at the same time as WT control. Though they form no to few eggs.
  - "Successfully molted" have gonads and vulva similar to adults.
- Have gonad development defects that reduce fertility, gonads may lose integrity and oocytes' content may spill outside the gonad. It does not differ between "arrested" and "successfully molted".
- Dauer stage does not suppress the phenotype.

cbr-lin-46

In *C. elegans*, *lin-46* acts downstream of *lin-28*. Mutations in this gene suppress the precocious developmental phenotype of *lin-28*(lf). When outcrossed, *lin-46*(lf) mutants have a slightly retarded phenotype with an increased number of seam cells and gaps in the adult alae.

I obtained three mutant alleles of *C. briggsae lin-46*.
C. briggsae had a more severe retarded phenotype in contrast to *C. elegans*. At 20°C, 75% of *cbr-lin-46* mutants had alae gaps vs. 35% in *C. elegans*. The number of seam cells was slightly increased. The phenotype was also temperature sensitive similarly to *C. elegans*. At 15°C, the number of seam cells was higher than at 20°C. *cbr-lin-46* also had vulval development and egg-laying defects that were not observed in *C. elegans*.

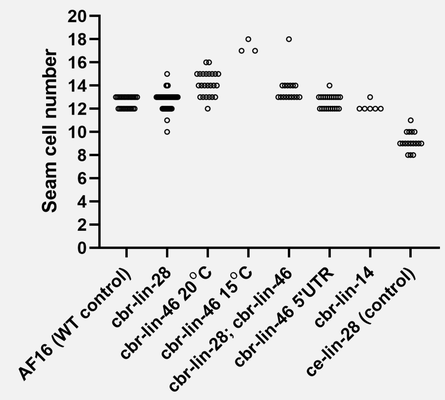

Figure 4. Seam cell counts for different mutant strains of C. briggsae. Counts might have slight variation because no fluorescent marker was used to identify seam cells.

cbr-lin-14

I obtained multiple alleles of *C. briggsae lin-14* by targeting its 6th exon to affect all isoforms.
The resulting phenotype closely resembles that of *C. elegans*.
Worms develop precocious alae and have a normal number of seam cells. The fertility is dramatically reduced.
C. elegans lin-14 mutants also have a defect in the tail innervation that is not obvious in *C. briggsae* and might be absent.

Figure 7. The phenotype of cbr-lin-14 mutant.

cbr-lin-46;cbr-lin-28

I crossed *cbr-lin-46* strain and *cbr-lin-28* strains to see if a suppression occurs.
Double mutants showed a partial suppression of both phenotypes. More worms started to undergo L4 molt successfully, though some individuals still became "arrested L4". 6 out of 10 of "successfully molted" worms had alae at the adult stage. Worms produced more eggs and reproduced better than the *cbr-lin-28* mutants.

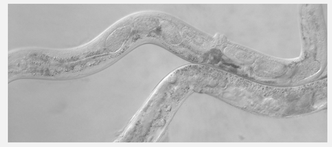

Figure 5. The phenotype of cbr-lin-28;cbr-lin-46 double mutant.

cbr-lin-46 5'UTR

Preliminary data suggest that *lin-28* binds the 5'UTR of *lin-46*. 5'UTR *lin-46* mutants have a precocious phenotype that resembles *lin-28*(lf). The sequence is also conserved *C. briggsae*.
I obtained a mutant allele of *cbr-lin-46* 5'UTR that has a 6bp deletion.

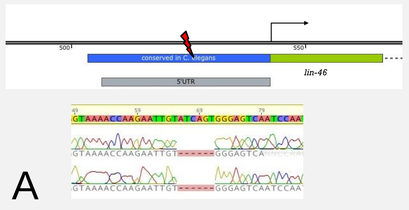

Figure 6.(A) CRISPR site and a sequence of the obtained mutation in the 5'UTR of cbr-lin-46. (B) The corresponding phenotype.

Mutant worms have a precocious alae at L4. They also have a protruding vulva and normal fertility. This phenotype is close *lin-28*(lf) of C. elegans. Though, the number of seam cells is wild-type in *cbr-lin-46* 5'UTR mutant.

Conclusions

1. *lin-14* is the most conserved functionally among the genes that I studied.
2. *lin-46* has conserved functions but the phenotype differs in the severity.
3. *lin-46* 5'UTR is likely to have conserved functions with the difference in its role in L2 cell fates.
4. *lin-28* remains the least conserved in *C. elegans* and *C. briggsae* and apparently has a completely different roles between species, though it is still antagonistic with *lin-46*.