Assessing the Role of the Transcriptional Repressor BLMP-1 in the Molting Timer of *C. elegans*

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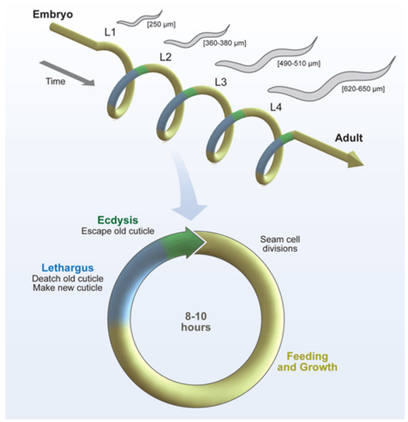
The Molting Cycle of *C. elegans*

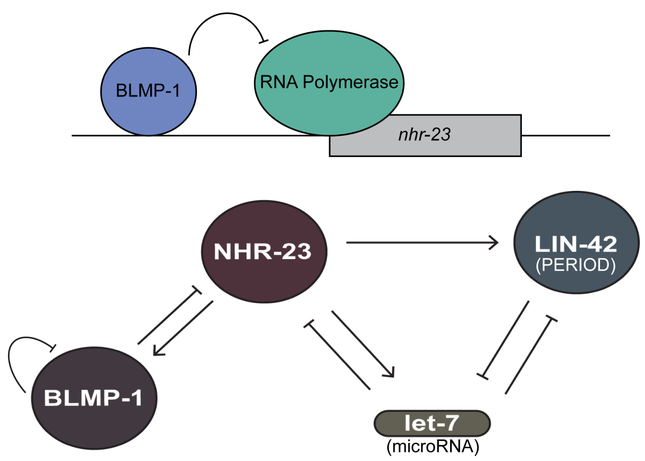
- *C. elegans* larvae molt 4 times at 8-10 hour intervals.
- A sleep-like state, known as lethargus, accompanies each molt during which locomotion is reduced and feeding ceases.
- The old cuticle is shed during the process of ecdysis, marking the beginning of a new life stage when feeding resumes.
- Adult animals do not molt.
- Findings about the molting timer may apply to biological clocks in mammals.



BLMP-1 as a Component of the Molting Timer in *C. elegans*

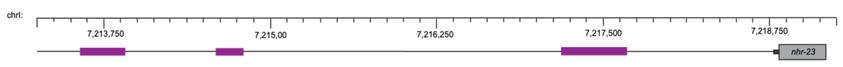
BLMP-1, the B lymphocyte-induced maturation protein, plays a role in cell specification and differentiation in other species. Furthermore, BLMP-1 acts as a transcriptional repressor. The *blmp-1* gene is a target of NHR-23, which is a nuclear hormone receptor known to activate genes involved in the process of molting.

I hypothesize that a transcriptional feedback loop between RORα/NHR-23 and BLMP-1 operates in the molting timer.



Interactions between NHR-23 and BLMP-1

- Chromatin Immunoprecipitation assays show BLMP-1 binds to the promoter region of *nhr-23.*



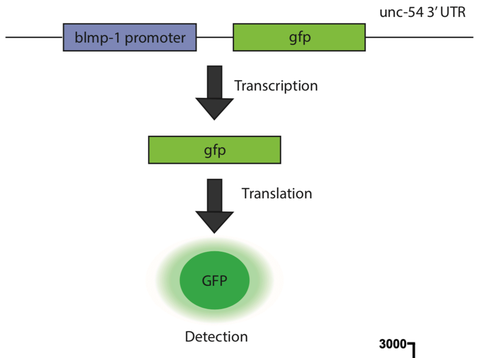
The genomic locus of *nhr-23* is depicted above. BLMP-1 binding sites, shown in purple, correspond to peaks in ChIP sequence data publicly available on WormBase.

- Chromatin Immunoprecipitation assays show both NHR-23 and BLMP-1 bind to the promoter region of *blmp-1.*

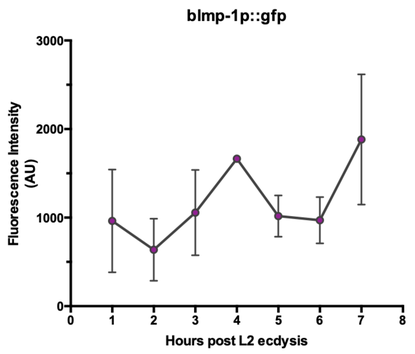
The genomic locus of *blmp-1* is depicted above. NHR-23 binding sites are shaded gray; BLMP-1 binding sites, purple.

The combined ChIP-seq data suggests BLMP-1 may repress expression of *blmp-1* itself and *nhr-23*, whereas NHR-23 activates *blmp-1.*

Expression Profile of a *blmp-1* Transcriptional Reporter



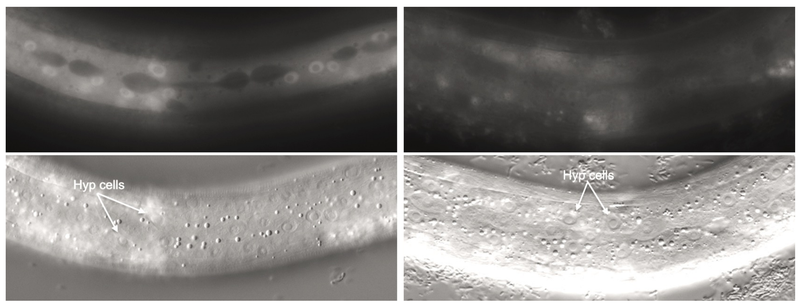
Expression of the *blmp-1* transcriptional reporter oscillates in the epidermal cells. In particular, fluorescence levels are more than double the lowest observed value, which occurs 2 hours after L2 ecdysis, as the animals are entering the L3/L4 molt.



Peak Expression of *blmp-1p::gfp* Depends on NHR-23

- The epidermal cells consist of the seam and hyp cells.
- Together these cells function in replenishing the cuticle between each molt.
- BLMP-1 expression is known to peak during the L3/L4 molt and during the L4/Adult molt.
- Lower fluorescence intensity is observed in *nhr-23*(*RNAi*) animals during the L3/L4 molt.

A) Worms grown on *vec*(RNAi) B) Worms grown on *nhr-23*(RNAi)

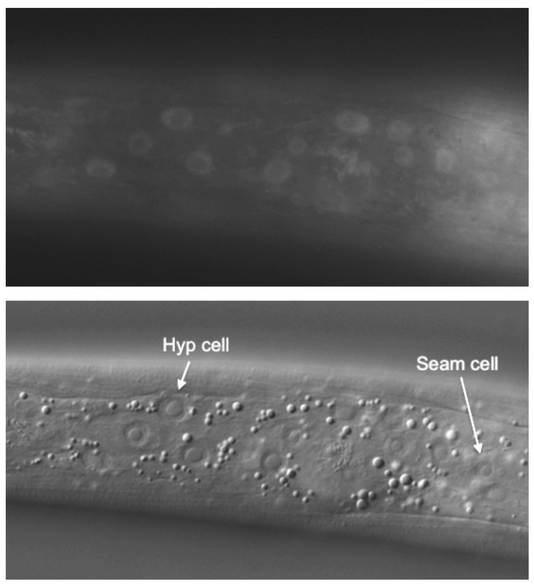


BLMP-1::GFP expression in epidermal nuclei

- BLMP-1::GFP was detected in both hyp and seam nuclei.
- The detectable signal intensity was highest in animals undergoing the L3/L4 molt.

Top: Fluorescence image show GFP tagged BLMP-1.

Bottom: Corresponding DIC image shows surface of the epidermis.

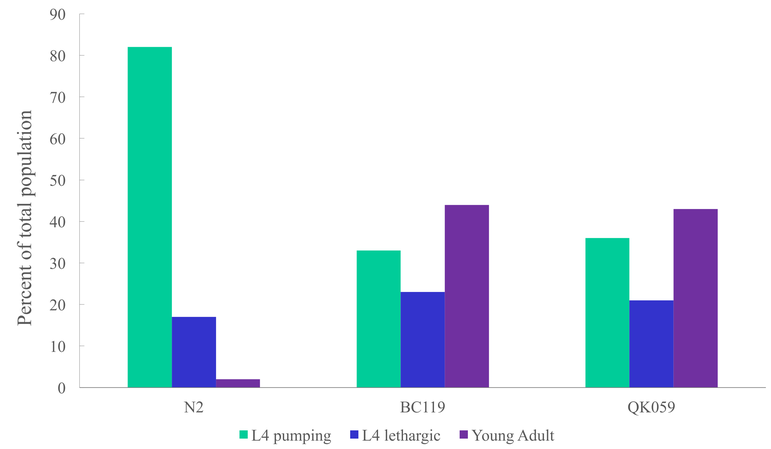


BC119 the *blmp-1* Mutants

BC119 is a strain with the *blmp-1 s(71)* mutation, which specifies the substitution of leucine with a stop codon at position 281 of BLMP-1.

Duration of the Molting Cycle in *blmp-1* Mutants

- 42 hours after being released from starvation, almost 50% of *blmp-1* mutants have completed the molting cycle and reached adulthood. Meanwhile, more than 80% of the wild-type population are found active in the L4 stage.
- The QK059 strain is a *let-7* mutant that develops at an accelerated pace.



Conclusions and Discussion

In order to determine whether BLMP-1 is involved in the timing of the molting cycle in *C. elegans*, I examined how it alters the pace in mutants, how it oscillates in expression, and how it interacts with other components of the molting timer, namely NHR-23.

- *Blmp-1* mutants reach adulthood earlier relative to wild type animals, when both are released from starvation at the same time.
- *Blmp-1* expression oscillates in the epidermis, similar to known components of the molting cycle timer.
- *Blmp-1* levels are influenced by the presence of NHR-23.

Biological rhythms are key features of animal development. A deeper understanding of the molting timer may lead to insights about the biological clocks pertinent to human circadian rhythms and aging.

Future Directions

In order to better understand other functions of BLMP-1 in the molting timer it is useful to consider the following:

- How does BLMP-1 interact with NHR-23 and how does it fit in the regulatory circuit?
- How does *blmp-1* interact with *let-7* and other components of the molting timer?
- Using higher resolution confocal laser microscopy is it possible to detect BLMP-1 expression in other cells?

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