Regulation of *engrailed* and *invected* expression in the *Drosophila* central nervous system

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Background

Neurodevelopmental Disorders

- Approximately 20% of children under the age of 17 are diagnosed with a neurodevelopmental disorder
- Gaining a better understanding of how the nervous system develops will help elucidate some of the mechanisms that govern neurodevelopmental disorders

The Engrailed Protein is Important For Neurodevelopment

- The *engrailed (en)* gene is found in many bilaterians and encodes a homeodomain protein and nuclear transcription factor
- Engrailed protein plays important roles during neurodevelopment

The Model Organism *Drosophila Melanogaster*

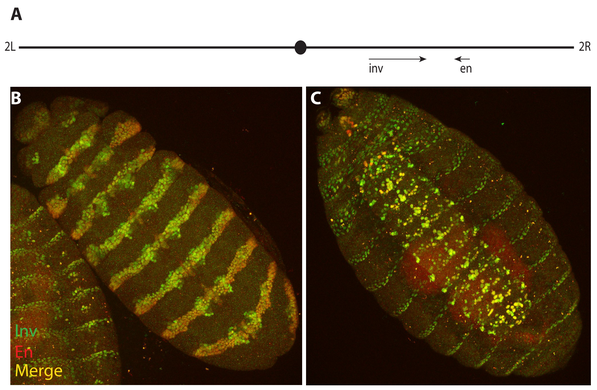


Figure 1. *engrailed (en)* and its genomic neighbor, *invected (inv),* are co-expressed in the central nervous system (CNS) throughout development, beginning during embryonic development. **A.** Schematic of *inv/en* gene locus, located on the right arm of chromosome 2 **B.** Maximum projection confocal image of wild-type (WT) stage 12 whole embryo and **C.** stage 17 whole embryo. Anterior upper left, ventral view.

How is the inv/en gene complex regulated in the central nervous system?

Polycomb Group Proteins Bind to Inv/En Regulatory DNA

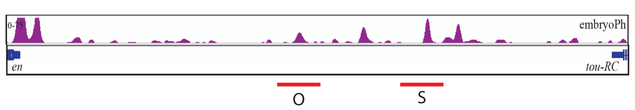


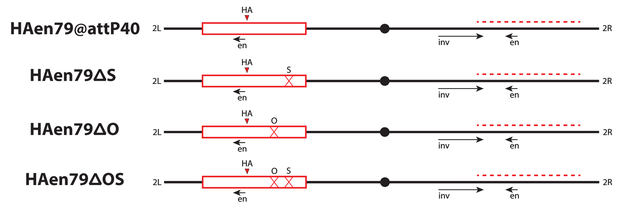
Figure 2. ChIP-seq for Polyhomeotic (Ph), a Polycomb group (PcG) protein. O and S fragments within *inv/en* regulatory DNA bind Ph in embryos.

- PcG complexes modify chromatin to repress transcription of target genes
- Polycomb Response Elements (PREs) are *cis*-regulatory elements that recruit PcG complexes to DNA
- O and S fragments were shown to contain enhancers for *Drosophila* imaginal discs

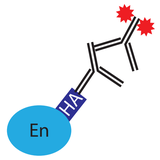
Do the O and S fragments play a role in PcG-mediated silencing?

Methods

Using Large Transgenes to Study Regulation of *engrailed*



Collect embryos
- HAen79@attP40
- HAen79ΔS
- HAen79ΔO
- HAen79ΔOS



Conduct immunofluorescence assay

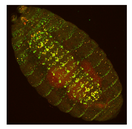


Image
- Stage 12
- Stage 17

Results

Misexpression of HA-En is Observed Only in Late Stage Embryos

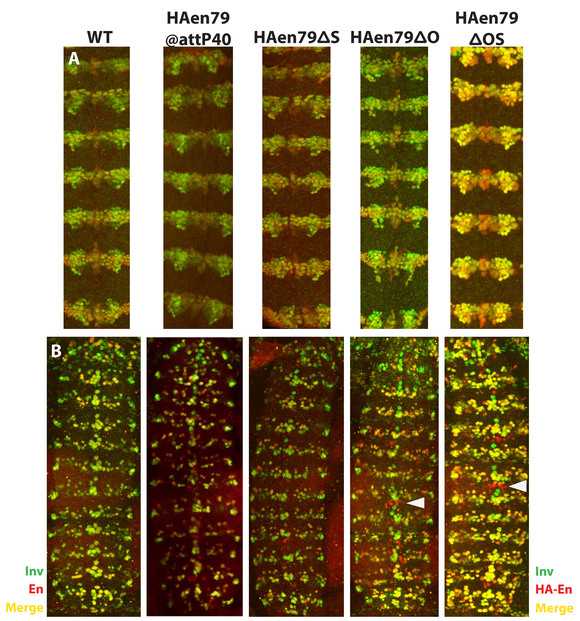


Figure 3. Misexpression of HA-En occurs in all late stage HA lines, but is exacerbated in the midline of HAen79ΔO and HAen79ΔOS.
Maximum projection confocal images of the CNS in **A.** Stage 12 embryos, and **B.** Stage 17 embryos. Arrowheads highlight clusters of cells misexpressing HA-En near the midline and within the CNS segments. WT embryos stained with Inv and En, all HA line embyros stained with Inv and HA-En. Anterior up, ventral view.

Loss of Expression of HA-En Accompanies Gain of Expression in Neighboring Cells

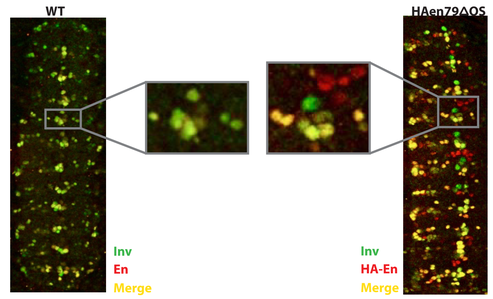
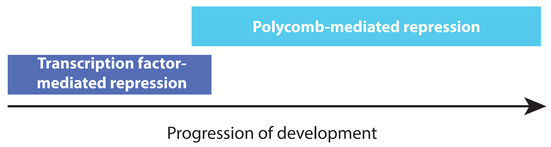


Figure 4. Some midline cells normally expressing HA-En and Inv lose expression of HA-En, while adjacent cells in the segment gain expression of HA-En. Confocal image of stage 17 WT and HAen79ΔOS CNS, focused on midline cells.

Why does misexpression occur only in late embryonic development?

Exacerbated Misexpression of HA-En in HAen79ΔO and HAen79ΔOS Could Be Due to Loss of Polycomb Silencing

Gene Repression Cascade



Conclusions

- Fragment O could contain a CNS-specific PRE
- Fragment S has a Ph peak but does not appear to have a CNS-specific PRE

Future Directions

- Examine *en* expression in CNS of Polycomb mutants
- Delete O fragment from endogenous locus
- Determine cell types that lose and gain expression of HA-En in HA lines using eNeuro atlas

Acknowledgements

My deepest appreciation goes out to the Kassis lab for maintaining a collaborative and enriching lab environment. I would like to thank NICHD and the NIH Postbaccalaureate Intramural Research Training Award program for supporting my research.