Deciphering the Regulatory Roles of the Transcription Factors Midline and Extramacrochaetae in *Drosophila* Embryonic CNS Development

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Abstract

We showed that the *midline* (*mid*) transcription factor gene functions genetically within the Notch/Delta signaling pathway of neuronal cell fate specification within the peripheral nervous system (PNS) of developing Drosophila eye tissues (Das et al., 2013). Functioning downstream of the Notch/Delta pathway, we now predict that Mid and the Groucho protein function as co-repressors of the Enhancer-of-split gene complex to inhibit either the activity of Extramacrochaetae (Emc), a basic-loop-helix transcription factor lacking a DNA binding domain. Since the Notch/Delta signaling pathway plays an evolutionarily conserved role in mediating neuronal cell fate specification within the central nervous system (CNS) via the activities of Mid and Emc as demonstrated in the PNS, we expect to uncover co-regulation of Mid and Emc expression within the embryonic CNS. In this study, we are examining the wild-type expression patterns of Mid and Emc during all developmental stages of the ventral nerve cord of the embryonic CNS. We will then use *mid* or *emc* loss-of-function and gain-of-function studies to examine whether reciprocal co-regulation among *mid* and *emc* occurs as neurons acquire unique cell fates. This research will solidify the mechanism by which *mid* and *emc* regulate cell fate specification in the embryonic nerve cord of *Drosophila*.

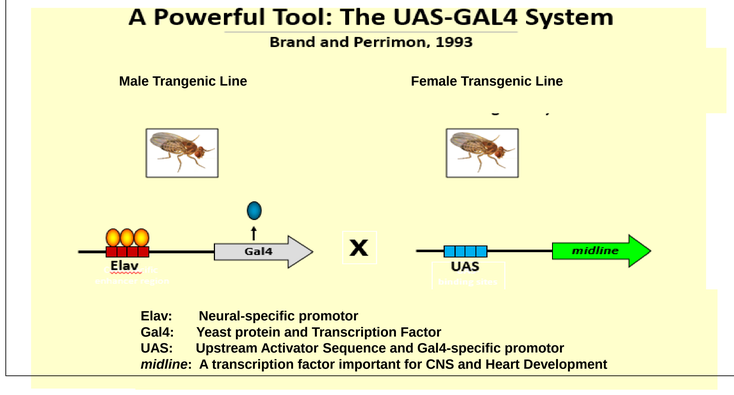
Update: The Emc antibody lost reactivity during interstate relocation of the Leal lab. In addition, this project had to be initiated in January of 2018. The *UAS-mid* gain-of-function study was carried out to first examine the potential genetic interaction between *mid* and the *Engrailed (En)* genes as published studies suggest a genetic interaction may exist between these genes to regulate neuronal cell fates in the embryonic CNS. Dr. Yuh-Nung Jan (UCSF, San Francisco) has kindly sent an aliquot of anti-Emc antibody to complete the originally planned studies. The preliminary results are encouraging and we are now poised to utilize confocal microscopy to analyze the data in greater detail.

Hypothesis

midline interacts with *Engrailed* to specify neuronal cell fates in the developing embryonic CNS.

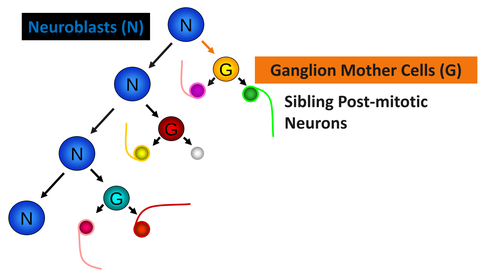
Materials and Methods

We used the *UAS-Gal4* system and the CNS-specific driver *Elav-Gal4* balanced on chromosomes II and III to express the following transgenes in Wild-type (WT) and specific mutant backgrounds: *UAS-mid*. *Drosophila melanogaster* strains were maintained at 25°C on standard cornmeal-yeast-agar media. *Oregon-R* flies were used as WT controls. Dr. Rolf Bodmer and Dr. James Skeath oprovided the transgenic fly stocks. The cartoon below summarizes the UAS Gal4 binary expression system, a powerful technique developed by Brand and Perrimon, 1993.



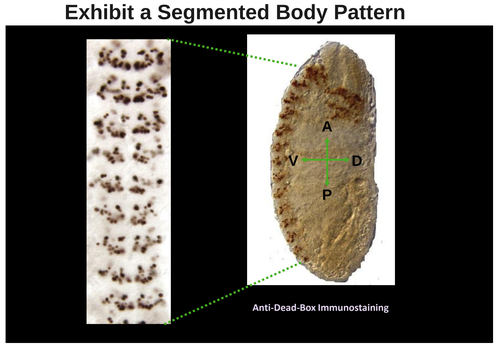
Introduction

Neuroblasts Divide Asymmetrically to Generate Neuronal Diversity



During Stage 9 of embryonic development, neuroblasts (NBs) establish their cell lineages by dividing asymmetrically to regenerate themselves and to produce smaller, secondary neural precursors, ganglion mother cells (GMCs). GMCs, in turn, divide asymmetrically to produce a pair of distinct post-mitotic neurons. As a result of lineage-specific gene expression patterns (intrinsic cues), extrinsic cues, and asymmetric division, individual post-mitotic cells in the CNS acquire individual fates.

The *Drosophila* Embryo and Ventral Nerve Cord Exhibit a Segmented Body Pattern

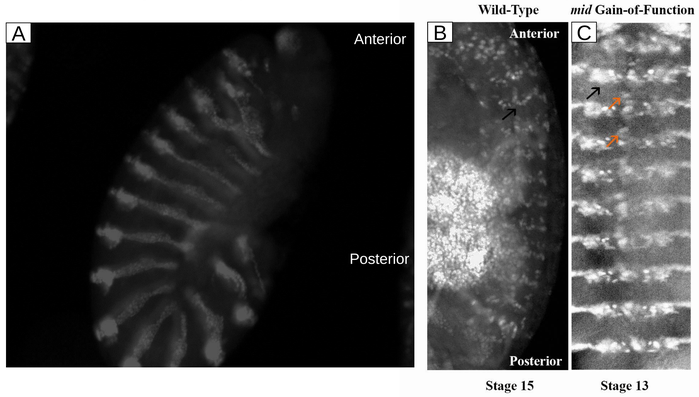


Engrailed is Expressed within Specific Neuroblast Lineages of the CNS

During Stages 9-11 of early CNS embryonic development, neuroblast precursors 6-1,7-1,1-1, 7-4, 6-2, 7-2 expressed Engrailed and establish their cell lineages (Doe, 1999). Median Neuroblasts (MNB) within each segment also express Engrailed (Bossing and Brand, 1999). In late CNS development (Stages 15-17), post-mitotic neurons expressing Engrailed have been identified as interneurons (Siegler and Jia, 1999). Midline is also expressed exclusively within subsets of interneuron populations (Leal et al., 2009).

Results

Engrailed Expression in WT and Mutant Embryos



A) Engrailed (En) exhibits a classical staining pattern within 14 segments of the early-staged WT *Drosophila* embryo. B) In late-staged WT embryos, ectodermal expression is evident superficially while the CNS and midline below show a distinct pattern of neuronal expression. C) The *mid* gain-of-function mutants (*Elav-Gal4;UAS-mid*) also express En. The *black* arrows are pointing to posterior-placed En-expressing neurons along the segment of the nerve cord. The *orange* arrow is depicting a faint median neuroblast expressing En. The resolution of single, En-expressing neurons requires enhancement using confocal imaging analyses before making accurate conclusions. Plans are underway to utilize a confocal microscope at Saint Louis University or Washington University Medical School.

Conclusions

We have successfully carried out a key experiment testing genetic fly stocks and other reagents (antibodies) in order to initiate detailed studies to determine whether *midline (mid)* interacts genetically with either *engrailed (en)* and/or *extramacrochaetae (emc)* to specify neuronal cell fates within the embryonic CNS. Interestingly, these genes/proteins share one property in common: all are expressed within distinct populations of interneurons based upon published studies.

Future Directions: We will use the confocal microscope to reanalyze these results and to accurately assess whether the expression pattern of En was altered under *mid* gain-of-function conditions. We will also conduct the originally planned studies using new, working antibody stocks from the Jan Lab (UCSF) and reciprocally, investigate changes in gene/protein expression under *mid* loss-of-function conditions.

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