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Regulation of mRNA Stability During Drosophila Neural Development

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Abstract:

Neural development is a complex process requiring generation of diverse and highly specialized cell types. Drosophila melanogaster studies in developmental biology have implicated a vast array of transcriptional regulators in controlling neural development. However, assessing only differential transcription of mRNA is not sufficient to explain the intricate regulatory scheme required for proper neural development. Regulation of gene expression is complex and involves both RNA synthesis and decay pathways. The importance of mRNA decay is exemplified from the implications of misregulation of mRNA decay in neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Our objective is to measure and evaluate mRNA decay in cells of the embryonic central nervous system (CNS) and larval peripheral nervous system (PNS). We used TU-decay methodology to measure transcript decay during Drosophila development. TU-tagging analysis of neural stem and progenitor cells of the embryonic CNS revealed an enrichment of the Pumilio recognition element (PRE) motif in neural cell fate transcription factors. TU tagging uses the UPRT gene to convert 4-thiouracil (TU) to 4-thiouridine in order to incorporate this into a newly synthesized RNA. This allows for the cell type specific tagging of mRNAs at particular times during development. This method involves TU pulse to tag the RNA and it is then subsequently exposed to excess uridine. Results from this can be quantified with a microarray to model decay kinetics. RNAi knockdown of the RNA binding protein Pumilio in these cells of the CNS followed by TU-decay analysis showed stabilization of mRNAs with a PRE. We then confirmed that the PRE is sufficient to direct mRNA decay though a 3'UTR reporter assay in the embryonic CNS. Applying cell-type specific TU-decay, RNA binding protein knockdown, and reporter assays reveals pertinent information allowing us to understand the regulation of mRNA decay.

Lay Abstract:

Precise regulation of gene expression is important for development. Genes are encoded in our DNA and eventually will be transcribed into a messenger RNA molecule. RNA is then translated into a functional protein. Traditionally, research in the biomedical field has focused on the production and abundance of RNA. However, the regulation of RNA stability and translation is also critical for our understanding. RNA stability is important for gene expression in the nervous system. In our research, we study the mechanisms of RNA decay during neural development in the fruit fly, Drosophila melanogaster. It is important to understand how mRNA decay is regulated in the nervous system because this is what drives proper neuronal functioning as well as proliferation and differentiation of neuronal precursors. There is evidence that misregulation of RNA stability can contribute to the pathophysiology of complex neurological disease, including Alzheimer's, Lou Gehrig's disease, and Fragile X syndrome.

In order to gain a full understanding of these mechanisms, we have developed techniques to evaluate genome-wide decay mechanisms in the central and peripheral nervous system of Drosophila. We use a methodology that involves chemically tagging mRNA to estimate the amount that is present and how much still remains at particular time points during development. With genome wide information on RNA decay kinetics in specific tissues we can work toward establishing innovative regulatory mechanisms. We are interested in how proteins that bind RNA might affect transcript stability. Pumilio is a well-studied RNA binding protein that is known to play a crucial factor for establishing anatomical positions of the early Drosophila embryo. We have shown that Pumilio is also able to regulate RNA stability by binding targets in the central nervous system as well as promoting decay. The goal of this study is to provide insight on the regulation of RNA stability and gene expression which will help our understanding of neural deficiencies.

Crystal Bakhaj is from Los Angeles, CA. She is currently a fourth year Human Biology major at the University of California, Merced. Throughout her college career, she has been actively involved on campus in which she partakes in volunteering, clubs, and research. Crystal has worked for several years as an undergraduate researcher in Dr. Michael Cleary's lab. She is also employed at the university as an Academic Advising Mentor for the School of Natural Sciences. All of these opportunities have taught her leadership skills, time management, and responsibility. After graduation, she hopes to gain more research experience with the ultimate goal of attending medical school.