

Wayne Sossin- Scientific Summary

Project Title: Determining how stalled polysomes are generated for transport in RNA granules and regulated local translation.

Statement of Purpose: We will determine how polysomes are stalled in RNA granules and what determine how neurons choose which mRNAs to transport in stalled polysomes.

Project Summary:

Local translation in the axons and dendrites of neurons is critical for wiring up a functioning nervous system. Neuronal RNA granules are densely packed clusters of mRNA and ribosomes that transport mRNAs for local translation in synapses and mutations in proteins necessary for the function of neuronal RNA granules lead to mis-wiring and neurodevelopmental disorders. Learning how these granules are generated, structured and function is necessary to develop strategies to ameliorate neurodevelopmental disorders caused by dysregulation of these granules. To accomplish this, we have adopted a multidisciplinary approach combining cryo-EM, ribosome profiling of purified RNA granules, and cellular imaging of RNA granules in developing neurons. Our hypothesis is that mRNA in RNA granules is repressed at the elongation step in stalled polysomes, allowing neurons to swiftly translate synaptic mRNAs into functional proteins, bypassing the rate-limiting step of initiation. The following aims will significantly increase our understanding of RNA granules.

In Aim 1, we will establish how the RNA granules are stalled using cryo-electron microscopy (cryo-EM). Our analysis has identified ribosomes in purified RNA granules that contain unidentified densities not found in cryo-EM of normal polysomes. We will generate high-resolution cryo-EM maps that will allow us to identify the stalling factors triggering the formation of the RNA granules (Aim 1A). RNA granules will also be analyzed by cryo-electron tomography (cryo-ET). These data will be used to reveal how the different ribosome subpopulations are positioned in 3D within the RNA granule (Aim 1B). We will determine if identified stalling factors are lacking in models of neurodevelopmental diseases, such as the Fragile X syndrome, where stalled polysomes are thought to be affected (Aim 1C).

In Aim 2, we will use ribosome profiling on purified RNA granules to determine where on the mRNA stalling occurs. Using novel strategies to cleave the tightly clustered polysomes in RNA has allowed us, for the first time, an examination of the mRNA sequences protected by ribosomes in RNA granules. Notably, there is a significant enrichment of protected fragments on mRNAs encoding proteins shown to be mutated in neurodevelopmental diseases and also mRNAs previously implicated in regulation by elongation. We will use bioinformatics techniques to determine the specific sequences in or around the protected fragments that determine stalling and determine if these motifs can identify RNA binding proteins important for stalling (Aim 2A). We will evaluate the role of putative stalling factors by comparing the ribosome profiling results in wild type mice from those lacking these proteins (Aim2B).

In Aim 3, we will determine the mechanism for reactivation of RNA granules. The X linked RNA helicase, DDX3 is the most common protein mutated in girls presenting with

neurodevelopmental disorders. We will test the hypothesis that dominant-negative DDX3 mutations alter the structure and liquid-liquid phase state of RNA granules, preventing the dissociation and unstalling of the stalled polysomes in these granules.

Together these studies will piece together a complete picture of how RNA granules are generated and how they are dysfunctional in neurodevelopmental disorders suggesting new therapies for intervention.