

Scientific Summary- David Kaplan & Freda Miller

Project Title: Translating neural precursor cells to neurons and glia during normal and abnormal murine brain development

Statement of Purpose: This project will determine the importance of translational repression for cell genesis during normal and abnormal brain development, focusing on repressive complexes that include proteins implicated in human ASD.

Project Summary:

The identification of genes associated with intellectual disability and autism spectrum disorder (ASD) are providing important insights into neurodevelopmental disorders (NDDs). Some of these genes encode proteins that are components of translational repression complexes and since these complexes coordinately silence many different mRNAs, then their disruption would have broad-ranging consequences for neural development. We recently demonstrated the importance of translational repression for development of the neural precursor cells (NPCs) that build the cerebral cortex, the site of many higher cognitive functions. These studies led to the overarching hypothesis of this proposal, that translational repression regulates developmental genesis of neurons and glia and that genetic disruption of these repressive mechanisms in NDDs causes aberrant cell genesis and ultimately perturbed circuitry and cognition. Here, we will test this hypothesis, focusing on the translational repressor 4E-T and two 4E-T-associated proteins that are implicated in NDDs, the P-body protein and RNA helicase Ddx6 and the RNA binding protein (RBP) Cpeb4. Specifically, we propose:

Aim 1: To determine whether 4E-T and NDD-associated Ddx6 regulate developmental cell genesis, longterm brain development and, ultimately, cognition. Our previous work showed that 4E-T and Ddx6 are essential for maintaining transcriptionally primed embryonic NPCs in an undifferentiated state ensuring appropriate cortical neurogenesis. Our scRNA-seq analyses indicate that transcriptional priming also occurs in forebrain NPCs during an important neonatal developmental window when they generate glia and inhibitory interneurons. We therefore propose to ask (i) if 4E-T and Ddx6 regulate cell genesis in the neonatal forebrain and (ii) if loss of 4E-T/Ddx6 interactions in embryonic NPCs, as is seen in human NDD, has adverse neuroanatomical and ultimately behavioral consequences. Our initial data show that loss of 4et in postnatal NPCs causes stem cell depletion, aberrant glial and neuronal differentiation and derepression of transcription factor mRNAs.

Aim 2: To determine whether ASD-associated Cpeb4 collaborates with 4E-T to regulate translational repression and developmental cell genesis. We previously showed that 4E-T associates with Pum2 in embryonic cortical NPCs to repress proneurogenic mRNAs and thereby regulate neurogenesis. Intriguingly, both Pum2 and 4E-T are known to associate with Cpeb4, and Cpeb4 is deregulated in human idiopathic ASD brains. We will therefore test the idea that 4E-T, Pum2 and Cpeb4 collaboratively repress key target mRNAs to regulate cell genesis and normal versus abnormal brain development. Specifically, we will ask (i) if Cpeb4, Pum2 and 4E-T are associated in Ddx6-positive RNA granules in neural precursors and (ii) if

Cpeb4 is important for developmental cell genesis. We will also (iii) identify Cpeb4/Pum2/4E-T repressed target mRNAs that are important for cell genesis and that when deregulated cause NDDs, taking advantage of 4E-T-associated mRNAs we previously identified. Our initial data indicate that Cpeb4 is important for embryonic cortical neurogenesis and that 16 predicted shared 4E-T/Cpeb4/Pum2 targets are already known to be associated with NDDs.

These studies will define transcriptional repression mechanisms important for developmental genesis of neurons and glia and will help us understand how mutation of genes involved in translational repression can cause long-term cognitive perturbations in NDDs.