

Title: A phagocyte-specific ribosome profiling approach to identify novel targets of *pumilio* regulating sleep in *Drosophila melanogaster*

Sleep problems add approximately \$16 billion annually to the cost of healthcare in the U.S., and result in \$50 billion annually lost in productivity. Furthermore epidemiological studies indicate that sleep deprivation is dramatically increasing in modern society. However, the mechanisms underlying sleep need or sleep homeostasis are still largely unclear. Studies in our laboratory have shown that the translational regulator *pumilio* plays an important role in the regulation of sleep homeostasis. Moreover, we have substantial evidence indicating that the anatomical loci of *pumilio* effects involves the immune system, and specifically, the phagocytes. However, *pumilio* regulates a wide range of proteins and it is unknown which ones are mediating the sleep effects. The goal of this project is to identify *pumilio* targets in phagocytes using a tissue-specific ribosome profiling approach. This approach takes advantage of the wide range of tissue-specific promoters available in *Drosophila* and combines it with the tagging of a key ribosomal protein that allows the pull down of all transcripts being actively translated in the tissue of interest (phagocytes). To accomplish this goal we will perform the following steps: 1) recombine the transgene containing the tissue-specific promoter (*hml-gal4*) with the tagged ribosomal protein transgene (*UAS-RpL3-Flag*); 2) cross the recombinant fly with either the *pumilio* RNAi fly or the control transgene; 3) pull down all RNAs being actively translated using the RpL3-Flag protein and magnetic beads with anti-Flag and; 4) identify *pumilio* targets by examining differentially expressed genes between control and *pumilio* knockdown flies containing *pumilio* binding sites. Our studies not only will bring insights into the mechanisms of sleep homeostasis, but also identified *pumilio* targets may serve for the development of novel drugs for sleep disorders.