Activity-Dependent Regulation of the RNA-Induced Silencing Complex in Mammalian Neurons

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Introduction. Synaptic plasticity in learning and memory depends on the precise spatiotemporal control of protein synthesis. As ribosomes and some messenger RNAs (mRNAs) exist in dendrites, the local activity-dependent translation of specific mRNAs could orchestrate plasticity at the synapse. An intriguing model suggests that mRNAs for proteins involved in long-term memory translocate to synapses in a repressed state and are translated upon activity[1].

A novel avenue of posttranscriptional regulation at the synapse involves microRNAs (miRNAs), short (~22 nt) noncoding RNAs that regulate the translation or stability of their mRNA targets[2]. Each mature miRNA strand recognizes the 3' untranslated region (3' UTR) of its target mRNA within the RNA-induced silencing complex (RISC). A diverse repertoire of brain-specific miRNAs could precisely regulate the expression of their targets[2].

While increasing evidence suggests the importance of specific miRNAs in key neuronal events, we still lack an overall understanding of how synaptic stimulation affects RISC itself in mammalian systems. This is important because RISC compartmentalizes the miRNA:mRNA duplex to allow spatiotemporal regulation. **I hypothesize that neuronal activity regulates RISC to free mRNA from miRNA-mediated translational repression in mammalian neurons.** This is based on the following observations: **(1)** The core mammalian RISC component Argonaute 2 (Ago2) is enriched in the postsynaptic density of dendritic spines, where most excitatory synapses occur[3]. **(2)** Neuronal activity coincides with the degradation of the RISC component MOV10 and the translation of dendritic mRNAs[4]**. (3)** Brain-derived neurotrophic factor (BDNF) releases ribonucleoproteins (RNPs) from Ago2[5] and relieves translational repression of *Limk1*, a spine development-enhancing protein kinase, by the miRNA miR-134[6].

Aim 1: How does activity remodel RISC at the synapse? Neuronal activity could induce dendritic mRNA translation by altering RISC composition in a miRNA-dependent or independent manner[7]. For each experiment, I will stimulate mature mouse hippocampal neuron cultures with KCl, glutamate, or BDNF, leaving the control group unstimulated, and collect whole-cell and synaptoneurosome (SN) fractions from each group before and after stimulation; comparisons between these fractions will indicate whether any activity-dependent changes I observe are synapse-specific. a) Observe changes in RISC composition. I will transfect neurons with HA-tagged Ago2 and co-immunoprecipitate (co-IP) associated proteins. I will visualize proteins by SDS-PAGE and silver staining, and analyze major bands by mass spectrometry to obtain RISC compositions before and after stimulation. For those components whose abundance or molecular weight changes after activity, I will discover their possible regulators through yeast two-hybrid assays. b) Determine whether translational activation is miRNA-dependent. I will transfect neurons with luciferase reporters fused to 3' UTRs of known dendritic mRNAs, and knock down their respective miRNAs at the time of stimulation using antisense oligonucleotides. I will measure relative luciferase activity to see whether translation still occurs after stimulation. These results will identify RISC profiles and regulators required for activity-dependent translation, and determine whether miRNAs remain within RISC as translational activators[7].

Aim 2: What signaling pathways participate in activity-dependent RISC regulation? Neuronal stimulation could also activate signaling pathways that modulate RISC's interaction with translation initiation factors. BDNF-activated reversal of miR-134 mediated *Limk1* translational repression requires the mTOR signaling pathway by an unknown mechanism[6]. A

possible clue exists in *Drosophila*, where Ago2 bound to target mRNA competes with eukaryotic translation initiation factor 4G (eIF4G) for eIF4E and represses target mRNA translation[8]. Since phosphorylation by mTOR dissociates eIF4E-binding protein (4E-BP) from the eIF4G binding site on eIF4E and activates translation[9], BDNF activation of the mTOR signaling pathway could dissociate RISC from eIF4E by a similar mechanism. I will treat neurons with BDNF or BDNF + rapamycin (an mTOR inhibitor), and IP Ago2 from SN fractions. If eIF4E co-IPs with Ago2 in the absence of BDNF and in the presence of rapamycin, mTOR signaling could disrupt eIF4E-Ago2 interaction. I would then coexpress phosphorylation mutants of Ago2 with *Limk1* reporter mRNAs in hippocampal neurons, and repeat the above treatments. If the relative luciferase activities of the *Limk1* reporters do not differ significantly between the three conditions, BDNF-activated mTOR signaling could relieve miR-134 mediated repression of *Limk1* translation by disrupting RISC interaction with eIF4E, allowing eIF4G to bind and initiate translation.

Aim 3: How does activity affect RISC's interaction with other RNA granules in dendrites?
RISC resides in RNA granules called cytoplasmic processing bodies (PBs), sites of translational repression that disassemble and enter dendrites after neuronal stimulation[5]. At the basal state, PBs briefly dock to other repressed mRNA-containing granules, such as stress granules (SGs) and transport ribonucleoproteins (RNPs)[10]. Since one can infer an mRNA's fate by its localization to a particular RNA granule, understanding how activity affects PBs' interaction with other RNA granules would reveal the state of RISC after activity. I will transfect neurons with fluorescently tagged Ago2 and markers of SGs, transport RNPs, or polyribosomes. At Harvard's Nikon Imaging Center, I will track their dendritic localization by live cell fluorescence microscopy to determine whether stimulation changes the frequency of their respective docking and colocalization. Since all RNA granules except PBs contain translation initiation factors[11], stimulation may increase PBs' frequency of interaction with other RNA granules.

Broader Impacts. Understanding RISC regulation during neuronal activity will elucidate both the role of miRNAs in local posttranscriptional regulation and the molecular processes underlying memory maintenance. I will publish major findings in journals and present them at the SfN Annual Meeting. Since rodents with impaired hippocampal RISC activity have learning and contextual memory deficits[12], my work will benefit the public by providing means to improve cognitive performance in students and the elderly. Through Harvard's Summer Honors Undergraduate Research Program, I will mentor students who are underrepresented in science and teach them how to present their contributions to this work to others. As a tutor at Cambridge Rindge and Latin School, I will also present my work to students in clubs such as the Biotech Club, Girls Preparing to Succeed, Minority Student Achievement Network, and Science Team. Finally, I will give a public talk through Harvard's Science in the News student seminar series.
This proposal is my own original work. At Harvard I propose to learn from the expertise of Gary Ruvkun and Michael Greenberg, who respectively study miRNA-mediated gene regulation and activity-dependent gene expression[6]. The NSF Fellowship would also allow me to attend the Marine Biological Laboratory's Neurobiology summer course, where I will learn imaging and molecular biology techniques directly applicable to this study.

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