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Ribosomal Trafficking Patterns in Myelinating Schwann Cells in Response to Neuronal Injury
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Trafficking of ribosomes in oligodendrocytes, myelinating cells of the central nervous system, has been noted to occur from the cell body to the myelinating processes during development and myelination. Additionally, recent studies implicate Schwann cells, myelinating cells of the peripheral nervous system, as a source of ribosomes for injured axons. Developing an understanding of the rates of ribosomal trafficking following injury will provide a more thorough illustration of the response mechanism of the Schwann cell. Specifically, whether ribosomes may be prepared for transfer prior to injury or supplemented after the fact.
In order to quantify this phenomenon, Schwann cells are harvested from the sciatic nerves of neonatal Sprague-Dawley rats and transfected with a plasmid encoding RPL4-GFP, a fluorescently tagged ribosomal subunit. These cells are purified and transferred to cultures of embryonic DRG neurons that and induced to myelinate through the addition of ascorbic acid (50 μg/ml). Two weeks following treatment, time-lapsed fluorescent microscopy is utilized to obtain images of ribosomal distributions in myelinating Schwann cells. Subsets of neurons are injured through axonal severance and ribosomal trafficking is subsequently observed. Preliminary results have identified trafficking of ribosomes to localizations of axonal contact. Additionally, notable increases in adhesion proteins have been identified at axonal contacts. Future experiments will look to identify how these rates relate to those following injury and possibly the role of adhesion in this process.

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In Vivo Force Measurements Reveal that GSK-3 Regulates Axonal Transport by Altering the Number of Active Motors
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Neurons rely on microtubule motor proteins such as kinesin-1 and dynein to transport essential cargos along the axon. Defective transport is connected to neurodegenerative diseases, including Alzheimer's disease. Glycogen Synthase Kinase 3(GSK-3) has been proposed to be a central player in Alzheimer's. We show that GSK-3 is a required negative regulator of both kinesin-1-mediated and dynein-mediated transport of the Amyloid Precursor Protein, a key contributor to Alzheimer's pathology. By measuring the forces motors generate in vivo, we find that GSK-3 regulates transport by altering the activity of kinesin-1 motors but not their binding to the cargo leading us to propose that this regulation occurs via a mechanism of changing motor-microtubule interactions.

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Understanding mRNA Transport during Developing Hippocampal Neurons and during Neuron Regeneration
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Translation of mRNA in axons and dendrites enables a rapid supply of proteins to specific sites of localization within the neuron. Distinct populations of mRNA-containing cargoes, including granules and mitochondrial mRNA, are transported with neuronal projections. The distributions of these cargoes appear to change during neuronal development, but details on the dynamics of mRNA transport during these transitions remain to be elucidated. The goal of this project is to characterize transport of mitochondrial and non-mitochondrial mRNA in neuronal projections during the development of hippocampal neurons. Hippocampal neurons of one day old rat neonates were cultured on poly-lysine-coated glass cover slips. The neurons were co-labeled with dyes marking mRNA and mitochondria, to distinguish mRNA from mitochondrial mRNA. Live fluorescence imaging were performed on three different days, corresponding to different stages of development. Maturity of the neurons was determined via immunolabeling with PSD95, a post-synaptic marker. Immunolabeling with SMI-31, an axon-specific marker, was performed to differentiate axon and dendrites. Parameters of mRNA trafficking were quantified via kymograph (graph of spatial position over time). Statistical analysis was performed by Kolmogorov-Smirnov (K-S) test and ANOVA : Tukey. The results suggest differences in the transport pattern of mitochondrial and non-mitochondrial mRNA, and also indicate significant differences in transport parameters at different time points. Higher mRNA velocity in mature neurons was observed. In addition, the mRNA flux increases during growth as it forms mature synapse, presumably because local protein synthesis is essential for long term potential. To better understand the logic underlying altered mRNA transport, we are currently exploring transport mechanism of mRNA, and its importance in regeneration. This work has important implications for the regulation of neuronal plasticity during neuronal development and in response to neuronal injury.
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Amyotrophic Lateral Sclerosis IgGs Enhance the Mobility of Lysotracker-Labelled Vesicles in Cultured Rat Astrocytes
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We examined the effect of purified immunoglobulins G (IgG) from amyotrophic lateral sclerosis (ALS) patients on the mobility and exocytotic release from Lysotracker-stained vesicles in cultured rat astrocytes. Time-lapse confocal images were acquired and vesicle mobility analyzed before and after the application of ALS IgG. The vesicle counts were obtained to assess cargo exocytosis from stained organelles. At rest, when mobility was monitored for 2 minutes in bath with Ca^{2+}, two vesicle populations were discovered: i) non-mobile vesicles (6.1%) with total track length (TL)$<$1 μm, averaging at 0.33 ± 0.01 μm (n=1305) and ii) mobile vesicles (93.9%) with TL$>$1 μm, averaging at 3.03 ± 0.01 μm (n=20200). ALS IgG (0.1 mg/ml) from 12 out of 13 patients increased the TL of mobile vesicles by ~24% and maximal displacement (MD) by ~26% within 4 minutes, while the IgG from control group did not alter the vesicle mobility. The mobility enhancement by ALS IgG was reduced in extracellular solution devoid of Ca^{2+}, indicating that ALS-IgG vesicle mobility-enhancement involves changes in Ca^{2+} homeostasis. To examine, if enhanced mobility relates to elevated Ca^{2+} activity, cells were stimulated by 1 mM ATP, a cytosolic Ca^{2+} increasing agent, in the presence (2 mM) and in the absence of extracellular Ca^{2+}. ATP stimulation triggered an increase in TL by ~7% and ~12%, and a decrease in MD by ~11% and ~1%, within 4 minutes respectively. Interestingly none of the stimuli triggered the release of vesicle cargo. It is concluded that ALS IgG-enhanced vesicle mobility in astrocytes engages changes in calcium homeostasis.

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3D Simulation of Filament-Filament Switching in Intracellular Transport
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Long-distance intracellular transport is predominantly microtubule-based, but shorter local transport often depends on actin and an unconventional myosin such as Myosin-V. A key factor in regulating actin transport involves regulating the probability that cargos switch at intersections between actin filaments. We previously postulated that by controlling the number of motors on the cargo, one might be able to control what occurs at intersections. Here we investigate this hypothesis using 3D Monte Carlo simulations, and discover that cargo behavior at intersections is much less sensitive to motor number than expected. Thus, simply controlling the number of active motors on the cargo cannot account for the in vivo observations, suggesting the existence of an additional form of regulation.

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Visualizing Bacterial DNA Segregation in a Cell Free System
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DNA segregation (or partition) is of central importance, but the mechanism in bacteria remains elusive. Bacterial genomes ensure inheritance using three components: a centromere (*sopC*), a centromere binding protein (SopB), and an ATPase (SopA). Intriguingly, the ATPase has been observed to form patterns on the nucleoid. We hypothesize this ATP-driven transport system uses nucleoid-mediated patterns to properly localize DNA in the cell.