Ribosomal Trafficking Patterns in Myelinating Schwann Cells in Response to Neuronal Innervation

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Tracing the movement 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. Subsequently, 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.

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.

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 cargos, including granules and mitochondrial mRNA, are transported with neuronal projections. The distributions of these cargos 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 by SMI-31, an axon-specific marker, 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.

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 Ca2+, 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 Ca2+, indicating that ALS-IgG vesicle mobility-enhancement involves changes in Ca2+ homeostasis. To examine, if enhanced mobility relates to elevated Ca2+ activity, cells were stimulated by 1 mM ATP, a cytosolic Ca2+ increasing agent, in the presence (2 mM) and in the absence of extracellular Ca2+. 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.

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.

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.
A well-studied model of partition is that of the E. coli sex factor, F plasmid. We reconstituted its partition system by introducing fluorescent SopA, SopB and plasmid (encoding sopC) into a DNA capped flowcell (nucleoid biomimetic) and visualized the system dynamics using TIRFM. We found SopA-ATP dynamically bound the DNA carpet, and the steady-state carpet density was lowered by SopB. When all components were infused onto the DNA carpet with ATP, the plasmids transiently bind the carpet with colocalized SopA and SopB. Over time, the static plasmids released SopA, began to wiggle, and finally popped off when little to no SopA remained. The results suggest that plasmid movement and release are coupled to SopB stimulated ATP-hydrolysis by SopA. The tethered particle motion of some wiggling plasmid clusters produced SopA craters on the DNA carpet, suggesting that plasmid-bound SopB not only releases SopA on the plasmid but also releases nearby SopA on the DNA carpet. We propose SopB communicates with both SopA on the plasmid and nucleoid to control the transport of plasmid via an ATP-driven diffusion-ratchet mechanism.

Study of this unique transport system is vital because surface-mediated patterning as a means for cargo carrying is inherently different from the classical motor protein or the actin/microtubule-mechanisms of transport.

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Dynamic Self-Organization of Bacterial DNA Segregation Machinery in a Cell-Free Reaction
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Bacterial plasmids have evolved segregation machineries to partition replicated DNA to the daughter cells at cell division. Plasmid phage lysogenizes as a low-copy-number plasmid in Escherichia coli. Its partition system consists of three components, a centromere-like region, parS, an adaptor protein that binds to the centromere, ParB and a partition ATPase, ParA. In general, it is known, that a ParB-parS partition complex is formed when ParB oligomerizes onto the centromere. This large nucleoprotein complex interacts with ParA and is thought to couple ATP hydrolysis to drive the movement and segregation of plasmids to opposite cell-halves. To understand ATP-driven DNA segregation, we reconstituted the plasmid partition system in a cell-free reaction and visualized the spatiotemporal dynamics using TIRF microscopy. We flowed a cell flow surface with non-specific DNA to mimic the bacterial nucleoid surface and flowed in the three-component reaction system. We found that ParA coats the artificial nucleoid creating a reference scaffold for plasmid movement. ParA assemblies onto the ParB/parS complexes and anchors them onto the ParA-coated nucleoid surface. ParB stimulates ParA disassembly leading to vigorous Brownian motion of the plasmid as the complex loses bridging interactions with the nucleoid. The plasmid detaches from the nucleoid surface leaving a hole devoid of ParA, which is refilled rapidly with ParA rebinding onto the nucleoid. FRAP experiments demonstrate the dynamic exchange of proteins on the nucleoid surface and the partition complex. We present a Par partition model of ParB-stimulated ParA assembly/disassembly triggering dynamic instability leading to plasmid segregation and movement.

Neutron & X-Ray Scattering

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Accurate Structures, Conformations, and Assemblies of Macromolecules in Solution by X-Ray Scattering (SAXS) using Quality Control Parameters John Tainer.
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Conformations and assemblies of proteins, DNA, and RNA, plus their detailed structural chemistry, encode key information needed to define biological outcomes in cell biology. We are developing SAXS combined with crystallography as a premiere tool for defining macromolecular conformations and connections suitable to join proteins to pathways and at the proteomic scale1. Crystallography supplies unsurpassed structural detail for mechanistic analyses. Yet, advances in SAXS are making this technique increasingly powerful and robust for efficiently examining complexes in solution, as aided by interfaces allowing biologists to do these experiments in 3. Structures of flexible filamentous the XLF vs XRCC4-Ligase IV-A5; the conformational and flexible Rad50 link to Mre11, as well as of DNA-PKcs in complex with Ku and DNA7 support the promise of SAXS for examining the assemblies and conformations of dynamic complexes in solution8. In principle, SAXS can provide reliable solution data on small and large macromolecules1-2. In practice, SAXS can be limited by problems in samples and analyses, which can be reduced or avoided. SAXS has not been perceived as a legitimate structural technique largely due to previous limitations from data collection and interpretation. Our emerging results show that SAXS has great potential to provide accurate shapes, conformations, and assembly states in solution and inform biological functions in fundamental ways1-7. Our results furthermore show that many SAXS limitations can be overcome by improved sample characterization, quality control parameters and methods to assess model accuracy.

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Data Collection and Processing for Simultaneous Small- and Wide-Angle Protein Solution Scattering
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The procedures used at beamline X9 of National Synchrotron Light Source for collecting and processing protein solution scattering data are described. The scattering data are collected simultaneously on two CCD detectors, one (SAXS) located at the end of the ~2.5m-long flight path and the other (WAXS) at ~0.4m from the sample in vacuum. A package of python scripts, named pyXS, are used to apply data corrections pertain to the scattering geometry and reduce the 2D SAXS and WAXS CCD images to a combined 1D curve spanning the full q-range, typically from ~0.007 Å-1 to 2.0 Å-1. The subtraction of buffer scattering is performed based on either the intensity of water scattering peak near 2.0 Å-1, or the combination of the protein volume fraction and transmitted beam intensity. Fluorescence from a salt solution is utilized to calibrate the X-ray energy as well as to determine the flat field response of the detector and the geometric corrections. Details of the data conversion and corrections, as well as example data, will be presented.

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Solution Conformation of Extracellular Matrix Proteins
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The extracellular matrix (ECM) is composed of large, multi-domain proteins with varying degree of shapes and post-translational modifications. Therefore, it is challenging to study such proteins with a single technique to gain reliable information about their size, shape and conformation. We have employed a multidisciplinary approach using a variety of techniques to investigate the solution conformation of laminin gamma-1 short arm, netrin-4 and G3 domain of agrin fused with human IgG-Fc (G3Fc). Briefly, the hydrodynamic properties of entire multi-domain proteins were studied using analytical ultracentrifugation, dynamic light scattering, and small angle X-ray scattering (SAXS). Further, the information from SAXS for entire multi-domain protein was then either used to determine ab initio structure or combined with high-resolution data for individual domains to obtain a detailed solution structure of desired multi-domain protein. Additionally, the SAXS models were verified by comparing the experimentally determined hydrodynamic parameters with the parameters calculated from solution structures. The ab initio structures for laminin gamma-1 short arm revealed an extended and curved assembly. We could also combine small portion of high-resolution structure from X-ray crystallography data to obtain rigid-body models. Further, we found that the netrin4 has an elongated shape in solution using ab initio methods. Remarkably, we found that the G3Fc chimera is T-shaped and not Y-shaped like an antibody in solution by rigid body modelling. Thus, we conclude that by combining various methods, it is possible to explore large multi-domain protein and protein-protein assemblies that will help enable us to understand structure-function relationship of these proteins with various forms of diseases.