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overall similar to dsDNA. Employing our novel magnetic torque tweezers assays [1], we have probed the torsional response of dsRNA and again find a behavior that is generally similar to dsDNA. Surprisingly, measurements of the twist-stretch coupling reveal a striking difference between dsRNA and dsDNA. While DNA lengthens when overwound, RNA shortens. In addition, we have studied the dynamics of the buckling transition and discovered that the characteristic time scale of the transition is about two orders of magnitude slower for RNA than for DNA.
We expect that these measurements of the fundamental properties of dsRNA can help refine our models for twist-storing polymers and inform quantitative models of RNA function in vivo.

[1] Lipfert, et al. Nature Methods (2010)
[2] Lipfert, Wiggin, et al., Nature Communications (2011)
[3] Janssen, Lipfert, et al., Nano Lett. (2012)

Subgroup: Bioenergetics

8-Subg

The Mitochondrial Uniporter: From Molecular Discovery to Physiology

Vamsi Mootha.
Harvard Medical School, MA, USA.

Calcium uptake via the mitochondrial ''uniporter'' was documented nearly 50 years ago, and has been studied extensively at the physiological level. This channel is hypothesized to be crucial to disease pathogenesis, yet its molecular identity has remained elusive. In this talk I will present our work over the past few years that has combined mitochondrial proteomics and comparative genomics to identify MCU (the putative pore-forming subunit) and MICU1 (a key regulatory partner). The molecular characterization has enabled us to gain new insights into the function and physiology of this channel complex.

9-Subg

Molecular Definition and Functional Role of the Mitochondrial Calcium Uniporter

Rosario Rizzuto.
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Mitochondria rapidly accumulate Ca^{2+} through a low-affinity uptake system (the mitochondrial Ca^{2+} uniporter, MCU) because they are exposed to high $[Ca^{2+}]$ microdomains generated by the opening of ER Ca^{2+} channels. These rapid $[Ca^{2+}]$ changes stimulate Ca^{2+}-sensitive dehydrogenases of the mitochondrial matrix, and hence rapidly upregulate ATP production in stimulated cells. Ca^{2+} also sensitizes to cell death mediators, e.g. ceramide. Accordingly, we demonstrated that Bcl-2 reduces the state of filling of ER Ca^{2+} stores, and this alteration is effective in reducing the sensitivity to apoptotic challenges. I will discuss our recent discovery of the molecular identity of the MCU, i.e. the key molecule of mitochondrial Ca^{2+} homeostasis. I will present the strategy, and the experiments, that allowed to identify the protein, that remained elusive for 50 years. Then, I will present data that clarify the composition and the regulatory mechanisms of this highly sophisticated signaling machinery. Finally, I will show that molecular targeting of MCU allows novel insight into the regulation of cellular metabolism and cell death processes.
References

D. De Stefani, A. Raffaello, E. Teardo, I. Szabo, R. Rizzuto (2011) A forty-kilodalton protein of the inner membrane is the mitochondrial calcium uniporter. Nature 476:336-340

10-Subg

Multiple Mitochondrial Calcium Influx Mechanisms: Physiological and Pathological Implication

Shey-Shing Sheu, Ph.D.
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Mitochondrial Ca^{2+} homeostasis is crucial in balancing cell survival and death. Especially mitochondrial Ca^{2+} uptake mechanism across the inner membrane is important for the regulation of ATP synthesis, the amplitude and spatiotemporal patterns of intracellular Ca^{2+} transients, the mitochondrial fission/fusion, and movement, the opening of permeability transition pores, and the generation of reactive oxygen species. Commonly, mitochondrial Ca^{2+} uniporter (MCU) has been considered to be the sole Ca^{2+} influx mechanism. However, several studies have also identified additional Ca^{2+} uptake pathways including rapid mode of uptake (RaM) and type 1 ryanodine receptor (mRyR1) from our and collaborators' laboratory. In this talk, I will focus on the relative contribution of MCU and mRyR1 in mitochondrial Ca^{2+} uptake. By using genetic approaches of knock-down, knock-out, or over-expression of MCU and RyR1, we were able to delineate the differential role of MCU and mRyR1 in regulating mitochondrial Ca^{2+}, energetics, and morphology. Furthermore, the pathophysiological implications of distinct characteristics of MCU and mRyR1 in cardiac excitation and contraction will be discussed.

11-Subg

Mitochondrial Dynamics and Quality Control

Heidi McBride, Ph.D.
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One of the most critical emerging functions for mitochondrial plasticity is the contribution to quality control and the cellular stress response. Mitochondrial hyperfusion is triggered in response to cellular stress or starvation, a process the we recently showed to be activated by the presence of oxidized glutathione. This transient hyperfusion is thought to protect the cell from stress-induced apoptosis. On the other hand, mitochondrial fragmentation is important in the segregation of dysfunctional organelles that have lost their electrochemical potential. These non-respiring mitochondrial fragments recruit the ubiquitin E3 ligase Parkin, which mediates delivery to the autophagosome. The identification of this pathway has cemented our understanding of the intimate links between fission and mitochondrial turnover. Our own lab has recently demonstrated that respiring, tubular mitochondria generate small vesicles that carry selected, damaged proteins to the lysosome. We are working on dissecting the molecular machinery that governs the generation of mitochondrial-derived vesicles, which will be the topic of discussion within this session. Together, the dynamic cycles of fusion, fission and vesicle generation operate at different levels to isolate oxidized proteins, lipids or entire organelles that are targeted for degradation.

12-Subg

Mitochondrial Division Prevents Neurodegeneration

Hiromi Sesaki.
Department of Cell Biology, Johns Hopkins University School of Medicine, Baltimore, MD, USA.

Mitochondria divide and fuse continuously, and the balance between these two processes regulates mitochondrial shape. Altered mitochondrial dynamics is linked to many neurodegenerative diseases. In this talk, I will discuss our recent findings on the physiological and cellular functions of mitochondrial division in postmitotic neurons using in vivo and in vitro gene knockout for the mitochondrial division protein Drp1. When mouse Drp1 was deleted in postmitotic Purkinje cells in the cerebellum, mitochondrial tubules elongated due to excess fusion, became large spheres due to oxidative damage, accumulated ubiquitin and mitophagy markers, and lost respiratory function, leading to neurodegeneration. Ubiquitination of mitochondria was independent of the E3 ubiquitin ligase parkin. Treatment with antioxidants rescued both mitochondrial swelling and cell death in Drp1KO Purkinje cells. Moreover, hydrogen peroxide converted elongated tubules into large spheres in Drp1KO fibroblasts. Our findings suggest that mitochondrial division serves as a quality control mechanism to suppress oxidative damage and thus promotes neuronal survival.

Subgroup: Biopolymers in vivo

13-Subg

DNA-Mediated Signaling

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Many experiments have now shown that double helical DNA can serve as a conduit for efficient charge transport over long distances. We have seen, for example, that oxidative damage to DNA can be promoted from a distance as a DNA-mediated redox process. Photophysical, electrochemical and biochemical experiments have been conducted to characterize this chemistry. Uniquely, this chemistry is exquisitely sensitive to perturbations in the DNA base stack, such as arise with base mismatches, lesions, and protein binding. We have explored how this chemistry may be used within the cell for long range signaling. Studies are described where DNA charge transport is utilized in signaling DNA-bound proteins, both to regulate transcription and to activate repair of base lesions under conditions of oxidative stress. DNA charge transport chemistry provides an opportunity to carry out redox chemistry at a distance.

14-Subg

Small Changes in Enzyme Function can Lead to Surprisingly Large In Vivo Effects during Evolution

Yousif Shamoo.
Rice University, Houston, TX, USA.

In principle, evolutionary outcomes could be largely predicted if all the relevant physicochemical variants of a particular protein function under selection were known and integrated into an appropriate physiological model. We have tested this principle by generating a family of variants of the tetracycline resistance protein TetX2 and identified the physicochemical properties most correlated with organismal fitness. Surprisingly, small changes in the $K_{m(MCN)}$, less than 2-fold, were sufficient to produce highly successful adaptive mutants over clinically relevant drug concentrations. We then built a quantitative model

directly relating the *in vitro* physicochemical properties of the mutant enzymes to the growth rates of bacteria carrying a single chromosomal copy of the *tet(X2)* variants over a wide range of minocycline (MCN) concentrations. Importantly, this model allows the prediction of enzymatic properties directly from cellular growth rates as well as the physicochemical-fitness landscape of TetX2. Using experimental evolution and deep sequencing to monitor the allelic frequencies of the seven most biochemically efficient TetX2 mutants in 10 independently evolving populations, we showed that the model correctly predicted the success of the two most beneficial variants $tet(X2)_{T280A}$ and $tet(X2)_{N371I}$. The structure of the most efficient variant, $TetX2_{T280A,}$ in complex with MCN at 2.7 Å resolution suggests an indirect effect on enzyme kinetics. Taken together, these findings support an important role for readily accessible small steps in protein evolution that can, in turn, greatly increase the fitness of an organism during natural selection.

15-Subg

New Directions for Vectorial Protein Folding

Patricia L. Clark.
Department of Chemistry & Biochemistry, University of Notre Dame, Notre Dame, IN, USA.

In vivo, protein folding typically proceeds vectorially, from one end of the polypeptide chain to the other. N- to C-terminal vectorial folding occurs during polypeptide chain synthesis by the ribosome, and transport of unfolded polypeptides through the Sec translocon. C- to N-terminal vectorial folding, while less common, occurs during the maturation of some classes of secreted proteins. For example, members of the autotransporter (AT) family of virulence proteins from Gram-negative bacteria are first secreted across the bacterial inner membrane from N- to C-terminus, and then across the outer membrane from C- to N-terminus. Here we demonstrate that these different folding vectors significantly alter the folding mechanism for pertactin, an archetypal AT protein. Moreover, it is now clear that pertactin and other AT proteins exploit these different folding mechanisms to increase secretion efficiency.

16-Subg

Nanoscopy with Focused Light

Stefan W. Hell.
Max Planck Institute for Biophysical Chemistry, Goettingen, Germany.

Since the 19th century it has been widely accepted that a light microscope cannot see details that are finer than half the wavelength of light (>200 nm). However, in the 1990s it was discovered that this diffraction resolution barrier can be effectively overcome, such that fluorescent features can be resolved virtually down to molecular dimensions. Here we discuss the simple yet powerful physical principles that allowed us to break the resolution limit, with special emphasis on STED and RESOLFT microscopy. We exemplify the relevance of this ‘optical nanoscopy’ to the neurosciences.[1-3]

17-Subg

Structures and Dynamics of the E. Coli FtsZ-Ring and its Associated Proteins during Cell Division Revealed by Superresolution Imaging

Jackson Buss, Carla Coltharp, **Jie Xiao**.
Johns Hopkins University, Baltimore, MD, USA.

Cell division is essential for all organisms. In prokaryotes, the FtsZ protein, a tubulin-like GTPase, plays a central role. FtsZ is highly conserved across bacterial species and has been identified as a novel drug target for antimicrobial therapy. In vivo FtsZ assembles into a supramolecular ring-like structure (Z-ring) at the leading edge of the invaginating membrane. The Z-ring serves as an essential scaffold to recruit all other division proteins and generates contractile force for cytokinesis. Despite the essential role of the Z-ring in bacterial cell division, it is not known what the structure of the Z-ring is or how the contractile force is generated.

We employ the newly developed single-molecule-based superresolution imaging technique to characterize the in vivo structure of the cell division apparatus in E. coli. We discovered that the Z-ring of E. coli adopts a compressed helical conformation in addition to the expected ring-conformation. The Z-ring is likely composed of a loose bundle of FtsZ protofilaments that randomly overlap with each other in both longitudinal and radial directions of the cell. We also observed the structural dynamics of the Z-ring during cell division and found that the Z-ring goes through a condensation phase before it finally collapses toward the end of septum closure . Furthermore, we extend the work into other cell division proteins that play important roles in maintaining the structural integrity of the cell division ring. We imaged two Z-ring binding proteins, ZapA and ZapB, and characterized their influence on the structure of the Z-ring. Our results provide significant insight into the spatial organization of the bacterial cell division apparatus and open the door for further investigations of structure-function relationships and cell cycle-dependent regulation of the Z-ring.

18-Subg

Solving Structure in Situ using Cryo-Electron Tomography and Correlative Methods

John A.G. Briggs.
EMBL, Germany.

Cryo-electron tomography can be used to generate 3D reconstructions of parts of cells in a close to native state. By applying image processing methods to these 3D reconstructions it is possible to obtain structures of protein complexes at a resolution of ~2 nm in situ, within the cell. These methods provide a unique opportunity to solve the structures of complexes from within cells, and at the same time obtain contextual information on the cellular environment around the complexes. I will describe the application of cryo-electron tomography and sub-tomogram averaging methods to understand the structure and assembly of coated vesicles and of enveloped viruses.

Correlative light and electron microscopy methods can be used to link structural information from electron tomography with dynamic information from fluorescence microscopy. I will describe the application of these methods to generate a virtual ultrastructural movie of membrane deformation during clathrin-mediated endocytosis.

Subgroup: Motility

19-Subg

The Mechanism of Cytoplasmic Dynein Motility

Ahmet Yildiz, Ph.D.
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Cytoplasmic dynein is a homodimeric AAA+ motor that transports a multitude of cargos towards the microtubule minus end. The mechanism of dynein processivity and interhead coordination remain unclear due to its large size (2.6 MDa) and the complexity of its structure. In contrast to kinesins and myosins, we directly observed that dynein heads move independently along the microtubule. Stepping behavior of the heads varies as a function of interhead separation and establishing the basis of high variability in dynein step size. By engineering the mechanical and catalytic properties of the dynein motor domain, we show that a rigid linkage between monomers and dimerization between N-terminal tail domains are not essential for processive movement. Instead, dynein processivity minimally requires the linker domain of one active monomer to be attached to an inert MT tether retaining only the MT-binding domain. The release of a dynein monomer from the MT can be mediated either by nucleotide binding or external load. However, nucleotide dependent release is inhibited when force was applied to the linker domain. Force dependent release is significantly asymmetric, with faster release towards the minus-end. On the basis of these measurements, we developed a model that describes the basis of dynein processivity, directionality and force generation.

20-Subg

Path-Finding on the Microtubule

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In long-range transport of cargo, prototypical kinesin-1 steps along a single protofilament on the microtubule, an astonishing behavior given the number of theoretically available binding sites on adjacent protofilaments. Using a laser trap assay, we analyzed the trajectories of several representatives from the kinesin-2 class on freely suspended microtubules, mimicking cargo transport as accurately as possible. In stark contrast to kinesin-1, heterodimeric kinesin-2 motors from diverse organisms displayed an astounding range of rotational pitches, expanding the list of torque generating kinesins to include processive representatives of the kinesin-2 family. We provide direct evidence that neck region of kinesin determines the torque generating properties by reversibly manipulating the properties of the neck of the human kinesin-1 and the heterodimeric kinesin-2 from sea urchin. Disrupting the stability of the neck by inserting flexible peptide stretches results in pronounced left-handed spiraling. Mimicking neck stability by crosslinking significantly reduces the spiraling of the motor up to the point of protofilament tracking.

21-Subg

Mechanisms of Mechanochemical Coupling in Low and High Duty Ratio Myosins

Ralph P. Diensthuber, Falk K. Hartmann, Daniela Kathmann, Katharina Stahl, Manuel H. Taft, **Georgios Tsiavaliaris**.
Hannover Medical School, Hannover, Germany.

All myosins undergo the same ATP dependent biochemical cycle but with variations in the lifetime of the individual states of the cycle and the fraction of the total cycle time spent in each state. E.g., processive class-5 myosins are high duty ratio motors that stay most of the ATPase cycle time (> 0.5) attached