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.
Montreal Neurological Institute, Montreal, QC, Canada.

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