Chemistry-Biology Interface Summer Retreat!

July 26th, 2019

at the University of the Sciences

The McNeil Science and Technology Center Auditorium

4308 Woodland Ave, Philadelphia PA 19026

Guest Speakers

Martin Wuhr (Princeton)

"Proteomic clues to cellular organization"

Daniel Hebert (UMass Amherst)

"The endoplasmic reticulum: the cellular hub for protein maturation and quality control"

Ralph Kleiner (Princeton)

"Chemical approaches to illuminate RNA biology"

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Departments of Chemistry & Biochemistry and Molecular Biophysics

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Chemistry-Biology Interface Summer Retreat

Martin Wuhr (Princeton)

"Systems biochemistry of the metaphase spindle"

The metaphase spindle is composed of chromosomes, microtubules, and an unknown number of proteins. Spindle microtubules are dynamic with an average lifetime of ~20 seconds making isolation of native spindles extremely challenging. We developed methods to isolate metaphase spindles in less than 5 seconds from undiluted Xenopus egg extract via a rapid filtration approach. Using quantitative multiplexed proteomics, we determined the partitioning between spindle and cytoplasm for ~5,500 proteins. We observed over 100 new spindle proteins and confirmed the localization for a subset via microscopy. We were able to globally measure the spindle's proteome turn-over by adding cell lysate from a different frog species and follow the equilibration of these proteins with quantitative proteomics. Lastly, we determined the absolute amount of each protein bound to the spindle. This systems level measurement allowed us to compare the concentration of spindle microtubules with bound microtubule associated proteins (MAPs). In contrast to standard textbook cartoons, microtubules seem to be saturated with MAPs. We demonstrate that MAPs are competing for microtubule binding sites in the spindle suggesting a simple model how spindle composition and morphology could adapt to the drastically changing cell sized in early embryonic development. Thus, we present the first measurement of the composition of a metaphase spindle with endogenous dynamics at molecular resolution. This generated new insight into spindle architecture and might provide a framework to understand how the spindle can adapt its size for different developmental contexts.

Daniel N. Hebert (University of Massachusetts Amherst)

"The endoplasmic reticulum: the cellular hub for protein maturation and quality control"

One third of the proteome is targeted to the secretory pathway in mammalian cells. The endoplasmic reticulum (ER) is the site where proteins cotranslationally enter the secretory pathway after translocation through the ER membrane. The ER houses a number of maturation factors that assist with the folding, modification and assembly of nascent proteins. We are interested in understanding how these factors assist in the efficient maturation of secretory and membrane proteins to their native and functional state. Protein maturation is however an error-prone process. A quality control process is in place in the ER that monitors the successfulness of the maturation process. Properly folded or native proteins are packaged for further trafficking through the secretory pathway, while aberrant products are targeted for destruction. Studies in the lab also focus on understanding the mechanism for the quality control decision and how these proteins are targeted for degradation. By using biochemical and cell biological approaches, we are exploring many of the key roles for this important eukaryotic organelle.

Ralph E. Kleiner (Princeton)

"Chemical approaches to illuminate RNA biology"

Chemical modifications on biological macromolecules play a critical role in cellular physiology. In particular, RNA is extensively modified with a diverse array of post-transcriptional modifications, including "epitranscriptomic" modifications that occur on mRNA. A major challenge is to identify the functional consequences of these modifications and elucidate the molecular mechanisms by which they affect gene expression programs in cells. To address this gap in our knowledge, we are developing chemical biology strategies to study the effects of mRNA modifications on cellular processes. First, we describe a chemical proteomics approach relying upon photocrosslinkable diazirine-containing synthetic RNA oligonucleotide probes and quantitative proteomics to profile cellular readers of RNA modifications. We apply this approach to identify readers of N6-methyladenosine (m6A) and find that m6A disrupts RNA binding by the stress granule proteins G3BP1/2, UPS10, CAPRIN1, and RBM42, providing a link between mRNA modifications and the integrated stress response. Second, we describe a strategy for the metabolic incorporation of non-canonical nucleotides into cellular RNA. We apply protein engineering to uridine-cytidine kinase 2, an enzyme in the pyrimidine nucleotide salvage, to alter its substrate specificity. Remarkably, introduction of this mutant enzyme into mammalian cells enables the incorporation of bulky C5-modified pyrimidine nucleosides into RNA. We have used this approach for the metabolic incorporation of novel biorthogonal and photoactivatable nucleotides for visualizing RNA synthesis and turnover and profiling RNA-protein interactions. Taken together, our work should improve our understanding of fundamental RNA regulatory mechanisms and provide powerful and general strategies for interrogating the function of mRNA modifications.