

## 2.1

**Ribosome Heterogeneity in Translating the Genetic Code**

Naomi Genuth<sup>1</sup>, Victoria Hung<sup>1</sup>, Zhen Shi<sup>1</sup>, Deniz Simsek<sup>1</sup>, Juan A. Osés-Prieto<sup>2</sup>, ALma Burlingame<sup>2</sup>, Maria Barna<sup>1</sup>

<sup>1</sup>Departments of Developmental Biology & Genetics, Stanford University, <sup>2</sup>Department of Pharmaceutical Chemistry, University of California, San Francisco

In the transmission of biological information, the ribosome has been perceived to serve an integral but largely passive role in the synthesis of proteins. Our research has changed this view, by demonstrating that not all ribosomes within each cell are the same and that ribosome heterogeneity provides a novel means for diversity of the proteins that can be produced in specific cells, tissues, and organisms. I will present our work developing a roadmap for the characterization of ribosome composition at a single cell level and during cellular differentiation. We employed a highly quantitative mass spectrometry-based approach to precisely quantify the abundance of each ribosomal protein (RP) as well as a large cohort of ribosome associating proteins (RAPs) belonging to actively translating ribosomes. This led to the identification of subsets of ribosomes that are heterogeneous for RP composition. To address the functional role of ribosome heterogeneity in translational control, we employed CRISPR/Cas9 to endogenously tag and purify heterogeneous ribosome populations. We then developed an adapted ribosome profiling method to precisely quantify and characterize the nature of mRNAs translated by distinct heterogeneous ribosomes genome-wide. This led to the identification of subpools of transcripts, critical for key cellular processes including cell signaling, metabolism, growth, proliferation and survival, which are selectively translated by specific types of ribosomes. Most interestingly, there are specific signaling pathways where almost every single component is selectively translated by specialized ribosomes demarcated by a single RP. I will further present recent findings on mass-spectrometry based proteomics to identify more than 150 phosphosites across 68 core ribosomal proteins. We are currently test whether these phosphosites on the ribosome are dynamic during cellular differentiation and cell signaling. Together, these findings suggest that a “ribosome code” at the level of dynamic regulation in ribosome composition, ribosome-associated proteins, and post-translational modifications endows a new level of regulation to gene expression.

## 2.2

**Deciphering cellular organization using protein thermal stability and solubility**

Mikhail Savitski<sup>1</sup>

<sup>1</sup>EMBL

Determination of proteome-wide thermal stability by the recently developed thermal proteome profiling technology (TPP) can be used to identify drug targets in living cells. This is achieved by combining the cellular thermal shift assay with multiplexed quantitative mass spectrometry. We have recently further developed this technology and substantially increased the sensitivity of detection of thermal stability changes and applied it to study fundamental biology. We have shown that TPP can be used to study complexes and the activity of metabolic enzymes inside cells. In our latest work we have studied the effects of adenosine triphosphate, ATP, and have revealed novel effects of this metabolite on protein thermal stability and solubility. ATP had a wide reaching effect on the stability of protein complexes and remarkably affected the solubility of a large cohort of disordered proteins. Our most recent work uses TPP to understand the proteome organization of *E. Coli*.

2.3

**A proximity biotinylation map of a human cell**

Christopher Go<sup>1,2</sup>, James Knight<sup>1</sup>, Geoffrey Hesketh<sup>1</sup>, Anne-Claude Gingras<sup>1,2</sup>

<sup>1</sup>Lunenfeld-Tanenbaum Research Institute, <sup>2</sup>University of Toronto

Compartmentalization is an essential characteristic of eukaryotic life that ensures cellular processes are partitioned to defined subcellular locations. Large-scale microscopy and biochemical fractionation coupled to mass spectrometry have helped define the proteome of multiple organelles and structures simultaneously. However, many compartments are difficult to quantitatively probe with classical methods, due to lysis and purification artefacts as well as loss of subcompartment resolution. Recently developed proximity-dependent biotinylation approaches such as BioID and APEX provide a new avenue for defining the composition of cellular compartments in living cells. Here we report an extensive BioID-based proximity map of the human cell that uses 192 markers from 32 compartments to identify 35,902 unique high confidence proximity interactions and localize 4,145 of the proteins expressed in HEK293 cells. The recall of our localization predictions is on par with or better than previous large-scale mass-spectrometry and microscopy approaches, but with greater localization specificity. In addition to generating compartment and sub-compartment localizations for many previously unlocalized proteins, our data contain fine-grained localization information that, for example, allowed us to identify proteins localized to ER-mitochondrial contact sites involved in mitochondrial fission/fusion. To aid the community we created the humancellmap.org, a website that allows others to explore our data in detail, and to assist with analysis of their own BioID experiments.

Here, we will describe the resource and its application to selecting BioID-sensors that can profile the recruitment of proteins to different cellular environments, such as the endolysosome surface.

2.4

**Rapid, Sensitive and Multiplexed Ubiquitylation Profiling in Cells and Tissues**

Namrata D. Udeshi<sup>1</sup>, Deepak Mani<sup>1</sup>, Philipp Mertins<sup>2</sup>, Shaunt Fereshetian<sup>1</sup>, Jessica A. Gasser<sup>1</sup>, Shankha Satpathy<sup>1</sup>, Tanya Svinkina<sup>1</sup>, Hasmik Keshishian<sup>1</sup>, Benjamin L. Ebert<sup>1</sup>, Steven A. Carr<sup>1</sup>

<sup>1</sup>Broad Institute, <sup>2</sup>Max Delbruck Center for Molecular Medicine

Global ubiquitylation profiling by mass spectrometry has been a key method for identifying and understanding how protein ubiquitylation sites are regulated in cellular systems. Almost all analyses of ubiquitylation to date have been carried out in cells grown in culture with quantification using SILAC. This approach has been enormously successful, but is limited by the requirement that samples be amenable to metabolic labeling, the need for relatively large amounts of sample (multi-milligram/sample), and that SILAC can only routinely be multiplexed to three. Here, we present a new method that allows for deepscale, quantitative and highly multiplexed ubiquitylome analyses in any biological system. The method is much faster and requires far less sample than prior approaches and permits the comparison of up to 11 conditions without a significant loss in total numbers of identified ubiquitylation sites. Using this method we identify >10,000 ubiquitin sites in cell line and tissue samples in TMT 10/11-plex samples

## 2.5

**Proteomic exploration of lysine acetylation signaling**Chunaram Choudhary<sup>1</sup><sup>1</sup>Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Denmark

Lysine acetylation is a key regulatory posttranslational modification. Function of lysine acetylation is most extensively studied in the context of epigenetic regulation of gene transcription via acetylation of histones. Our laboratory is using mass spectrometry (MS)-based quantitative proteomics to map the scope of acetylation as well as to investigate its dynamic regulation in response to genetic and environmental perturbations. Furthermore, we developed novel proteomic methods to accurately quantify stoichiometry of acetylation on a proteome-wide scale. Our results show that acetylation can occur through both enzymatic and non-enzymatic mechanisms and suggest an important function of sub-cellular compartmentalization in the evolution of acetylation signaling in eukaryotes. I will discuss our recently published and ongoing efforts in understanding acetylation signaling.

## 2.6

**Methods to interrogate the spatial relationship between the transcriptome and proteome on a cell wide scale**Kathryn S. Lilley<sup>1</sup>, Mohamed Elzek<sup>1</sup>, Rayner Queiroz<sup>1</sup>, Tom S. Smith<sup>1</sup>, Mie Monti<sup>1</sup>, Oliver M. Crook<sup>1</sup>, Eneko Villanueva<sup>1</sup><sup>1</sup>University of Cambridge, UK

The complexity of living organisms does not scale with the predicted number of protein coding genes. Many factors contribute to increasing complexity, including non-coding RNA mediated control mechanisms and post-transcriptional and post-translation processing. Additionally, the location of protein synthesis plays a key role in expanding protein functionality, with aberrant spatial translation being a driver in multiple diseases. A thorough understanding of the spatial relationship of the transcriptome, translome and proteome is necessary in order build cellular models of disease. Many methodologies capture subsets of cellular processes and their location. Holistic approaches, however, are required to construct cell-wide models that can give insight into the multi-purposing of components that leads to the expansion of cellular functions.

I will describe new approaches that we are developing to capture the spatial relationship between RNA and protein on a cell-wide scale. We have created several methods to capture the spatial proteome, (1,2), which are based on subcellular fractionation and quantitative mass spectrometry to give whole cell protein atlases. We have significantly modified these approaches in order to capture the transcriptome using subcellular fractionation. To enable proteins and RNA to be recovered from the same subcellular fractions, we have also developed the orthogonal organic phase separation (OOPS) protocol to recover cross-link RNA-protein complexes in an unbiased manner. OOPS is a highly efficient method that enables reproducible recovery of RNA-binding proteins or protein-bound RNA, compatible with downstream proteomics and RNA sequencing independent of polyadenylation status of RNA (3). When using OOPS in conjunction with subcellular fractionation, we can now determine the spatial interplay of the proteome and transcriptome on a cell-wide scale by creating three over-lapping maps:

1. Protein map (LOPIT)
2. RNA binding protein (RBP) map
3. Total RNA map (LoRNA – localization of RNA)

I will describe some unexpected findings from these maps including the RNA binding capacity of many metabolic enzymes and therapeutic targets and the steady state location of mRNA species that code for different protein families.

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