The ribosome is arguably the most important drug target in the bacterial cell, with seven distinct classes of drug inhibiting its action and assembly. These drugs include the macrolides, the safest known anti-bacterials, and the aminoglycosides, the most effective known anti-bacterials. Bacterial disease remains a global health problem not because we lack good drugs but because of the rise of bacterial resistance. The aminoglycosides also suffer from poor therapeutic indices, further limiting their use in the clinic.

We are therefore developing a proteomics approach to enhancing the lifetime and effectiveness of existing anti-bacterial drugs, especially the macrolides and aminoglycosides. We aim to identify targets whose inhibition will lead to synergy with aminoglycosides and macrolides, substantially reducing the likelihood of resistance and enhancing the effectiveness of the drugs. This requires that we can quantify the proteins of the translational machinery and determine the effect of sub-lethal antibiotic doses on the bacterial proteome.

To this end we have designed a flexible QconCAT for the quantification of the E. coli translational machinery by mass spectrometry. A QconCAT is an artificial protein, made up of signature peptides from each of the proteins under study. These peptides are concatenated together and expressed in labelled form using an artificial gene. Our initial QconCAT (the core) contains signature peptides from six central ribosomal proteins L2, L4 and L5 from the 50S subunit and S2, S7 and S8 from the 30S subunit. All these proteins are in place early in the ribosomal assembly process and their presence can be used as markers for ribosomes. The gene encoding the core contains restriction sites into which one or more cassettes encoding other peptides may be inserted. Thus the first cassette encodes signature peptides for the 30S ribosomal proteins.

Most of the ribosomal proteins are small and basic and a digestion protocol involving trypsin alone does not yield sufficient correctly-cleaved peptides for quantification. We therefore employ sequential digestion with endoproteinase Lys-C and trypsin to analyze ribosomal proteins, and the QconCATs are designed for this strategy.

Preliminary results on the effect of the aminoglycoside gentamycin on the bacterial proteome are described. The ribosome is an artificial protein, made up of signature peptides from each of the proteins under study. These peptides are concatenated together and expressed in labelled form using an artificial gene. Our initial QconCAT (the core) contains signature peptides from six central ribosomal proteins L2, L4 and L5 from the 50S subunit and S2, S7 and S8 from the 30S subunit. All these proteins are in place early in the ribosomal assembly process and their presence can be used as markers for ribosomes. The gene encoding the core contains restriction sites into which one or more cassettes encoding other peptides may be inserted. Thus the first cassette encodes signature peptides for the 30S ribosomal proteins.

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Preliminary results on the effect of the aminoglycoside gentamycin on the bacterial proteome are described.

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Acute myeloid leukemia (AML) remains a highly lethal malignancy with limited therapeutic options. Full transformation in AML requires coupling of aberrant molecular events associated with multiple cellular processes. For example, recent work has linked oncogenic FLT-3 kinase, the most common molecular abnormality in acute myeloid leukemia (AML), with functional disruption of otherwise normally expressed C/EBPα, a key transcription factor in hematopoiesis. Moreover, there is growing evidence that combined FLT-3 and C/EBPα karyotypes provide useful prognostic indicators for AML patients. Despite these strong molecular and clinical links, we know surprisingly little about the mechanisms that underlie normal C/EBPα gene activation, and how FLT-3 signaling may interfere with C/EBPα function in the context of AML. We are testing the hypothesis that FLT-3 mediated phosphorylation of C/EBPα governs the assembly of transcriptionally active or repressed protein complexes. Towards this end we have established inducible expression of dual-affinity tagged C/EBPα in myeloid cells that also exhibit constitutive FLT-3 activity. We observe that inhibition of FLT-3 signaling modulates C/EBPα phosphorylation in a dose-dependent manner. Next, quantitative proteomics methodology, including iTRAQ labeling and nanoflow LC coupled with online multidimensional RP/RP fractionation was used to monitor remodeling of C/EBPα protein complexes as a function of FLT-3 mediated phosphorylation. Our data significantly expand upon the known repertoire of C/EBPα interactors, including more than 100 proteins involved in chromatin organization, transcriptional modulation, and cell cycle regulation. Furthermore, our quantitative proteomics data demonstrate that (i) C/EBPα interacts with proteins genetically linked to leukemia; and (ii) many of these interact with C/EBPα in a phosphorylation-dependent manner. Genetic depletion of newly-identified, leukemia-associated protein interactors reduced the ability of C/EBPα to drive expression of granulocytic target genes. In addition we have confirmed co-localization of C/EBPα and proteins identified in our proteomics analysis at the promoters of early, myeloid-specific genes. Collectively our data demonstrate direct physical and functional links between the tumor suppressor C/EBPα and other leukemia-associated, putative oncogenes. Our ability to quantitatively monitor multiple leukemia-related gene products in the context of C/EBPα protein complexes provides valuable insight into the mechanisms by which oncogenic kinase activity disrupts transcription and leads to leukemogenesis.
8.3 Rapid, Near Proteome-wide, Quantitative Analysis of Aneuploid Budding Yeast

N. Dephoure1, E. M. Torres2, J. Villén1, A. Amon2, and S. P. Gygi1

1Harvard Medical School, Boston, MA; 2Howard Hughes Medical Institute, MIT, Cambridge, MA

Mass spectrometry based proteomics holds the promise to extend the global analysis of gene expression afforded by DNA microarrays to the measurement of cellular proteins. Recent advances in methodology and instrumentation have brought the elusive goal of facile global protein measurement closer. However, the extensive analysis time required to achieve high protein coverage renders routine experimentation impractical for most researchers. Using stable isotope labeling with amino acids in cell culture (SILAC) and strong cation exchange chromatography we are able to routinely and reproducibly quantify ~3,000 budding yeast proteins in less than two days of instrument analysis time. We have used this method to characterize protein level changes in a collection of aneuploid yeast strains, harboring two copies of a single chromosome (disomic). In all strains examined to date, proteins coded on the disomic chromosome were enriched ~2-fold, while levels of other proteins remained constant. Such small changes can be extremely challenging to detect by traditional methods such as immunoblotting, but were easily discernible by our method. We also observed a subset of disomic gene products whose levels were unchanged and are trying to understand the mechanisms that allow these proteins to escape the increase in gene dosage.

8.4 Advancing Epigenetics Research by Proteomics: Technologies, Applications and Perspectives

O. N. Jensen

Centre for Epigenetics and Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

“An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence” (Berger et al. 2009).

Histone proteins plays a major role in maintaining chromatin structure, regulating gene activity and DNA integrity. Post-translational modifications of histone proteins that constitute the nucleosome modulate the interactions of these proteins with DNA, transcription factors and chromatin modifying enzymes. There is emerging evidence that combinations of post-translational modifications are key regulators of cellular development and differentiation programs, including epigenetic mechanisms, and that errors in these systems lead to a variety of diseases.

Mass spectrometry plays a prominent role in mapping and quantifying histone modifications, including multi-site, cooperative modifications of histone tails. In this lecture I will describe some of our analytical strategies that are aimed at detailed characterization of histone proteins in the context of pathogenic microorganisms (Salcedo-Amaya et al., 2009; Trelle et al., 2009), drug development (Beck et al., 2006), stem cell research and cancer (Jung et al., submitted). I will also discuss some of the current bottlenecks in functional proteomics and emerging areas of research where proteomics is likely to play a major role.

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