Organellar and signaling proteomics by quantitative mass spectrometry

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Mass spectrometry has emerged as the central technique for the identification and quantification of proteins (Aebersold and Mann, 2003, Steen and Mann, 2004). It is becoming an ideal complement to other large scale functional genomics techniques which tend to be focused at the oligonucleotide or whole organism level. The technology continues to advance rapidly as will be briefly described. In particular, I will describe perspectives opened up by a new linear ion trap Fourier Transform instrument with very high accuracy and sequencing speed (LTQ-FT). Importantly, the advent of quantitative proteomics with stable isotope incorporation now makes it possible to directly compare levels of large numbers of proteins in mass spectrometric experiments.

In one of these quantitative techniques - SILAC (Stable Isotope Labeling by Amino acids in Cell culture) - , cells are completely metabolically labeled with a 'heavy' analog of an essential amino acid. This makes the labeled proteome distinguishable for the unlabled one by mass spectrometry without otherwise changing the cells. Peptides then occur in pairs and the peak ratios indicate the ratio of proteins in the two states. We have employed these techniques in a wide range of signaling applications, for example, to determine low affinity as well as signal dependent interactions. One cell population is stimulated and a phospho-binding entity, such as a SH2 or PTB domain, is used as a bait to specifically enrich activated, phosphorylated signaling molecules (such as receptor tyrosine kinases) and associated proteins (Blagoev et al, 2003). Antibodies directed against phosphotyrosine can be used in a similar but more generic method to reveal quantitative changes in phosphotyrosine signaling in response to stimuli or disease. A similar technique was used to define SH3 – proline rich domain interactions (Schultze and Mann, JBC, 2004).

Time resolved profiling of global phosphorylation states is also beginning to be possible. In HeLa cells stimulated with EGF, we have sequenced several hundred proteins which were immunoaffinity purified with anti-phosphotyrosine antibodies, including virtually all previously described tyrosine phosphorylated proteins. SILAC was used to determine activation state both as a function of time and as the result of different stimuli and to quantitatively follow activation of these substrates over time as a result of phosphorylation(Blagoev 2004).

Organelles can also be studied in much more depth by novel proteomic technologies. We will discuss the examples of the centrosome, mitochonria and nucleolus; in all these cases, novel insights have emerged from proteomics analysis (Andersen et al, Nature, 2003; Mootha et al Cell, 2003).

Department of Biological Sciences

Structural Biology and Functional Genomics Lecture Series 2004

Seminar Announcement

Date: 19 Nov 2004, Fri
Time: 4 pm
Venue: LT 20
Host: A/P Maxey Chung

All are welcome

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