6.1

Global Approches to the Analysis of PTMs

Helmut E. Meyer[1], Katrin Marcus[2], Heike Schaefer[2], Daniel Chamrad[1], Joerg Reinders[2], and Albert Sickmann[2]

[1]Protagen AG, Emil-Figge-Str. 76A, D-44227 Dortmund, Germany; and [2]Medical Proteom-Center, Ruhr-University Bochum, ZKF E 0.43, D-44801 Bochum, Germany

Analysis of posttranslational modifications (PTMs) in proteins is an important step in understanding the changed functional properties of many proteins upon such a modification. Thus, one of the major challenges in proteome studies nowadays is the detection, analysis and localization of these PTMs in vivo. Due to their unique properties during the usually applied analytical techniques the analysis of many of such PTMs is quite difficult. Additionally, in most cases only a small part of protein is modified. Anyhow, the advancement of different technologies like mass spectrometry, 2D-PAGE or 2D-HPLC potentiated the successful identification of different PTMs (Sickmann et al. (2001); Ficarro et al. (2002); MacCoss et al. (2002)).

Despite these promising results another problem arises: in fact it is possible to get information if and how the analysed proteins are modified. Indeed, the functional characterisation of the respective PTMs in all these proteomic-approaches is complicated. Therefore, for a complete protein characterisation more starting material is needed allowing to elucidate the functional properties of distinct PTMs. Beyond this, proteomic approaches have to be combined with molecular biological techniques to get answers to global questions. However, such analyses need a lot more time and effort to become completed.

Sickmann A, Marcus K, Schafer H, Butt-Dorje E, Lehr S, Herkner A, Suer S, Bahr I, Meyer HE (2001) Identification of post-translationally modified proteins in proteome studies, *Electrophoresis* May;22(9):1669–76

Ficarro SB, McCleland ML, Stukenberg PT, Burke DJ, Ross MM, Shabanowitz J, Hunt DF, White FM (2002) Phosphoproteome analysis by mass spectrometry and its application to *Saccharomyces cerevisiae*, *Nat Biotechnol.* Mar;20(3):301–5

6.2

A Novel Approach to the Quantitation of Differential Protein Expression

Gavin E. Reid[1], Richard A. J. O'Hair[2], and Richard J. Simpson[1]

[1]Joint Proteomics Laboratory, The Ludwig Institute for Cancer Research and The Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, 3050, Australia; and [2]School of Chemistry, University of Melbourne, Parkville, Victoria, Australia

With completion of the first draft of the human genome sequence, the challenge now facing medical research is to understand gene function. However, the biological function of a gene cannot be determined from a simple examination of its sequence. Comprehensive analysis of the proteins expressed by the genome, therefore, promises to bridge the gap between the gene and its biological function. The term proteomics has become synonymous with the high-throughput identification and characterization of all proteins secreted by a particular cell type, and quantitation of global changes in protein expression between healthy versus diseased cells (i.e., expression proteomics), and with the identification of components of functionally active protein complexes and characterization of the intricate protein-protein interactions involved in intracellular protein trafficking and signaling pathways (i.e., cell-mapping proteomics). Taken together, these approaches allow comprehensive examination, at the protein level, of the complex cellular changes that occur following transformation of cells from a normal to a diseased state.

Over the last few years, a variety of approaches for quantitative measurement of differential protein expression, exemplified by the isotope-coded affinity tag (ICAT) technique, have been demonstrated. However, there remains a need for the development of improved technologies in expression proteomics, particularly for the quantitation and identification of trace-abundance proteins.

In this presentation, an overview of recent advances in the use of mass spectrometry based methods for quantitation of differential protein expression will be presented. Additionally, a novel derivatization strategy will be described that, when coupled with tandem mass spectrometry, provides enhanced selectivity and up to two orders of magnitude greater sensitivity over existing methods.

6.3

Proteomics as a Process: Using Technology Optimally

Walter Blackstock, Jyoti Choudhary, and Toby Mathieson

Cellzome AG, 160 Centennial Park, Elstree, Herts WD6 3SH, United Kingdom

It is widely assumed that proteomics is more complex than genomics, and that today's tools, while adequate, are not optimum, and so the search must continue for the ultimate proteomics analyzer. This focus on technological salvation is inappropriate. Proteomics is a process involving many steps, from asking a good biological question, to experimental design, sample preparation, analysis, validation and informatics. Asking well thought out biological questions and using the best tools available to answer them is more important than throughput alone. Mass spectrometry has become the method of choice to characterize proteins, and progress in instrument design over the past decade has been outstanding, so much so, that today it is arguable whether mass spectrometry limits proteomic endeavour. We will consider the key "break points" in proteomics, and assess whether effort is directed in the right places by considering sample introduction, operating conditions, and use of protein databases. We conclude that while proteomics is already very effective, further substantial improvement can come at by thinking about the process in its entirety.

6.4

Gel-free Quantitative Proteomics

Joël Vandekerckhove, Jozef Van Damme, Marc Goethals, An Staes, Grégoire Thomas, Lennart Martens, Hans Demol, Magda Puype, and Kris Gevaert

Ghent University, Ghent, 9000 Belgium

The peptide-based proteome approach presented here, is based on the concept of "diagonal chromatography" and has been adapted in order to sort subsets of peptides from mixtures containing more than 50,000 components (COmbined FRActional DIagonal Chromatography: COFRADIC™).

In a first application we generated the *E. coli* K12 proteome using only 50 million cells and we selected for methionine-containing peptides. Using two different search algorithms we identified 872 different proteins. The method is 10 to 100 times more sensitive than conventional 2D-gel analysis. We identified abundant and low copy number proteins as well as a large number of membrane proteins. Using trypsin-mediated oxygen-16 or oxygen-18 incorporation or using differential *N*-terminal labelling COFRADIC™ was used as quantitative approach.

COFRADIC™ is highly versatile and can be used to sort for Cys-peptides, phospho-peptides etc . . . Examples of each of these applications will be shown.

6.5

Flow Cytometric Isolation of Human Antibodies from a Nonimmune *Saccharomyces cerevisiae* Surface Display Library

Michael Feldhaus

Battelle/Pacific Northwest National Laboratory, 790 6th Street, Richland, Washington 99352

A nonimmune library of 10^9 human antibody scFv fragments has been cloned and expressed on the surface of yeast, and nanomolar-affinity scFvs routinely obtained by magnetic bead screening and flow cytometric sorting. The yeast library can be amplified 10^{10}-fold without measurable loss of clonal diversity, enabling effectively indefinite expansion of the library. The expression, stability, and antigen binding properties of more than 50 isolated scFv clones were assessed directly on the yeast cell surface by immunofluorescent labeling and flow cytometry, obviating separate subcloning, expression, and purification steps and thereby expediting the isolation of novel affinity reagents. The ability to use multiplex library screening demonstrates the utility of this approach for high throughput antibody isolation for proteomics applications. Immuno-precipitations, westerns blots, cell surface labeling, and ELISA have all utilized the purified scFv antibodies. Furthermore, we have used molecular evolution techniques to rapidly increase the affinity and specificity of several novel scFv antibodies.