Meeting report

Lipids join the post-genomic era

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A report of the meeting 'From Proteomics to Lipidomics - Basics, Advances and Applications', Bonn, Germany, 30 June-1 July 2006.

The global analysis of biomolecules such as proteins, lipids and carbohydrates within a tissue, cell or organelle under specific physiological situations is a major challenge of the post-genomic era. Experts from the fields of proteomics, lipidomics and metabolomics discussed this challenge at a recent meeting on these topics in Bonn.

Our ability to determine large numbers of proteins from a biological sample has dramatically increased through the development of soft-ionization techniques and the growing availability of state-of-the-art mass spectrometers, even in non-specialist laboratories. One of the pioneers in applying mass spectrometry to biomolecules, Franz Hillenkamp (University of Münster, Germany), gave an excellent introduction to the basic mechanisms of matrix-assisted laser desorption/ionization (MALDI). He discussed the use of MALDI-MS for the imaging of biological tissue and proposed the use of infrared-laser rather than the more commonly used UV-lasers for this aim. Using this different setting, the critical matrix preparation step can be avoided and analytes can be directly ionized, thereby preventing artificial results. Boris Macek (Max Planck Institute of Biochemistry, Munich, Germany) gave an introduction to electrospray-ionization (ESI) mass spectrometry and described the use of high-resolution Fourier-transform mass spectrometers to perform top-down mass spectrometry of intact proteins and high-throughput analysis of whole proteomes. In both instances, internal calibration on an ion with a known mass (known as the lock mass) resulted in a resolution power of more than 60.000 and mass deviations of less than 2 ppm. This new degree of accuracy will boost the level of confidence with which proteins can be identified and is therefore crucial for future proteomic research. Alexandre Shvartsburg (Pacific NW National Laboratory, Richland, USA) showed that the analysis of ions using field-asymmetric waveform ion-mobility spectrometry (FAIMS) in conjunction with mass spectrometry could be used to perform ultrahigh-throughput studies on conformational isomers of proteins even within complex mixtures.

Although many proteins in complex samples can currently be identified using these approaches, the description of a full proteome of a whole organism such as yeast still provides a great challenge. This is, as Rudi Aebersold (Institute of Molecular Systems Biology, Zürich, Switzerland) pointed out, due to considerable under-sampling by the mass spectrometers now in use and the enormous complexity of the samples studied. A possible solution to the complexity problem and the resulting under-sampling may be offered by the technique presented by Joel Vandekerckhove (University of Gent, Belgium). The method, which he termed combined fractional diagonal chromatography (COFRADIC), relies on the analysis of a subset of specific peptides from a given protein such as the amino-terminal peptide or cysteine-containing peptides. The information gathered from this approach is sufficient to identify proteins from the analysis of a single peptide and thus considerably reduces the degree of complexity within a sample.

To get quantitative information on the amount of a protein and its interactions and relationships with other proteins, an enormous amount of information not only has to be recorded but also has to be analyzed. Peer Bork (European Molecular Biology Laboratory, Heidelberg, Germany) introduced the protein network analysis tool STRING developed in his lab (available from the STRING website [http://string.embl.de/]), which integrates data from more than 30 million predicted and experimentally derived protein interactions and offers a variety of interaction and network-analysis options. For the display of network data,

the Bork lab has created MEDUSA, a graph viewer [http://coot.embl.de/medusa/]. A further step towards a full description of a simple biological system was provided by Uwe Sauer (Institute of Molecular Systems Biology, Zürich, Switzerland), who described his work on the metabolic flux of ^{13}C among different metabolites within a bacterial cell. His work revealed that metabolic networks within bacteria are surprisingly robust and rigid, with the relative flux of carbon not being affected by random genetic perturbations. This stability sheds new light on our view of how metabolic pathways evolve.

Despite the limitations in current technologies, mass spectrometry is frequently used to improve the diagnosis of human diseases and to aid drug discovery. One big hope is that high-throughput comparative proteomics will be able to detect biomarkers that have a diagnostic value. However, as Denis Hochstrasser (Geneva University Hospital, Switzerland) pointed out, any marker that is to be useful in clinical practice has to have low false-positive and false-negative rates - a criterion that has so far not been met by any marker detected purely by mass spectrometric methods. The use of mass spectrometry in drug discovery is showing more promise. For example, Bernhard Küster (Cellzome AG, Heidelberg, Germany) who reported the use of immobilized small-molecule kinase inhibitors to purify their target kinases from cellular extracts and the subsequent identification of the kinases by liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods. These experiments revealed new specificities for the kinase inhibitors for enzymes they were not initially targeted to. These surprising results can be used to improve inhibitor specificities and to predict possible new applications for these small molecules.

The important roles of lipids in cell, tissue and organ physiology have been demonstrated by a large number of genetic studies. The cellular lipidome comprises more than 1,000 different lipids, and they are generally accepted to be key factors in the regulation of cell function and to be important players in diseases such as cancer, diabetes and neurodegenerative diseases. New techniques such as LC-MS/MS and the coupling of triple quadrupole and hybrid quadrupole ion-trap instruments to high-resolution LC are being applied to lipidome analysis. Jasna Peter-Katalinic (Institute of Medical Physics and Biophysics, Münster, Germany) discussed methods that allow the efficient ionization of glycolipids using either ESI and/or MALDI. She showed that ESI, in combination with accessory tools such as capillary electrophoresis, could be very useful for the analysis of complex mixtures from body fluids, tissue culture and human tissues. Used with automated sample delivery such a combination would then allow high-throughput screening. Andrei Shevchenko (Max Planck Institute, Dresden, Germany) provided an interesting insight into the high mass resolution LTQ-Orbitrap mass spectrometer (Thermo Electron Corporation). LTQ-Orbitrap machines are very useful for qualitative applications such as shotgun lipidomics; for example, under pathophysiological conditions altered lipidome profiles can be analyzed very rapidly, providing qualitative and quantitative data. Alfred Merrill (Smithgall Institute, Georgia, USA) addressed the problem of visualization the enormous amounts of data being generated. This requires models that facilitate pattern recognition, considers changes with time, and allows a comparison among groups (see the lipidmap and sphingomap websites [http://www.lipidmaps.org] [http://www.sphingomap.org]). Describing the application of these tools to sphingolipid metabolism, Merrill reported that when serine palmitoyltransferase was stably overexpressed (this is the first enzyme of the sphingolipid biosynthesis pathway that is thought to induce ceramide biosynthesis), galactosylceramide instead of glycosylceramide was formed.

It will be a challenge to understand how cells use their thousands of different lipid molecules to regulate their structure and function. As an approach to this goal, Gerrit van Meer (Bijvoet Center and Institute of Biomembranes, Utrecht, The Netherlands) emphasized the concept of cellular lipidomics. This field embraces the enzymes of lipid metabolism, and their transport, specificity, localization and regulation. He described an example of how Golgi ceramide pools might regulate the pH of intracellular compartments. He and his colleagues have studied the transmembrane flippase that is responsible for flipping glycosylceramide continuously to the exoplasmic surface of a membrane. Glycosylceramide binds to V-ATPase in the *trans* Golgi network, which can be stimulated by some glycolipids. In mutants unable to synthesize glycosphingolipids the pH in the compartments was one unit higher than in controls.

Mechanisms of protein-mediated translocation of lipids across cellular membranes, their intracellular transport to distinct organelles, and protein-mediated lipid transport were also the focus of the talk by Thomas Pomorski (Humboldt University, Berlin, Germany). His group studies protein mixtures enriched in flippase members of the family of plasma membrane P4-ATPases (Dnf1p and Dnf2p) in yeast, which are most probably involved in inward ATP-driven lipid transport. Their studies have established yeast as a promising model for the analysis of protein-mediated translocation of lipids. Nevertheless, better methods are needed to study this process at the subcellular level. Pomorski uses photoactivatable and fluorescent lipids that mimic natural lipids as promising tools to study lipid translocation.

In vivo studies give insight into the role of lipids in development, differentiation, signaling and disease. Julie Saba (Children's Hospital and Research Center, Oakland, USA) reported her analysis of the role of sphingosine 1-phosphate (S1P) and the enzyme responsible for its

catabolism, S1P lyase (SPL), in *Drosophila* development and in colon cancer. S1P is a sphingolipid metabolite that regulates cell proliferation, migration and apoptosis through specific signaling pathways. Using *Drosophila* as a model system, Saba showed that complex developmental pathologies can be directly linked to a disruption of sphingolipid catabolism. Extending her studies to mice, she showed that SPL is downregulated in early adenomatous lesions of the Min mouse model for intestinal tumorigenesis. In addition, SPL expression was significantly downregulated in human colon cancer tissues, the first example of altered SPL expression in a human tumor. On the basis of these findings she suggested that endogenous SPL might play a physiological role in stress-induced apoptosis. The role of lipids, especially sphingolipids, in disease was further emphasized by Michaela Dragusin (Kekulé Institute of Organic Chemistry and Biochemistry, Bonn, Germany). She showed that the function of intestinal smooth muscle cells is particularly affected by the activity of both ceramide 1-phosphate (C1P) and S1P. Thus, sustained raised concentrations of the two bioactive sphingolipids in this tissue after intestinal surgery could, at least in part, explain postoperative intestinal dysmotility.

The emerging field of systems biology tries to integrate all information that can be gathered for a particular biological system in order to derive predictive models for its reaction to external or internal disturbances. Meetings like this symposium contribute to the progress of systems biology by bringing together researchers with expertise in the multiple '-omics' technologies. The formation of such research networks will ultimately help us to better understand the network of life.