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Proteomics and Mass Spectrometry

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Editorial

Proteomics is a brand-new 'post-genomic' science with a lot of promise. Proteomics, unlike gene expression studies using oligonucleotide chips ('transcriptomic'), directly addresses the level of gene products present in a particular cell state and can characterise protein functions, relationships, and subcellular distributions. Proteomics has been used to investigate the protein composition of organelles, the systematic elucidation of protein-protein interactions, and the large-scale mapping of protein phosphorylation in response to a stimulus, among other things. Proteomics is now undergoing significant development, particularly as a tool for displaying and detecting all cellular proteins in a format appropriate for further research. Separation science for the separation of proteins and peptides, analytical science for the identification and quantification of analytes, and bioinformatics for data management and analysis are all used in these analyses. Its first version combined high-resolution two-dimensional gel electrophoresis (2DE) with IEF (isoelectric focusing)/SDS-PAGE gel for the separation, detection, and quantification of individual proteins present in a complex sample with mass spectrometry and sequence database searching for the identification of the separated proteins [1-3].

Quantitative proteomics

The use of DNA chips to profile the transcriptome is a common and powerful tool of functional genomics. Although still unsatisfactory, current technology allows for the monitoring of entire transcriptomes and the processing of huge quantities of samples in common experimental formats. Quantitative proteomics, on the other hand, is still far from fully describing complete proteomes and has a poor throughput. Biological processes, on the other hand, are largely governed by proteins, and mRNA is only a proxy for estimating protein abundance [2,3]. Protein levels and activities are determined by a variety of variables other than mRNA abundance, including controlled protein degradation and post-translational modification. The old technology of stable-isotope labelling has been adopted for protein analysis to give a quantitative dimension to non-2DE-based proteome investigations. The procedure entails adding chemically similar but stable isotopically tagged internal standards to a sample. Because the ionisation efficiency of different peptides varies greatly, the only internal reference for a candidate peptide is the same peptide labelled with stable isotopes. When a protein mixture (reference sample) is compared to a second sample having the same proteins at varied abundances and labelled with heavy stable isotopes, quantitative protein profiling is achieved.

Mass spectrometry's function

Whole genome sequencing has defined all proteins that exist in an organism at the gene level for a growing number of animals. As a result, rather than requiring comprehensive covalent characterisation to identify a protein, partial fragment or sequence information is sufficient to identify the protein at the gene level within a genome sequence database. Mass spectrometric approaches, which are generally based on MALDI-TOF and/or electrospray ionisation with subsequent peptide fragmentation, can now provide the information needed to identify the protein at sensitivities as low as 1 pM. Two mass spectrometric approaches for identifying proteins at the gene level using samples of the quantities commonly available on 2D gels are currently widely used [4,5].

Analysis of protein complexes Individual proteins do not perform the majority of cellular tasks; rather, protein assemblies, also known as multi-protein complexes, do. It is reasonable to presume that proteins that interact specifically also perform the same function. Because it is directly related to protein function throughout biological processes, the discovery of precisely interacting proteins is a vital component of proteomics. In general, the methods for analysing protein mixtures mentioned above are equally suitable for analysing protein complexes. Indeed, this field has produced some of the most scientifically rewarding applications of protein mass spectrometry. The technological advances described in this review, particularly the ability to accurately measure the quantitative changes induced by perturbations on large numbers of proteins and the ability to analyze functional protein complexes, have significantly improved our ability to study biological processes and systems on a global scale. In the subject of proteomics, the following year is going to be both amazing and interesting [1,2].

Interaction proteomics

Mapping protein interaction partners is a great place to start when trying to figure out what a protein does. These interaction partners could put the protein at the centre of a biological process. MS-based proteomics has already produced interaction maps of the yeast proteome. However, because interacting partners may be expressed endogenously at low levels in cells, interactions may be ephemeral, and a significant background of co-precipitating proteins may be present, such tests can be difficult. When it comes to biochemical purification, the trade-off between sensitivity and specificity is always present. Briefly, the bait and a similarly related control are treated separately with cell lysate. Proteins that interact with the bait and control are labelled differently (chemically or metabolically) and combined. Background proteins bind to bait and control in the same way, resulting in one-to-one ratios, but particular interactions with the bait produce differential ratios. Any type of affinity purification, including protein-protein, protein-oligonucleotide, and protein-drug interactions, can use this approach [2].

Mass spectrometry's consequences

We believe that genomics methodologies will be unable to analyze substantial areas of cellular networks and uses, and that technologies like functional proteomics will be a vital and essential element of this effort. Although the area of functional proteomics is still in its early stages, we feel that the results and methods that have previously been published show that it has a promising future, and that current technical advancements hold even more potential for more effective functional proteomics methodologies. Those of you who are more closely involved with mass spectrometry technical development than we are, we hope that this review will inspire you to keep working on the urgently needed technical developments in absolute sensitivity of MS analyses, rapid collection of MS data suitable for phenotypic characterization of proteins, and automation of MS data interpretation on peptides and proteins [4,5].

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Conflict of Interest

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