Fresenius J Anal Chem (2000) 366 : 517–524

REVIEW

Roland Kellner

Proteomics. Concepts and perspectives

Received: 8 September 1999 / Revised: 2 November 1999 / Accepted: 14 November 1999

Abstract Within the last five years the field of proteomics has changed the understanding of molecular biology. Proteins manifest physiological as well as pathophysiological processes in a cell or an organism, and proteomics describes the complete protein inventory in dependence on *in vivo* parameters. Disease mechanism or drug effects both affect a protein profile and, *vice versa*, characterising protein profiles reveals information for the understanding of disease and therapy. Analytical methods for proteomics are based on conventional tools for protein characterisation. The technical challenge is the complete coverage of physico-chemical properties for thousands of proteins. Nucleic acids display a relative chemical homogeneity and therefore genomics was considered more promising in the past than proteomics. Further improvements in proteomics technologies will likely change this course with proteomics complementing genomics as a tool to study life sciences.

A new view of proteins: historical perspective

The US Patent and Trademark Office rejected Craig Venter's application to patent 1350 partial gene sequences just a few years ago. Nevertheless, this was the start of genomics activities and resulted in the founding of numerous genome companies, e.g., Human Genome Sciences HGS. Genomics initiated multimillion-dollar funding not only from the pharmaceutical industry, and now the same holds true for the field of proteomics.

The new term "proteome" or "proteomics" was first introduced in 1995 [1]. Proteome analysis is an attempt to describe the molecular basis of (patho)physiological processes. A proteome is like a snapshot of a physiological scenario. Life is the translation of the static genome into highly dynamic proteomes. Mounting these single snapshots to make a movie, it would describe the dynamics of a living cell. Proteome analysis supplements gene sequence data with protein information about where and in which ratio and under what conditions proteins are expressed. The word "proteome" was designed to denote the protein complement of a genome. The demanding fields of proteins and genomics are remarkably different and nevertheless they are basically interlinked – here single compounds displaying extremely different properties and requiring labour intensive studies, and there a total set of information with high-through put techniques gaining enormous attention. Proteomics and genomics are synergetic (Fig. 1).

The historical perspective of proteome analysis comes from protein characterisation methods [2–5]. A powerful set of protein analytical tools was developed over the years. Techniques became available to separate thousands of proteins in one run, and their identification could be achieved with minute amounts. The prerequisite for the study of biologically relevant questions was fulfilled. Simultaneously the dogma "one gene – one protein" was no longer accepted. The simple view of protein biosynthesis, where a DNA sequence found in a chromosome translates into a corresponding RNA sequence which serves as a blueprint for the ribosome to manufacture an amino acid sequence and the resulting protein develops a three-dimensional structure to play a particular role at a particular place in an organism, was obsolete. In addition, molecular

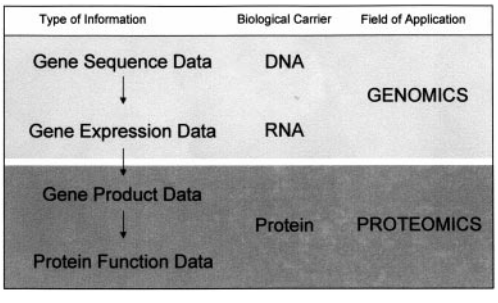

Fig. 1 Genomics and proteomics are synergetic

R. Kellner
Preclinical R&D, Merck KGaA, 64271 Darmstadt, Germany
e-mail: roland.kellner@merck.de

biology has taught us that a gene can undergo different splicings, and that posttranslational modifications can result in several active forms of a protein. Sequence information in DNA is not sufficient to describe life. Proteins are the virtual actors in the life sciences.

What is a proteome and what does proteomics do: a definition

A proteome indicates the quantitative protein expression profile of a cell, an organism, or a tissue under exactly defined conditions. The human genome comprises about 100 000 genes and this gene inventory is applied for a cell type-specific expression of a set of 10 000 genes. One gene will result in multiple protein products, on average 1.3 proteins per gene in *E. coli*, 3 proteins per gene in *S. cerevisiae* and perhaps more than 10 proteins per gene in humans if one also considers body fluids [6]. Therefore, a proteome consists out of about 100 000 proteins. These simple numbers demonstrate the enormous variability in the composition of a proteome and the ability to form an infinite number of phenotypes. Slight modifications in the expression parameters, due, for example, to stress or drug effects, will change the protein pattern and cause the presence or absence of a protein or gradual variations in abundances. Proteomics analyses and compares protein expression profiles and links the observed protein pattern changes to the causal effects. The key steps in proteomics are the rational design of a proteome project, the quantitative determination of expressed protein patterns by biochemical characterisation techniques, and data interpretation and data mining by bioinformatics.

Correlation between gene expression and protein products

Proteomics and genomics are complementary. Genomics has fundamental restrictions in so far that gene sequence data contains insufficient information to understand the function of the gene products. Neither DNA nor mRNA encodes the arrangement for, e.g., a signalling pathway or for a metabolic cascade. The lack of correlation between gene expression and protein function can be described by several features: The cell locus as well as the time point for gene expression can be different to that of the actual protein product, and the activity displayed by the protein will become effective unpredictable by the gene. Posttranscriptional splicing and recombination of RNA can lead to various protein products, and this will multiply the number of components and functions. The rate for synthesis and degradation varies for nucleotides and proteins, and the individual stability gives rise to discrepancies in their correlation. In addition, proteins undergo several co- and posttranslational events. Different functional groups can be added to a protein, e.g., sugars or phosphate. A premature protein is enzymatically cleaved to become active or intra- or intermolecular interactions form a functional oligomer or a protein complex. All these modifications contribute to the complexity of protein products.

The different types of biological information cannot be linked quantitatively. In an attempt to correlate mRNA and protein abundances in human liver [7] a correlation coefficient of 0.48 was determined. A poor correlation between protein and mRNA levels was also reported by a recent comparison for yeast proteins. Some 150 protein spots from *Saccharomyces cerevisiae* were identified by applying 2DE (2-D gel electrophoresis) and mass spectrometry and quantified by using metabolic labelling and scintillation counting [8]. Corresponding mRNA levels were calculated after SAGE (serial analysis of gene expression) analysis [9]. It was not possible to predict protein expression levels from mRNA transcript data. Posttranscriptional regulation of gene expression is a frequent phenomenon in higher organisms.

Proteome strategy and technology

The description of a biological system requires the characterisation of a complex mixture of thousands of proteins, which differ greatly in their physico-chemical properties. There are proteins of very high abundance together with some of very low abundance, and a dynamic range of 10^5–10^6 in protein concentration has to be handled; small proteins (< 10 kD) are present together with large ones (> 200 kD); soluble proteins beneath membrane-bound ones; acidic beneath basic proteins. The sample preparation techniques as well as the protein identification tools must be safeguarded regarding the sample transfer steps and the risk of contamination. Sample transfer steps must be quantitative and prevent unspecific protein adsorption onto surfaces. Major contaminations are introduced as dust through the air or by pipetting tips or sample tubes. The solubility of proteins is tremendously different, ranging from secreted compounds dissolved in their native milieu up to membrane proteins nearly insoluble even in harsh chemicals. Trying to collect and analyse as many proteins as possible from a cell, several solubilisation steps are required, which will give solubility fractions such as buffer-soluble cytosolic proteins, detergent-soluble membrane attached proteins and membrane proteins remaining in the pellet.

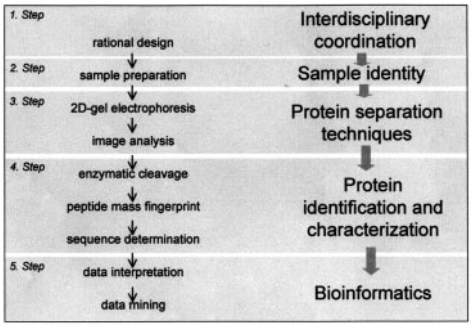

Fig. 2 General strategy for proteome projects

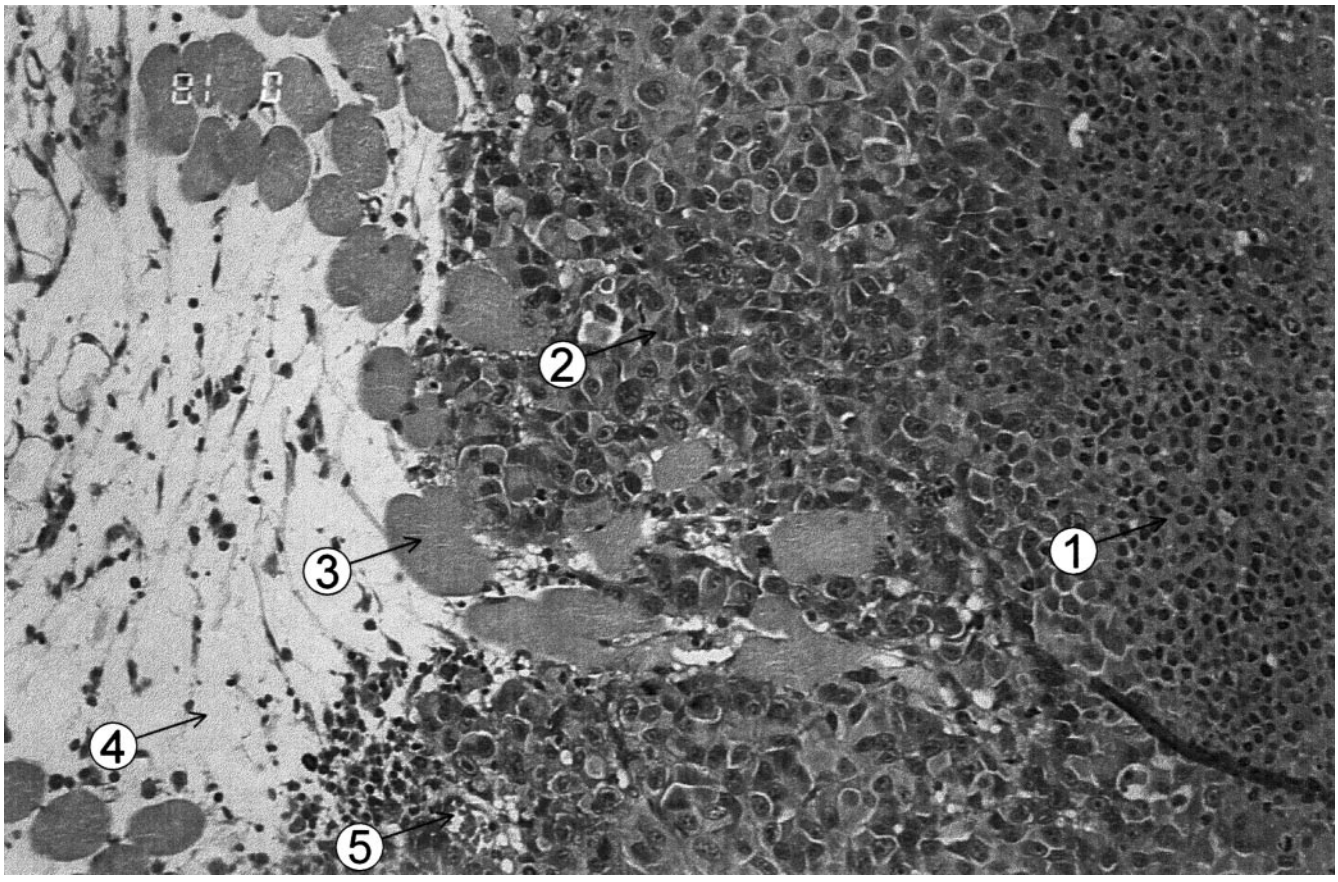

Fig. 3 Heterogeneity of cancer tissue material is demonstrated by numerous cell types present in the sample: *(1)* apoptotic tumour cells; *(2)* tumour cells infiltrating muscles; *(3)* muscle fibres; *(4)* adipose and connective tissue; *(5)* blood vessel containing erythrocytes. (By courtesy of Dr. Fittschen, Merck Darmstadt)

A broad consensus exists about the strategy used for a proteome approach and the techniques, which are involved (Fig. 2) [10, 11].

First step. An interdisciplinary discussion should define the rational design for a proteome project and several questions have to be answered. What is the biological source used for a study? Cultured cell lines are preferred, because manipulations are possible to investigate experimental parameters in the laboratory and reproducibility guarantees samples with sufficient amounts of protein. Tissue specimens display the *in vivo* situation but often they are highly heterogeneous because of various cell types or cell states (Fig. 3). In such a case protein profiles will display a very high background and differences in the protein abundances or low-abundant proteins may not be identified. Is a subcellular fractionation opportune? Is the complete determination of all proteins required or is there a focus on a special group of proteins? Is it necessary to identify the very low-abundant ones?

Second step. Sample preparation will ensure the reproducibility of the experiment. An exact handling, avoiding any quantitative or qualitative change in protein composition, must follow the strictly controlled origin of a proteome. Any prefractionation steps must ensure that no protein decomposition occurs by cellular proteases and that the sample is quantitatively transferred onto the separation device [12].

Third step. Two-dimensional polyacrylamide gel electrophoresis (2DE) is used to separate the protein mixture [13, 14]. Isoelectric focusing (IEF) is applied in the first dimension to resolve proteins by their net charge. The availability of strips with immobilised pH gradients (IPG) was an important step for the accessibility and reproducibility of this technique [15]. In the second dimension polyacrylamide gel electrophoresis (SDS-PAGE) sieves the components according to their apparent molecular weight. 2DE is a sensitive tool enabling one to separate thousands of proteins in one run, but it is a very time and labour intensive method with difficulties in reproducibility. 2DE is the only technique capable of separating complex protein mixtures of more than a thousand components. The protein spots on a 2DE gel are visualised by staining either with Coomassie, silver or a fluorescent dye. A protein profile map is then drawn up by image analysis, displaying and matching the spot pattern on a computer screen. To investigate a new, yet unknown proteome, the spots have to be determined and a 2DE database is built up as a reference map. In comparison to this reference, related proteomes are analysed and the subtrac-

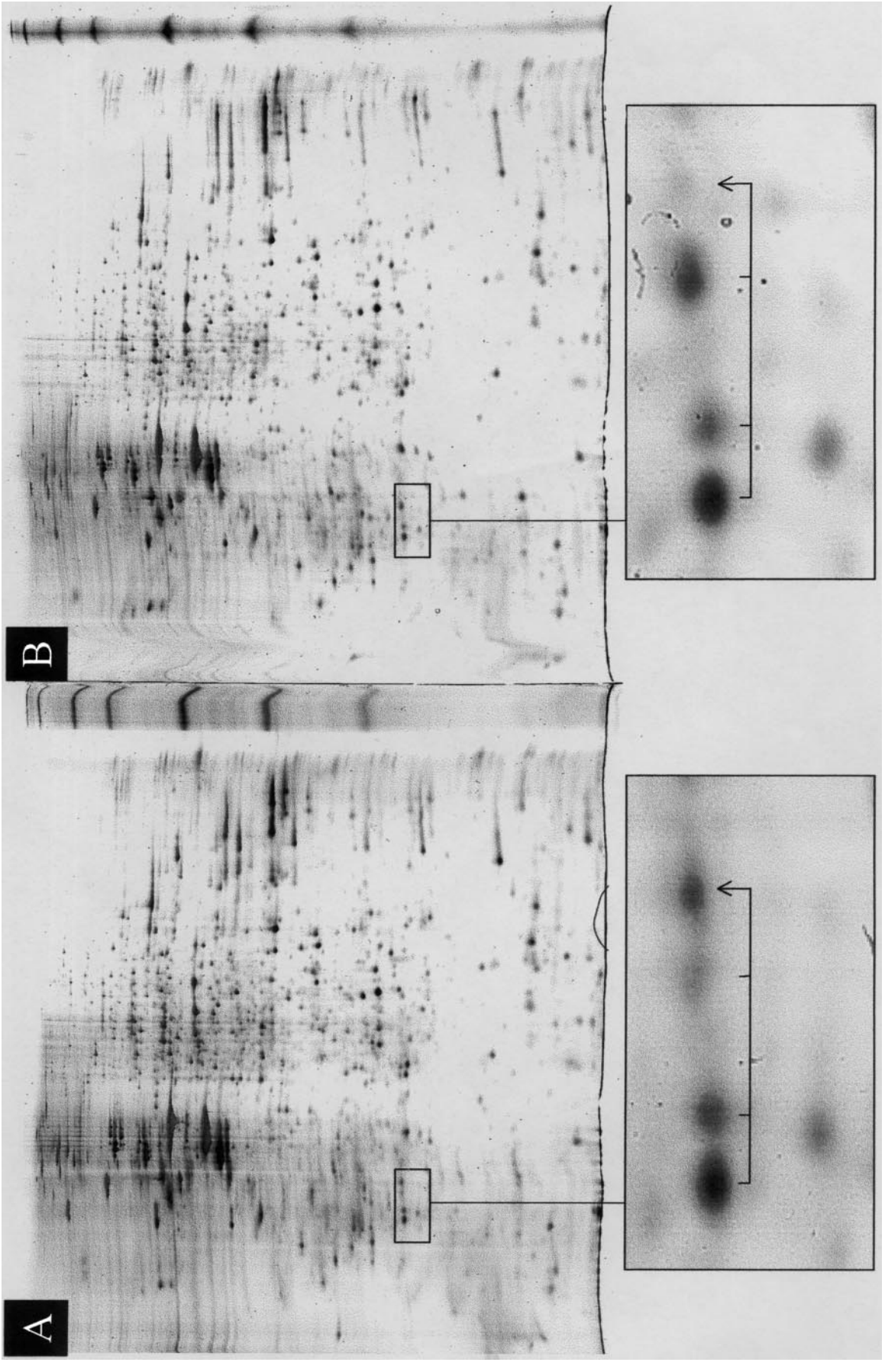

Fig. 4 Separation of cancer cell line lysates by 2D-gel electrophoresis. Cancer cells were grown under standard condition *A* and stimulated by a specific drug *B*. The zoom region shows differentially expressed protein spots that are market by an arrow

tive interpretations will highlight differentially expressed proteins (Fig. 4).

Fourth step. Biochemical methods are used to identify the proteins [16]. The protein spots embedded in the polyacrylamide gel matrix are excised and each spot is digested in-gel using, e.g., trypsin or any other proteolytic enzyme. The resulting peptide fragments are like a fingerprint for their parent protein. Therefore, the peptide masses given in a digest mixture can be determined, and the mass fingerprint obtained is used in a database search in order to identify the protein. A more detailed secondary screening of proteins will become necessary for not readily identified spots, for proteins not yet listed in the database, or for posttranslational modified proteins. For these purposes the chromatographic separation of peptide fragments and sequence determination by MS/MS techniques and Edman degradation are appropriate tools [17–20].

Fifth step. Large volumes of proteomics data are generated and subsequent database searching must keep pace [21]. Peptide mass maps have to be searched against protein and nucleotide databases that require an on-line link of mass data acquisition and submission of the mass list for a search. The specificity of the proteolytic enzyme used for the digest and potential peptide modifications, e.g. cysteine alkylation, are examples that will support an optimised search strategy. Bioinformatics is responsible for classification and mining of the informations acquired. Some helpful software tools are available via the Internet (Table 1).

As such, the procedure and the applied methods do not differ from conventional protein analysis. However, for a proteome study hundreds of proteins have to be handled instead of single compounds and, hence, capacities must be adapted. Because of the exploding workload, it is necessary to automate sample handling [22, 23] and data interpretation [21]. Skilled operators are still required.

The number of separations and analysis necessary to perform a proteome study is flexible, and the sample preparation protocol has a tremendous influence on the calculation of effort and costs:

– a subcellular fractionation can reduce the complexity of the protein mixture;
– narrow range isoelectric focusing will result in an array of gels per sample and, subsequently, the total number of analytical separations is increased;
– the identification of low-abundant proteins requires more starting material and either sample enrichment or sensitivities in the sub-femtomole range are required;
– the characterisation of posttranslational modifications requires additional analytical work.

The quality of results is improved by a high resolution 2DE separation. An overview gel is run first in the pI-range of pH 3–10, and then additional gels are zooming in the "crowded" ranges of, e.g., pH 4–5 or 5.5–6.0. The total number of detected protein spots will increase because of higher resolution. Furthermore, the (quantitative) results are improved substantially by sample repeats of three or five gels per analysis. The total number of 2DE separa-

Table 1 Software tools for proteome data exploration available via the Internet

Tool	Description	Source	URL
DARWIN	Data analysis and retrieval with indexed nucleotide and peptide sequences	ETH Zürich, Switzerland	cbrg.in.ethz.ch
EXPASY	Molecular biology server dedicated to the analysis of protein sequences and structures as well as 2-D PAGE	SIB Swiss Institute for Bioinformatics Geneva, Switzerland	www.expasy.ch
LUTEFISK97	Searching sequence databases via de novo peptide sequencing to tandem mass spectrometry	Immunex Corp. Seattle, USA	www.lsbc.com:70/Lutefisk97.html
MASCOT	A search engine which uses mass spectrometry data to identify proteins from primary sequence databases	Matrix Sciences Ltd. London, UK	www.matrixscience.com
MOWSE	Searching the owl protein sequence database with protein fragment information, and return the protein(s) which most likely correspond to your peptide-data	ICRF Imperial Cancer Research Fund London, UK	srs.hgmp.mrc.ac.uk/cgi-bin/mowse
PEPTIDESEARCH	Search the non-redundant protein sequence database by a list of peptide masses or a peptide sequence tag or an amino acid sequence	EMBL European Molecular Biology Laboratory Heidelberg, Germany	www.mann.embl-heidelberg.de/Services/PeptideSearch/PeptideSearchIntro.html
PROSPECTOR	Tools for mining sequence databases in conjunction with mass spectrometry experiments	UCSF University of California, San Francisco, USA	prospector.ucsf.edu
PROWL	Provides an interactive environment for protein analysis. ProFound: a tool for searching a protein sequence database using peptide maps and peptide fragment masses	Rockefeller University New York, USA	prowl1.rockefeller.edu
SEQUEST	Correlates uninterpreted tandem mass spectra of peptides with amino acid sequences from protein and nucleotide databases	University of Washington Seattle, USA	thompson.mbt.washington.edu/sequest/

tions required to compare two different proteomes can be calculated, for example, one proteome gives three solubility fractions which are separated in an overview gel ranging from pH 3–10 and each fraction is analysed in triplicates. For the comparison of two proteomes it is necessary to run 18 2D-gels. As an alternative, to achieve better reliability for low-abundant proteins, the analysis of 5 gels per fraction should be performed. This requires running 30 2D-gels. The number of separations will furthermore increase with the number of gels for narrow pH fractions in the first electrophoretic dimension and by the number of different proteomes under investigation. These calculations demonstrate the enormous effort that is required for proteome studies regarding just the first two steps of sample preparation and separation. On the other hand, it is a challenge to cut down the number of analyses by improving the separation technique.

The necessity of enhanced protein characterisation gives rise to additional experiments. A protein may be present in low abundance or it may be posttranslationally modified: in both cases characterisation work is tedious and requires higher amounts of sample for analysis. An important point to consider is the knowledge about the genome of the investigated organism. Preferably a fully sequenced genome is known and represents a database to assign every amino acid sequence and to determine the corresponding open reading frame. If this is not the case, e.g. as in the human genome, novel proteins will be detected and additional analyses have to be performed in order to characterise yet unknown proteins.

The interfaces for the consecutive steps require special attention and automation needs to couple the separation and analysis for thousands of components. Once the proteins become visible after 2DE separation, the polyacrylamide gel spots have to be individually excised and transferred into a sample tube for the subsequent proteolytic digest. A so-called ‘spot picker’ or ‘spot cutter’ will do the job and handle some hundreds of gel pieces from multiple gels without human intervention. Commercial instruments are available [24]. Once the excised spots are ready for digestion, several pipetting steps have to be performed to deliver the buffer and protease and afterwards to remove the supernatant containing the peptide fragments, e.g. onto a MALDI target for mass analysis [25]. Finally, the technologies and their links have to be mirrored on the software side and data have to be traced, collected and interpreted. Sample characteristics on history and origin have to be filed; reports for every 2D gel must list spot positions, quantitation and identification; and results from mass analysis are evaluated for corresponding peptide masses and derived protein assignments. Proteomics needs massive bioinformatical support to manage these enormous amount of data.

Several proteomics service companies offer the analytical methods to study proteomes in form of contract research; for example, the Australian Proteome Analysis Facility (Sydney, Australia) [26], Large Scale Biology (Rockville, USA) [27], Oxford GlycoScience (Oxford, UK) [28], Protagen (Bochum, Germany) [29], Protana (Odense, Denmark) [30], Toplab (Munich, Germany) [31]. This list cannot be complete because new companies are continuously emerging, quite often as spin-offs from numerous universities or institutions, which also perform proteomics.

Proteomics in the pharmaceutical industry

Proteomics has gained much attention as a drug development platform because disease processes and treatments are often manifest at the protein level. Drugs ought to produce protein expression effects and, hence, the pattern of protein changes after drug application will give information about the mechanism of action, either for therapeutic or toxicological effects. Various drugs might be compared and grouped according to their signalling cascades or metabolistic pathways. Side effects can also be described if additional proteins are involved [32]. Comparative studies of generica could be of special interest (but it is in conflict with cost arguments). The correlation of the dynamic expression of a proteome and the physiological changes related to a healthy or diseased condition can help to

- support the understanding of disease mechanisms,
- design new ways for the discovery and validation of disease models,
- find new diagnostic markers,
- identify potential therapeutic targets,
- optimise lead compounds for clinical development,
- characterise drug effects,
- study protein toxicology.

In order to describe the potential and perspectives in proteomics some exemplary numbers are helpful [33]. From 30,000 known diseases there are ca. 100 diseases with high medical need. 5–10 genes contribute to a disease phenotype which means that there are 500–1,000 disease-related genes and this corresponds to about 5,000–10,000 protein targets. So far fewer than 500 protein targets are at work, and this means no more than 10% of potential protein targets are already exploited. Proteomics thus resembles a gold mine for target discovery.

Modern drug discovery is a highly competitive process. It is not sufficient to identify a disease-related protein target by proteomics. Required is the understanding of the biochemistry and the regulation of an appropriate protein pathway or cascade, in order to find the best possibility to interfere.

Applications

It is impossible to give a comprehensive description on proteome projects and the following examples can only provide some ideas for further reading.

Several groups started a global analysis of proteins to systematically construct 2DE databases and to apply them for the study of health and disease scenarios. They enable

one to display data on identified proteins by clicking on the protein spots of interest and allow searches by protein name, keywords, Mr and pI or organelle or cellular component. Protein files contain extensive links to other databases (GenBank, EMBL, Swiss-Prot, PIR, MEDLINE, PDB, OMIM, etc.) or Web sites [34].

Protein expression profiles are used in cancer research, for example, to identify tumour subtypes and to achieve a more reliable and objective classification. The identification of markers for bladder cancer was reported that defines the degree of differentiation [35]. The detailed study analysed 150 fresh bladder tumours by 2DE and protein characterisation. Of these, six showed protein expression patterns corresponding to squamous cell carcinomas (SCC), a fact that was later confirmed by the pathologists´s report. Furthermore, a potential marker for SCC was identified as psoriasin, a protein externalised to the urine.

Human heart proteins are filed in 2D-databases [36] to investigate heart disease and transplantation [37, 38]. Alterations in myocardial protein expression of biopsies should provide information about quantitative and qualitative changes for dilated cardiomyopathy compared with ischaemic heart disease and undiseased tissue. Overexpression of some of these proteins can be used to predict the onset of rejection at least 4 weeks prior to a positive histological diagnosis.

The cyclosporine A-mediated nephrotoxicity was investigated by a proteome study of kidney tissue from dogs, monkey and human [39]. Protein profiling using 2DE could show a marked decrease in renal calbindin-D 28 kD protein and suggested calbindin as a marker for cyclosporin A nephrotoxicity. Calbindin acts as a calcium buffer whose loss apparently accounts for failure to excrete calcium and consequent accumulation of calcium deposits in the kidney. This application demonstrates the potential of proteomics to study mechanistic toxicology.

Gene-related functional proteomics is approached by a mouse proteome [40, 41]. On the basis of protein polymorphisms hundreds of genes on mouse chromosomes were mapped, and the genotype and phenotype of proteins were studied. In conclusion it was found that protein modifications can be genetically determined and it may be possible to subdivide genetic diseases into different diseases according to different affected genes.

Alternatives and perspectives

Proteomics should guide us to functional proteomics. But before this can happen there is the need to improve and optimise existing proteomics technologies. Sample preparation, separation and characterisation have to be improved regarding analytical reproducibility from sample to sample, completeness in recording a complex protein mixture and the quantification in sample handling and protein determination. The level of sensitivity in proteome studies must become comparable to genetic techniques in order to study low abundance proteins. The links of the technologies for protein analysis and software for data handling have to be optimised for a complete proteomics approach. Instrumentation which is more user-friendly is necessary and non-expert systems may open proteomics to a broader community.

The general proteomics strategy uses 2D electrophoresis for protein separation and mass analysis for identification of peptide fragments as shown in Fig. 2. An alternative approach for the separation step would be some kind of multidimensional chromatography. Protein interactions with chromatographic matrices are very complex and vary from no retardation to irreversible adsorption. Chromatographic elution means that every fractionation needs a time window and eluting thousands of proteins needs an unacceptably long time. Furthermore, chromatographic separations are run in a serial mode that hamper a high through put. These inherent difficulties restrict the use of multidimensional chromatography in proteomics to some special applications.

2DE has the capacity to separate several thousand proteins on one gel. Nevertheless, conventional electrophoretic instrumentation is currently slow, labour intensive and expensive, and proteomics would benefit from new separation tools. The 2-dimensional electrophoretic separation on microfluidic chips has the potential to miniaturise 2DE separations and run multiple samples in parallel. A patent for these microfluidic chips was issued in February 1997 as a key application of Aclara Biosciences [42]. Although the technique has been shown to be readily compatible with analysis of oligonucleotides, little information is yet available regarding the application to proteins [43]. Protein studies are underway.

A promising tool for sample preparation might be developed at the single cell level. Many samples are mixtures of different cell types (Fig. 3), and appropriate cell separation tools were either non-existent or inefficient. The technology of laser microdissection was developed for the isolation of single cells from a microscopic section [44, 45]. Two laser technologies are used for cell capturing. At the NIH the LaserCaptureMicrodissection was developed where a plastic film covers a tissue on a microscopic slide and a laser beam activates and adheres to distinct regions [46]. The portion of the tissue section that is hot-melted to the film can be lifted off the slide, leaving the remainder of the tissue intact. Alternatively, the LaserPressureCatapulting technology uses the high photonic pressure force of the focused laser beam to select the sample from the object plane and to catapult it into a cap [47]. The use for cancer tissue material could be shown [48] and the potential for proteome studies is in discussion [49].

A view concentrated on parts of a proteome instead of a complete analysis will make proteome projects more effective. Subsets of proteins can be focused on, for example, macromolecular protein complexes. The *Saccharomyces cerevisiae* ribosome was investigated using LC/MS/MS, in which more than 100 proteins were analysed in a single run [50]. Affinity binding of proteins is an attractive way for screening and identification from crude samples. Ciphergen Biosystems (Palo Alto, USA) [51] in-

troduced surface enhanced laser desorption ionisation (SELDI) to analyse bound proteins by mass spectrometry. A real challenge would be a protein version of the DNA chip technology.

References

1. Wilkins MR, Sanchez JC, Gooley AA, Appel RD, Humphrey-Smith I, Hochstrasser DF Williams KL (1995) Biotechnol. Gene Eng Rev 13 : 19–50
2. Wilkins MR, Williams KL, Appel RD, Hochstrasser (1997) Proteome Research: New Frontiers in Functional Genomics. Springer, Heidelberg
3. Persidis A (1998) Nat Biotechn 16 : 393–394
4. Page MJ (1999) Drug Discovery Today 4 : 55–62
5. Dove A (1999) Nat Biotechn 17 : 233–236
6. Hochstrasser DF (1997) In: Wilkins MR, Williams KL, Appel RD, Hochstrasser DF (eds) Proteome Research: New Frontiers in Functional Genomics. Springer, Heidelberg, p 211
7. Anderson L, Seilhammer J (1997) Electrophoresis 18 : 533–537
8. Gygi SP, Rochon Y, Franza BR, Aebersold R (1999) Mol Cell Biol 19 : 1720
9. Velculescu VE et al. (1997) Science 270 : 484
10. Lottspeich F (1999) Angew Chemie 111 : 2630–2647
11. Blackstock WP, Weir MP (1999) Tibtech 17 : 121–127
12. Link AJ (1999) 2-D proteome analysis protocols, Humana Press, New Jersey
13. Klose J (1975) Humangenetik 26 : 231–243
14. O'Farrel PH (1975) J Biol Chem 250 : 4007–4021
15. Görg A, Postel W, Gunther S (1988) Electrophoresis 9 : 531–546
16. Kellner R, Lottspeich F, Meyer HE (1999) Microcharacterisation of proteins. Wiley-VCH, Weinheim
17. Wilm MS, Mann M (1994) Int J Mass Spectrom Ion Processes 136 : 167–180
18. Ducret A, Van Oostveen I, Eng JE, Yates JR, Aebersold R (1998) Prot Sci 7 : 706–719
19. Yates JR (1998) J Mass Spectrom 33 : 1–19
20. Roepstorff P (2000) Fresenius J Anal Chem 366 (this issue)
21. Eng JE, McCormack AL, Yates JR (1994) J Am Soc Mass Spectrom 5 : 976–989
22. Houthaeve T, Gausepohl H, Mann M, Ashman K (1995) FEBS Lett 376 : 91–94
23. Hsieh YL, Wang H, Elicone C, Mark J, Martin SA, Regnier F (1996) Anal Chem 68 : 455–462
24. http://www.proteomeworks.bio-rad.com, http://www.genomic-solution.com
25. http://www.pbio.com, http://www.bruker-daltonik.de
26. http://www.proteome.org.au
27. http://www.lsbc.com
28. http://www.ogs.com
29. http://www.protagen.com
30. http://www.protana.com
31. http://www.toplab.de
32. Anderson NL, Anderson NG (1998) Electrophoresis 19 : 1853–1861
33. Drews J (1996) Nature Biotechn 14 : 1516–1518
34. Celis JE, Ostergaard M, Jensen NA, Gromova I, Rasmussen HH, Gromov P (1998) FEBS Lett 430 : 64–72
35. Ostergaard M, Rasmussen HH, Nielsen HV, Vorum H, Orntoft TF, Wolf H, Celis JE (1997) Cancer Res 57 : 4111–4117
36. http://www.harefield.nthames.nhs.uk/nhli/protein
37. Pleissner KP (1997) Electrophoresis 18 : 480–483
38. Evans G, Wheeler CH, Corbett JM, Dunn MJ (1997) Electrophoresis 18 : 471–479
39. Aicher L, Wahl D, Arce A, Grenet O, Steiner S (1998) Electrophoresis 19 : 1998–2003
40. Klose J (1999) Electrophoresis 20 : 643–652
41. Gauss C, Kalkum M, Löwe M, Lehrach H, Klose J (1999) Electrophoresis 20 : 575–600
42. http://www.aclara.com
43. Effenhauser CS, Gerard JM, Bruin, Paulus A (1997) Electrophoresis 18 : 2203–2213
44. Emmert-Buck MR, Bonner RF, Smith PD, Chuaqui RF, Zhuang Z, Goldstein SR, Weiss RA, Liotta LA (1996) Science 274 : 998–1001
45. Schütze K, Lahr G (1998) Nature Biotechn 16 : 737–742
46. http://dir.nichd.nih.gov./lcm/lcm.htm
47. http://www.palm-mikrolaser.com
48. Suarez-Quian CA, Goldstein SR, Pohida T, Smith PD, Peterson JI, Wellner E, Ghany M, Bonner RF (1999) Biotechniques 26 : 328–335
49. Banks RE, Dunn MJ, Forbes MA, Stanley A, Pappin D, Naven T, Gough M, Harnden P, Selby PJ (1999) Electrophoresis 20 : 689–700
50. Link AJ, Eng J, Schieltz DM, Carmack E, Mize GJ, Morris DR, Garvick BM, Yates JR (1999) Nature Biotechn 17 : 676–682
51. http://www.ciphergen.com