

REVIEW

Proteomics: Taking over where Genomics Leaves off

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Abstract: The proteomic studies are simultaneously developed in several directions and significantly influence our notions on the capabilities of biological sciences. The need for proteomics research is necessary as there are certain genes in a cell that encode proteins with specific functions. Using a variety of techniques, proteomics can be used to study how proteins interact within a system or how the protein expression changes in different parts of the body, in different stages of its life cycle and in different environmental conditions as every individual has one genome and many proteomes. Besides the qualitative and quantitative description of the expressed proteins, proteomics also deals with the analysis of mutual interactions of proteins. Thereby, candidate proteins can be identified which may be used as starting-points for diagnostic or even therapeutic approaches.

Keywords: applications; methods; protein databases; proteome; proteomics

Proteins are molecules that carry out major functions in the cell. Since the proteins cannot be replicated nor do they have any complementary sequences like DNA or RNA, the study of the protein global expression is rather difficult. However, new technologies that reliably determine the type of proteins that are present in the cell and their level are making the task of studying proteins much more accessible. Thus the global study of the expression of genetic information at the protein level, assessment of their three-dimensional structure and their interactions is called proteomics. The term proteomics was coined in 1995 by an Australian postdoctoral fellow Marc Wilkins as a complement of genomics, the study of genes (WILKINS *et al.* 1996). While the genome is rather a constant entity, the proteome differs from cell to cell and is constantly changing through

its biochemical interactions with the genome and the environment. In the simplest term, it is the study of proteome that refers to the collection of the total set of proteins expressed in a cell at a given time, in an organism. Using a variety of techniques, proteomics can be used to study how proteins interact within a system or how the protein expression changes in different parts of the body, in different stages of its life cycle and in different environmental conditions as every individual has one genome and many proteomes.

The need for proteomics research is necessary as there are certain genes in a cell that encode proteins with specific functions. The large increase in protein diversity is thought to be due to alternative splicing and about 200 different post-translational modifications of the proteins. This discrepancy implies that protein diversity

cannot be fully characterized by the gene expression analysis alone, making proteomics a useful tool for characterizing cells and tissues of interest. The proteome in any individual has a collection of 30–50% different gene products which are expressed at low levels, and it is only proteomics that can convert proteins to peptides, followed by their conversion to amino acids which can be further determined and identified. Moreover, the genome is static whereas the proteome reflects cellular processes.

The study of proteomics is aimed to obtain a global integrated view of: measurement of the protein composition of organelles (spliceosome, phagosome, speckles, etc.); normal and abnormal cellular processes; protein-protein interactions; protein-drug interactions; determination of protein structure (NMR, X-ray, *in silico*, etc.) and all this will finally lead to the determination of the protein function.

In plants proteomics is used for the identification and quantification of stress-related proteins, mapping of dynamics of their expression and post-translational modifications. Thus the information on complexity of the plant response to various environmental stress factors can enable us to find the biomarkers of plant tolerance to various biotic and abiotic stresses which would be usable by breeders (VÍTÁMVÁS *et al.* 2007).

Generally, proteins are more difficult to study than DNA. One PCR allows the amplification of a DNA sample which could greatly benefit in its study. There is no analogous process in studying proteins and, therefore, we must rely on the small number of molecules that are produced *in vivo*. Thus, techniques that aim to study proteins must be sensitive and accurate.

Methods of studying proteins

Two-dimensional gels and mass spectrometry. The mass spectrometric identification of gel-separated proteins has been the most significant breakthrough in proteomics. This method was developed in the late 1970s, when high-performance mass spectrometry instrumentation coupled with highly efficient chromatographic and electrophoretic separations enabled the rapid qualitative and quantitative analysis of thousands of proteins from minute samples of biological materials. Here, proteins from a sample are separated on the basis of

charge and mass, and then analyzed by spectrometry (HENZEL *et al.* 1993) and the method is mainly used for cellular proteomics. This is one of the established technologies (it can resolve 10 000 spots per gel) used for studying proteins expressed at different levels. MS instruments consist of three modules: an ion source which splits the sample molecules into ions; a mass analyzer which sorts the ions by their masses by applying electromagnetic fields; and a detector which measures the value of an indicator quantity and thus provides data for calculating the abundances of each ion present. The technique has both qualitative and quantitative uses. MS can determine the isotopic composition of elements in a molecule, the structure of a compound by observing its fragmentation, can quantify the amount of a compound in a sample or study the fundamentals of gas phase ion chemistry. It is biased at detecting very small, very large and nuclear bound proteins and at the same time it is time consuming. The recent development of a novel mass spectrometer (Orbitrap) and new dissociation methods such as electron-transfer dissociation has made possible the exciting new areas of proteomic application. Stable isotope labelling strategies have transformed mass spectrometry from a merely descriptive technique to a tool for measuring dynamic changes in protein expression, interaction and modification (HAN *et al.* 2008). Finally, advances in mass spectrometric imaging allow the gathering of specific information on the local molecular composition, relative abundance and spatial distribution of peptides and proteins in thin tissue sections.

Similarly, MALDI, or Matrix-assisted laser desorption/ionization technique which was developed by Karas and Hillenkamp in the late 1980s (KARAS & HILLENKAMP 1988), is a soft ionization technique used in mass spectrometry in which protein fragments in a solid-phase sample are ionized by a laser beam. To generate the gas phase, protonated molecules, a large excess of matrix material is coprecipitated with analyte molecules (that is, the molecules to be analyzed) by pipetting a submicroliter volume of the mixture onto a metal substrate and allowing it to dry. The resulting solid is then irradiated by nanosecond laser pulses, usually from small nitrogen lasers with a wavelength of 337 nm (MANN *et al.* 2001). This technique allows the analysis of biomolecules (proteins, peptides, and sugars) and larger organic molecules (polymers and dendrimers). MALDI is

used to ionize these molecules because they are very fragile and will usually fragment when ionized by other, more conventional ionization methods. The ion molecule is protected by a matrix to prevent it from being destroyed, and to allow for optimal vaporization and ionization.

Protein microarrays. Protein microarrays are tools that can be used in many different areas of research, including basic and translational research. Protein chips have emerged as a promising approach because complete genome sequences of different organisms and their cellular processing are generally performed by proteins. Proteomic arrays are typically high-density arrays (> 1000 elements/array) that are used to identify novel proteins or protein-protein interactions, to identify the substrates of protein kinases, or to identify the targets of biologically active small molecules (MACBEATH & SCHREIBER 2000). Silicon chips are coated with specific surface chemistries or known protein ligands, and proteins from a sample are fractionated on the chip, and then analyzed by mass spectrometry. Alternatively, chips are prepared with different surface chemistries, much like traditional protein chromatography columns, but in a flat array. To detect proteins that are bound to the array, the samples must be labelled directly with a fluorophore or a hapten. Alternatively, in some applications antibodies can be used to detect binding events, where antibodies are spotted onto the protein chip and are used as capture molecules to detect proteins from cell lysate solutions. For immunoproteomics approaches, antigen microarrays are used to detect disease associated changes in the autoantibody profiles. Protein microarray technology provides a robust way to study the protein function in a rapid, economical, and system-wide fashion. The ultimate goal of proteomics is to study biochemical activities of every protein encoded by an organism or proteome. ZHU *et al.* (2001) made a landmark where they prepared the first proteome chip by cloning ~94% (> 5800 of 6200) of the yeast open reading frames in a yeast expression vector which expressed the proteins as N-terminal GST-His x6 double tagged fusions. A high-throughput yeast protein purification method was developed to individually purify proteins. This study demonstrated that an entire proteome can be immobilized on a glass surface to directly screen for interactions with proteins and small molecules.

Automated yeast two-hybrid screen. The yeast two-hybrid system is often the first method used to

identify protein interactions. It provides an ideal format for screening one individual bait protein against large prey cDNA libraries. Pioneered by FIELDS and SONG (1989), the technique was originally designed to detect protein-protein interactions using the GAL4 transcriptional activator of the yeast *Saccharomyces cerevisiae*. The GAL4 protein activated the transcription of a protein involved in galactose utilization which formed the basis of selection (HURT *et al.* 2003). Since then the same principle has been adapted to describe many alternative methods including some that detect protein-DNA interactions, DNA-DNA interactions. There are several varieties of the yeast two-hybrid systems. The two most commonly used systems differ in the nature of the DBD used to express the bait fusion protein (GAL4 or LexA) and the AD used to generate the prey fusion protein (GAL4, VP16 or B42). JOUNG *et al.* (2000) used *E. coli* instead of yeast which facilitated the rapid analysis of larger libraries (due to the higher transformation efficiency and faster growth rate). Such methods might also be faster than the phage display, which is an enrichment technique requiring multiple rounds of affinity purification and amplification and which also allows studies of sequences (of much larger proteins) that are not readily displayed on a phage surface.

In general, in any two-hybrid experiment a protein of interest is fused to a DNA-binding domain and transfected in a yeast host cell bearing a reporter gene controlling this DNA-binding domain. When this fusion protein cannot activate transcription on its own, it can be used as bait or as a target to screen a library of cDNA clones that are fused to an activation domain. The cDNA clones within the library that encode proteins capable of forming protein-protein interactions with the bait are identified by virtue of their ability to cause activation of the reporter gene. So the yeast two-hybrid system is devised to identify genes encoding proteins that are physically associated with a given protein *in vivo*. Since the emergence of the two-hybrid approach in 1989, a number of improvements have been incorporated that have increased its applicability. A laboratory robot performs a bioassay to find whether pairs or groups interact (ALBERS *et al.* 2004). But a successful technique for rapid results in global proteomics provides information only on binding partners and not on the biochemical function. In plants the system has been used to study signalling pathways including resistance

gene signals (INNES 1998), phytochrome and cryptochrome interacting factors (SANG *et al.* 2005) as well as stress and hormone responses (MIZOGUCHI *et al.* 2000). Interaction of orthologous proteins can be used to compare and validate the interactions between MADS domain proteins that are conserved in Arabidopsis, rice, petunia. The utility of the new yeast one-hybrid technology is demonstrated by the successful cloning in wheat of full-length cDNAs encoding several transcription factors from three different families (LOPATO *et al.* 2006).

High-throughput crystallography. The high-throughput protein crystallography beamline is a dedicated facility for determining the structure of protein crystals and undertaking the initial assessment of more complex crystals. Single crystals are analysed using multiple wavelength anomalous dispersion (MAD). Hypothetical proteins from a sequenced genome are expressed, purified, and crystallized to obtain a three-dimensional structure, then tested for their biochemical functions (KIM *et al.* 2003). This technique is important to determine the three-dimensional structures of nonmembrane proteins for which sequence-based methods have been unable to predict a function. Membrane proteins were excluded because it is technologically challenging to produce them on a high-throughput scale, so a subset of protein structures is designed that may be useful in elucidating the protein function and that will contribute to our understanding of the sequence/structure relationship. This technique can be useful to uncover certain protein functions and also to provide their structural information.

Shotgun proteomics. Shotgun proteomics using liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides the most powerful analytical platform for global inventory of complex proteomes. It is a method of identifying proteins in complex mixtures using a combination of high performance liquid chromatography and mass spectrometry. In this method, the proteins in the mixture are digested and the resulting peptides are separated by liquid chromatography. Tandem mass spectrometry is then used to identify the peptides.

Bottom up proteomics. Bottom-up proteomics is the most mature and most widely used approach to protein identification and characterization. A method to identify proteins, characterize their amino acid sequences and post-translational modifications by proteolytic digestion of proteins prior to analysis by mass spectrometry. The proteins

may first be purified by gel electrophoresis. Then the crude protein extract is digested directly, followed by one or more dimensions of separation of the peptides by liquid chromatography coupled to mass spectrometry. By comparing the masses of the proteolytic peptides or their tandem mass spectra with those predicted from a sequence database, peptides can be identified and multiple peptide identifications assembled into protein identification. Reversed-phase HPLC provides high-resolution separations of peptide digests with solvents that are compatible with ESI.

Top-down proteomics. In top-down proteomics, intact protein molecular ions generated by ESI are introduced into the mass analyzer and are subjected to gas-phase fragmentation. It is a method of protein identification that uses an ion trapping mass spectrometer so as to store an isolated protein ion for mass measurement and tandem mass spectrometry analysis. An obstacle to this approach is the determination of product ion masses from multiple charged product ions.

Applications

Protein as a diagnostic tool. One of the most promising developments is the interaction between human genes and proteins resulting in the identification of new drugs for the treatment of diseases. If a certain protein is implicated in a disease, its 3D structure provides the information to design drugs which then interfere with the action of the protein. A molecule that fits the active site of an enzyme, but cannot be released by the enzyme, will inactivate the enzyme. The identification of new drugs to target and inactivate the enzyme HIV-1 protease that cleaves a very large HIV protein into smaller, functional proteins could significantly contribute to suppression of HIV infection in humans. The virus cannot survive without this enzyme; therefore, it is one of the most effective protein targets for killing HIV (SULLIVAN *et al.* 1998). In Alzheimer's disease, targeting the enzyme beta secretase (which causes plaque to build up in the patient's brain) slows the progression of the disease (COLE & VASSAR 2007). Specific proteins can be used as biomarkers to diagnose the disease. A number of techniques like western blot, immunohistochemical staining, enzyme linked immunosorbent assay (ELISA) or mass spectrometry allow us to test for proteins

produced during a particular disease, which helps to diagnose the disease quickly.

Protein chips. Chip-based separation of proteins (DAVID *et al.* 2007) provides methods that are faster, automated and more convenient than conventional gel electrophoresis. Chips that hold thousands of immobilized proteins could be used to detect protein-protein interactions quickly and easily (KERSTEN & FEILNER 2007). Zyomyx developed high-density protein chips made of silicon, which are microfabricated to posts to create a 3-D substrate. This technology offers a profiling biochip TM for high-throughput, low sample volume protein studies. A human cytokine chip was the first product to be commercialized, while various other products and technologies are under development. The chips are expensive.

Determining the existence of proteins in complex mixtures. Earlier, antibodies to particular proteins or to their modified forms were used in biochemistry and cell biology studies or ELISA was used for quantitative determination of protein amounts, but now, more recent techniques such as Matrix-assisted laser desorption/ionization have been employed for rapid determination of proteins in particular mixtures.

Differential expression of proteins. Proteins expressed under normal conditions are compared to those under stressed conditions. The approach is quite similar to microarray analysis where the expression levels of proteins are significantly changed, indicating the potential role of the protein in the causation of the stress.

Proteins as messengers. The binding of one protein to other protein receptors so as to send a signal to the cell results in forming structural connections between cells and thus leading to the study of interactions between the proteins.

In vivo isotopic labelling. This is a common method used in comparative proteomics (SCHWENDER & OHLROGGE 2002). Here one set of samples is grown on a natural nitrogen source while the comparative sample is grown in the presence of a heavy isotope. The isotopic label can be introduced either as an amino acid (termed stable isotopic labelling in cell culture, SILAC) or by ^{15}N as the sole nitrogen source, typically in the form of K^{15}NO_3 . Due to a difference in the masses of peptides from two or three populations a direct comparison of MS peak intensities can be made for the two samples.

In vitro labelling. The two recent techniques for quantitation of proteins are ICAT and iTRAQ.

ICAT (isotope-coded affinity tag) is used to identify and quantitatively analyze many proteins at the same time in biological samples. This technique describes the simultaneous identification and quantitation of oxidant-sensitive cysteine thiols in a complex protein mixture using a thiol-specific, acid-cleavable isotope-coded affinity tag (ICAT) reagent. The approach is based on the fact that only free cysteine thiols are susceptible to labelling by the iodoacetamide-based ICAT and that mass spectrometry can be used to quantitate the relative labelling of free thiols.

iTRAQ (isobaric tag for relative and absolute quantitation). It is an improved approach analogous to ICAT. It is a non-gel based technique that uses isotope coded covalent tags. The technique is based upon chemically tagging the N-terminus of peptides generated from protein digests that have been isolated from cells in a single experiment.

Few limitations of technologies used (applied) in proteomic research

- 2-D gels only work for hydrophilic (soluble) proteins. So membrane proteins do not simply run into the gel.

- 2-D gels are resolution sensitive. It is possible to resolve only hundreds to a few thousand spots on each gel whereas low abundance proteins may be masked by nearby abundant proteins on the gel.

- No two gels are exactly the same. So computers stretch the gel images (rubber-sheeting) to try to make them look as similar as possible before comparing them but now in DiGE (difference gel electrophoresis) (UNLU *et al.* 1997; TONGE *et al.* 2001). A mixed sample labelled with Cy2TM fluorescent dye (when a minimum labelling technique is applied) is loaded on one IPG strip (one gel) together with two samples labelled with Cy3TM and Cy5TM fluorescent dyes. This mixed sample then serves as an internal standard and enables the researchers for more accurate protein quantification than in classical 2-DE (two-dimensional electrophoresis). The gel is then viewed by a fluorescence imager at three wavelengths. Although this technique is simple, it is very expensive.

Companies

Some selected companies focusing on proteomics are Ciphergen (developing silicon microarrays for

protein fractionation), Hybrigenics (developing automated large-scale yeast two-hybrid screen), Oxford Glycosciences (2D gel system, assembling database of pharmaceutical companies), Proteome (a database that organizes and updates all available information about known proteins).

Software and protein databases

Commercial software packages are now available. Some of them are the Melanie package (WILKINS *et al.* 1996) from the Swiss Institute of Bioinformatics (<http://www.expasy.ch/>), Phoretix2D software (<http://www.nonlinear.com/>) and Gellab II from Scanalytics (<http://www.scanalytics.com/>), which can be used to analyze 2D gel patterns. A variety of protein sequence databases exist which play an important role as central comprehensive sources of protein information (PRUESS *et al.* 2006).

- UniProt (the UniProt Consortium comprises the European Bioinformatics Institute (EBI), the Swiss Institute of Bioinformatics (SIB) and the Protein Information Resource (PIR) are the world's most comprehensive source of protein information.
- PIR (Protein Information Resource to support genomic and proteomic research).
- Swiss-Prot (manually curated biological database of protein sequences) (JUNKER *et al.* 2000).
- TrEMBL – computer-annotated supplement of Swiss-Prot that contains all the translations of EMBL nucleotide sequence entries not yet integrated in Swiss-Prot.
- PDB (Protein Data Bank is a repository for 3-D structural data of proteins and nucleic acids).
- NCBI (National Centre for Biotechnology Information houses genome sequencing data in GenBank and an index of biomedical research articles in PubMed Central and PubMed, and information relevant to biotechnology).
- Human Protein Reference Database (manually curated scientific information pertaining to the biology of most human proteins).
- Proteopedia (the 3D encyclopaedia of proteins and other molecules).

Summary

The proteomic studies are simultaneously developed in several directions and significantly

influence our notions of the capabilities of biological sciences. Using databases on complete nucleotide sequences of genomes, methods of protein sequencing *de novo* by mass spectrometry, and highly effective methods of protein separation by means of 2D-electrophoresis and chromatography significantly increases the abilities of certain biological experiments and gives a fast algorithm for decoding particular molecular mechanisms and new features of functioning of the living matter. Besides the qualitative and quantitative description of the expressed proteins, proteomics also deals with the analysis of the mutual interactions of proteins. Therefore, the classical proteomics approach compares two different states of a proteome, e.g. healthy – diseased or drug-treated – untreated. Such differential analyses yield a quantitative comparison of the expressed protein patterns and facilitate conclusions about direct or indirect interactions of the altered parameters. Thereby, candidate proteins can be identified which may be used as starting-points for diagnostic or even therapeutic approaches. The article has been written to present the information in a manner so that the inexperienced reader becomes familiar with the concept and tools of proteomics.

References

- ALBERS M., KRANZ H., KOBER I., KAISER C., KLINK M., SUCKOW J., KERN R., KOEGL M. (2004): Automated yeast two-hybrid screening for nuclear receptor-interacting proteins. *Molecular Cell Proteomics*, **4**: 205–213.
- COLE L.S., VASSAR R. (2007): The Alzheimer's disease β -secretase enzyme, BACE1. *Molecular Neurodegeneration*, **2**: 22–26.
- DAVID A.H., JASON P., MICHAEL S. (2007): Protein microarray technology. *Mechanisms of Ageing and Development*, **128**: 161–167.
- FIELDS S., SONG O. (1989): A novel genetic system to detect protein: protein interactions. *Nature*, **340**: 245–246.
- HAN X., ASLANIAN A., YATES J.R. (2008) Mass spectrometry for proteomics. *Current Opinion in Chemical Biology*, **12**: 483–490.
- HENZEL W.J., BILLECI T.M., STULTS J.T., WONG S.C., GRIMLEY C., WATANABE C. (1993): Identifying proteins from two-dimensional gels by molecular mass searching of peptide fragments in protein sequence databases. *Proceedings of the National Academy of Sciences of the United States of America*, **90**: 5011–5015.

- HURT J., THIBODEAU S., HIRSH A., PABO C., JOUNG J. (2003): Highly specific zinc finger proteins obtained by directed domain shuffling and cell-based selection. *Proceedings of the National Academy of Sciences of the United States of America*, **100**: 12271–12276.
- INNES R.W. (1998): Genetic dissection of R gene signal transduction pathways. *Current Opinion in Plant Biology*, **1**: 229–304.
- JOUNG J.K., RAMM E.I., PABO O.C. (2000): A bacterial two-hybrid selection system for studying protein–DNA and protein–protein interactions. *Proceedings of the National Academy of Sciences of the United States of America*, **97**: 7382–7387.
- JUNKER V., CONTRINO S., FLEISCHMANN W., HERMJAKOB H., LANG F., MAGRANE M., MARTIN M.J., MITARITONNA N., DONOVAN C., APWEILER R. (2000): The role SWISS-PROT and TrEMBL play in the genome research environment. *Journal of Biotechnology*, **78**: 221–234.
- KARAS M., HILLENKAMP F. (1988): Laser desorption ionization of proteins with molecular mass exceeding 10 000 daltons. *Analytical Chemistry*, **60**: 2299–2301.
- KERSTEN B., FEILNER T. (2007): Generation of plant protein microarrays and investigation of antigen-antibody interactions. *Methods in Molecular Biology*, **355**: 365–378.
- KIM S.H., SHIN D.H., CHOI I.G., SCHULZE-GAHMEN U., CHEN S., KIM R.J. (2003): Structure-based functional inference in structural genomics. *Journal of Structural and Functional Genomics*, **4**: 129–130.
- LOPATO S., BAZANOVA N., MORRAN S., MILLIGAN A.S., SHIRLEY N., LANGRIDGE P. (2006): Use of a modified Yeast One-Hybrid System for isolation of plant transcription factors. *Plant Methods*, **2**: 3–17.
- MACBEATH G., SCHREIBER S.L. (2000): Printing proteins as microarrays for high-throughput function determination. *Science*, **289**: 1760–1763.
- MANN M., HENDRICKSON R.C., PANDEY A. (2001): Analysis of proteins and proteomes by mass spectrometry. *Annual Review Biochemistry*, **70**: 437–473.
- MIZOGUCHI T., ICHIMURA K., YOSHIDA R., SHINOZAKI K. (2000). MAP kinase cascades in Arabidopsis: Their roles in stress and hormone responses. *Results and Problems in Cell Differentiation*, **27**: 29–38.
- PRUESS M., KERSEY P., KULIKOVA T., APWEILER R. (2006): Databases and resources for in silico proteome analysis. *Methods of Biochemical Analysis*, **49**: 397–414.
- SANG Y., LI Q.H., RUBIO V., ZHANG Y.C., MAO J., DENG X.W., YANG H.Q. (2005): N-Terminal domain-mediated homodimerization is required for photoreceptor activity of Arabidopsis. *Plant Cell*, **17**: 1569–1584.
- SCHWENDER J., OHLROGGE J.B. (2002): Probing in vivo metabolism by stable isotope labeling of storage lipids and proteins in developing *Brassica napus* embryos. *Plant Physiology*, **130**: 347–361.
- SULLIVAN N., SUN Y., BINLEY J., LEE J., BARBAS C.F., PARREN P., BURTON D R., SODROSKI J. (1998): Determinants of human immunodeficiency virus type 1 envelope glycoprotein CD4 and monoclonal antibodies. *Journal of Virology*, **72**: 6332–6334.
- TONGE R., SHAW J., MIDDLETON B., ROWLINSON R., RAYNER S., YOUNG J., POGNAN F., HAWKINS E., CURRIE I., DAVISON M. (2001): Validation and development of fluorescent two-dimensional gel electrophoresis proteomics technology. *Proteomics*, **1**: 377–396.
- UNLU M., MORGAN M.E., MINDEN J.S. (1997): Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis*, **18**: 2071–2077.
- VÍTÁMVÁS P., KOSOVÁ K., PRÁŠIL I.T. (2007): Proteome analysis in plant stress research. *Czech Journal of Genetics and Plant Breeding*, **43**: 1–6.
- WILKINS M.R., SANCHEZ J.C., GOOLEY A.A., APPEL R.D., HUMPHERY I., HOCHSTRASSER D.F., WILLIAMS K.L. (1996): Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. *Biotechnology and Genetics Engineering Reviews*, **13**: 19–50.
- ZHU H., BILGIN M., BANGHAM R., HALL D., CASAMAYOR A., BERTONE P., LAN N, JANSEN R., BIDLINGMAIER S., HOUFEK T., MITCHELL T., MILLER P, DEAN R.A., GERSTEIN M., SNYDER M. (2001): Global analysis of protein activities using proteome chips. *Science*, **293**: 2101–2105.

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