Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography B, 815 (2005) 3–9
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Combination of two-dimensional electrophoresis and shotgun peptide sequencing in comparative proteomics

Kazuishi Kubota*, Toshiyuki Kosaka, Kimihisa Ichikawa

Biomedical Research Laboratories, Sankyo Co. Ltd., 1-2-58 Hiromachi, Shinagawa-ku, Tokyo 140-8710, Japan

Received 31 May 2004; accepted 1 October 2004
Available online 5 November 2004

Abstract

Two-dimensional electrophoresis (2-DE) and shotgun peptide sequencing are the two major technologies to compare the expression profile of proteins, which is also referred to as comparative proteomics or quantitative proteomics. Although the methodologies, such as difference gel electrophoresis for 2-DE and isotope-coded affinity tags for shotgun peptide sequencing, have made rapid progress, these two approaches have their own strengths and weaknesses. Therefore, the combination of the two methodologies is beneficial for the purpose of better comparative proteomics, especially in comprehensive coverage of the proteome and protein information such as post-translational modifications.
© 2004 Elsevier B.V. All rights reserved.

Keywords: Comparative proteomics; Two-dimensional electrophoresis; Shotgun peptide sequencing

Contents

1. Introduction

Since the term “proteome” was firstly defined as “the total protein complement of a genome” by Wilkins et al. [1,2], the field of proteome analysis, referred to as “proteomics” has kept growing rapidly [3–5]. Proteomics includes protein expression profiling of biological samples in one state [1], comparison of the protein expression profiles in two or more states [6–8], protein–protein interaction analysis based on yeast two hybrid [9–13] or affinity purification using a tagged protein as a bait [14–16], protein localization [17] and three-dimensional structure determination [18,19]. Nowadays, all kinds of comprehensive or large-scale protein analyses are called proteomics. Among them, comparative expression profiling, also referred to as comparative pro-

* Corresponding author. Tel.: +81 3 3492 3131; fax: +81 3 5436 8587.
E-mail address: kkubot@sankyo.co.jp (K. Kubota).

1570-0232/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2004.10.030

teomics or quantitative proteomics, has been conducted most extensively since the proteomics field arose, where proteins from different biological states are compared to understand various biological processes, to find new diagnosis markers and to discover novel molecular targets of drugs.

There are numerous methodologies to achieve this goal. We can divide them into two major categories. One is where proteins are identified after extensive separation. Owing to extraordinary resolution, easy availability and abundant accumulated knowledge, two-dimensional electrophoresis (2-DE) is most widely used for this separation, though there are other methods, such as organelle fractionation, one-dimensional SDS–PAGE, various chromatographic techniques, methodology incorporating chemically modified solid surfaces, liquid-phase isoelectric focusing (IEF), free flow electrophoresis and any combination of these methods [20–25]. In most cases, separated proteins are quantified and compared using protein staining, etc. followed by protein identification using mass spectrometry (MS). The other category is where proteins are identified without extensive separation. A complex protein mixture is digested as it is with protease, and the resultant peptides are separated with various chromatographic techniques and introduced into the mass spectrometer. This shotgun peptide sequencing, also referred to as shotgun proteomics, has been rapidly emerging since Link et al. [26] first demonstrated it, and recent introduction of stable isotope labeling has made it possible for more precise comparison in shotgun peptide sequencing [27,28]. A further category is where protein separation and identification are conducted simultaneously such as by using protein chips [29,30]. However, these methods are still in the developmental stage.

This review focuses on the most prevailing and contrasting two methods, 2-DE and shotgun peptide sequencing in the context of comparative protein profiling. We would like to reveal the complementarity of these two methods with regards to comprehensiveness of proteome coverage and protein information through the comparison of their strengths and weaknesses.

2. Two-dimensional electrophoresis (2-DE)

Almost 30 years ago, high resolution 2-DE was developed independently by O'Farrell [31] and Klose [32]. As the first step of this method, proteins are separated by their isoelectric points (p*I*s) using gel-based IEF, followed by a second separation using their relative molecular mass with SDS–PAGE. In other words, proteins are separated by their orthogonal characteristics, charge and mass. Proteins in the gels are stained as spots, and the staining intensities of each spot are compared to determine the quantitative change of the protein expression profiling (Fig. 1).

During the early stages of 2-DE, it was difficult to reproducibly manufacture a large number of 2-DE gels because the pH gradients generated by the carrier ampholytes were

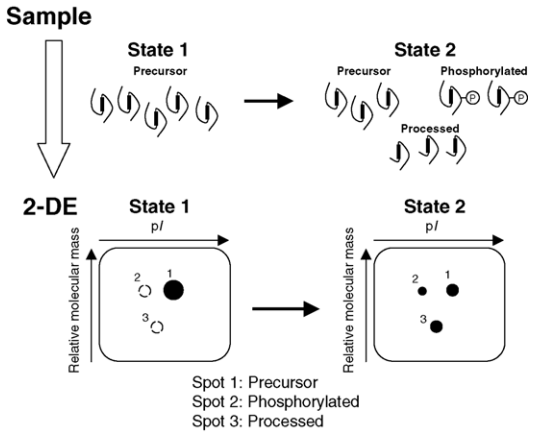

Fig. 1. Separation and obtained information by 2-DE. Proteins are separated by their isoelectric points (p*I*s) and their relative molecular mass in 2-DE. Therefore, proteins with different post-translational modifications, such as phosphorylation and processing, can be separated as different spots on 2-DE gel.

inherently variable [33]. However, Görg et al. [34] introduced an immobilized pH gradient (IPG) technique to 2-DE instead of using carrier ampholytes to make the pH gradient in the IEF gel, and achieved better reproducibility and higher protein load capacity. Novel development of fluorescent dyes for gel staining brought both high sensitivity comparable to silver staining and a wider range of quantitation than silver staining [35,36]. In other studies, two protein samples are labeled with two different fluorescent dyes separately and these labeled proteins are combined and subjected to the same 2-DE gel. This so-called difference gel electrophoresis (DIGE) technique eliminates gel-to-gel variation, and thus determination of subtle changes can be achieved [37]. Though 2-DE is sometimes treated as a classical technique, modification of this method is still going on now [38].

The primary strength of 2-DE is its extremely high resolution compared to other separation techniques. 2-DE can provide more than 10,000 detectable protein spots in a single gel [39]. Thus, proteins with post-translational modifications (PTMs), such as processing, phosphorylation and glycosylation, can be detected as separate spots on 2-DE gel (Fig. 1). As discussed later, this discrimination of proteins with PTMs is an obvious advantage over shotgun sequencing analysis. Since 2-DE has a long history and numerous users, many instruments and reagents are commercially available from several suppliers. This availability helps good person-to-person and laboratory-to laboratory reproducibility and decreases the burden on scientists starting to use this technology. In addition, 2-DE can be readily conducted in parallel. Up to twelve gels can be run and stained simultaneously by using commercial instruments. This high-throughput capacity easily, therefore, allows us to increase the number of experiments to detect subtle changes with significant differences. A spot separated by 2-DE should consist of an almost homogeneous protein, and thus the protein can be identified by pep-

tide mass fingerprinting (PMF) using single mass spectrometers. Tandem mass spectrometry (MS/MS) provides protein identification with higher confidence than PMF, however, tandem mass spectrometers practical for this purpose have been available only in the last decade and are more expensive than single mass spectrometers. Thus, most of the initial proteome work used matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) type mass spectrometers for protein identification [40–42], and PMF instead of MS/MS for protein identification is a further strength of 2-DE analysis.

In spite of these advantages, now it is accepted that 2-DE is a far from perfect methodology for analyzing a proteome [38,43]. The primary weakness of this method is difficulty of detecting low abundant proteins. Theoretically, we can only detect proteins at more than 1000 copies per cell due to the loading capacity of 2-DE gels [38,44], and experimentally, proteins identified on 2-DE gels exhibited strong bias to high abundant proteins [45]. Hydrophobic proteins are another group of proteins hardly observed on 2-DE gels [46,47]. In IEF, use of detergents to solubilize hydrophobic proteins is severely restricted, and these proteins tend to aggregate around their p*I* during electromigration, resulting in spots of less focus. Proteins having extremely acidic or basic p*I* are out of the range of one-dimensional IEF, and small proteins of less than 10 kDa are out of the range of two-dimensional SDS–PAGE. Naturally, these proteins cannot be observed in 2-DE analysis. Even if the p*I* of a protein is within the range of IEF, basic proteins tend to be less focused, and some optimization of experimental conditions might be needed [48,49].

There have been many attempts to overcome these shortcomings. To increase the ratio of low abundant proteins, prefractionation of samples, such as by sequential extraction [50] or microscale solution IEF [51], can be used. Modification of solubilization conditions gives better resolution and detection of proteins including hydrophobic ones [52]. Despite these considerable efforts, the comprehensiveness of 2-DE is insufficient to cover the whole proteome [38,46,47]. For example, membrane receptors, which are low abundant and very hydrophobic, have rarely been observed in 2-DE analysis.

3. Shotgun peptide sequencing

Development of MS/MS coupled with peptide sequencing or database searching provides capability of protein identification from a mixture of proteins [53–55]. In these methods, the protein mixture is digested by a protease to produce a large collection of peptides, and these complex peptides are then subjected to liquid chromatography tandem mass spectrometry (LC/MS/MS) analysis (Fig. 2). Proteins in the initial mixture are identified by using the MS/MS spectra of the digested peptides. Reliable protein identification is accomplished by using a fraction of the peptides produced from a protein. In

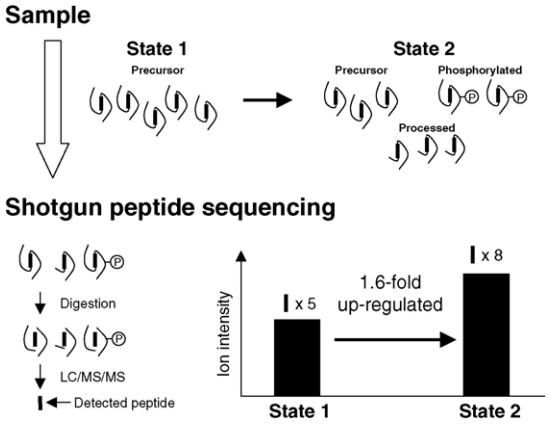

Fig. 2. Obtained information by shotgun peptide sequencing. Proteins are digested as a mixture in shotgun peptide sequencing and only a fraction of peptides are detected in LC/MS/MS analysis. Therefore, in most cases, the ion intensity of a peptide reflects the total amount of the corresponding protein, and proteins with different post-translational modifications, such as phosphorylation and processing, cannot be separated.

the case of a simple protein mixture, one-dimensional separation has sufficient capacity to separate the peptides.

However, in the case of highly complexed samples as in proteomics, single separation of peptides would be insufficient and multidimensional separation would be needed. Indeed, Link et al. [26] used two orthogonal chromatographic technologies, cation exchange and reversed-phase chromatographies, to separate a complex peptide mixture, and identified more than 100 proteins of yeast ribosome in a single run. Thereafter, Washburn et al. [56] identified approximately 1500 proteins from yeast lysate by optimization of the system described by Link et al. [26]. This would be the first application of shotgun peptide sequencing to large-scale proteome analysis. The number of 1500 identified protein species exceeds that identified in 2-DE analysis, moreover, many low abundant or hydrophobic integral membrane proteins were identified in this study [56]. In shotgun peptide sequencing, proteins are digested to peptides which are characteristically smaller and simpler and are thus easier to deal with than proteins. Therefore, the hydrophobicity, p*I* and relative molecular mass of a protein does not limit this technology, unlike 2-DE, and the high sensitivity of MS allows us to identify more low abundant proteins than in 2-DE. For example, Wu et al. [57] identified more than 1600 membrane proteins, a most unsuitable sample for 2-DE, using shotgun peptide sequencing.

By this methodology, we can investigate the presence of proteins, in other words, a simple expression profile, however comparative expression profiling needs additional technology. MS is used as a detector in all shotgun peptide sequencing analysis. Although ion intensities of peptides [7,58] or scores of database searching [59] correlate with protein quantity to some extent, quantitation by MS without internal standards is relatively unreliable because of the difference in ionization efficiency or suppression effect.

To circumvent this problem inherent in MS and compare the protein expression of two samples more precisely, several combinations of stable isotope labeling and shotgun peptide sequencing have been introduced [27,28]. In those methods, each protein mixture incorporates a different stable isotope by chemical [60] or metabolic labeling [61] and the isotopically derivatized samples are combined and subjected to shotgun peptide sequencing analysis [27,28]. Peptides labeled with light or heavy stable isotopes are observed as pairs of peptide ions in mass spectra, and the ratio of light and heavy ion intensities reflects the ratio of the initial protein amounts in the two states. Above all, isotope-coded affinity tags (ICAT) methodology, the first demonstration of chemical labeling, is the most utilized [62–68], in which cysteine residues of proteins are labeled with 8 Da separated light or heavy tags and the labeled peptides are purified by using biotin included in the tags [60]. Han et al. [69] compared two microsome fractions, which contain many membrane proteins, and determined the differences of approximately 500 proteins by using this technology. However, the first generation of ICAT reagents had some drawbacks. For example, deuterium affects the retention time of peptides in reversed-phase chromatography, or intense fragment ions from the biotin part of affinity tags hinders obtaining good MS/MS spectra for database searching. Development of modified ICAT reagents using cleavable tags and ^{13}C has been reported [70] and is commercially available now.

In addition, shotgun peptide sequencing is adequate for automation. Since this analysis requires detection by an expensive tandem mass spectrometer for each measurement, parallel measurements are not practical, unlike in 2-DE. However, all procedures in this method are conducted in solution, and thus, automation of experiments can be accomplished more easily than with the 2-DE approach by using on-line column switching and liquid handling robots, which are generally used and less expensive than the gel handling robots used for 2-DE analysis.

4. Combination of 2-DE and shotgun peptide sequencing

4.1. Proteome coverage

The human genome is predicted to encode 20,000–30,000 genes [71]. Though 2-DE could resolve more than 10,000 separate spots [39], this does not mean we can identify 10,000 proteins in 2-DE gels. As described previously, since the same protein with different PTMs is observed as separate spots, none of the 2-DE studies have identified more than 1000 protein species so far. With regard to shotgun peptide sequencing, more than 2000 protein species were identified in the most successful case [8]. Obviously, it would also be insufficient to cover the whole proteome, though the number of proteins expressed at one time is supposed to be much less than the gene number.

In addition, in the case of the comparative expression profiling, there is another problem with shotgun peptide sequencing. Precise comparison of the peptide amount needs isotopic derivatization using a stable isotope, as described in the previous section. Stable isotopes are incorporated into specific residues of proteins, and relative quantitation is based on the ion intensities of these labeled peptides. To generate good MS/MS spectra for identification, the labeled peptides must have appropriate sizes and properties after protease digestion. For example, cysteine residues are labeled in ICAT analysis. Since 8% of yeast protein does not contain cysteine residues, these proteins cannot be observed theoretically [60]. Among the 92% remaining proteins, only proteins that produce labeled peptides suitable for LC/MS/MS, namely, not too short, long, hydrophilic or hydrophobic, can be analyzed. That is, only proteins which have appropriate sequences are observed in these analyses. Indeed, all numbers of identified proteins reported in this kind of analysis were less than 1000 and smaller than non-comparative analysis. The coverage of the whole proteome in this approach looks similar to that of 2-DE.

Since both 2-DE and shotgun peptide sequencing strategies are insufficient in themselves, combination of the two strategies is promising to obtain a more comprehensive proteome. There were a few studies using both 2-DE and shotgun peptide sequencing for comparative expression profiling [67,68], one of which was conducted in our laboratory [68]. Our samples were conditioned media of cultured cells without serum and thus not very complex, like whole cell lysate. However, we found that the ratio of overlap of identified proteins was relatively small (35% for 2-DE and 42% for ICAT analysis) and that the two methods had their own preferences [68]. Therefore, combination of the two complementary methods would certainly provide better coverage of a proteome.

4.2. Protein information

The protein information obtained from 2-DE analysis is very different from that from shotgun sequencing. In 2-DE analysis, proteins with different p*I* or relative molecular mass are observed as separate spots, and owing to this information about the p*I* and relative molecular mass as well as the quantity and identity of the proteins, we can determine the change in the levels of PTMs as illustrated in Fig. 1. In contrast, it is very difficult to detect PTMs in shotgun peptide sequencing analysis. Identification and quantitation of protein in shotgun peptide sequencing depend on identified peptides, but sequence coverage of those peptides for each protein is mostly less than 10% [57]. Therefore, the possibility that a modified peptide would be observed and identified is very low. As a result, differences in protein expression in shotgun peptide sequencing analysis largely reflect changes in the total protein amount (Fig. 2). Even 2-DE can resolve proteins with PTMs; determination of the modification itself is accomplished only by direct detection

of the modification site. Thus, determination of a modification site is not an easy task for 2-DE analysis, either. As to shotgun peptide sequencing, development of new methodology aimed at determining PTMs by using purification of proteins with specific PTMs is attempted very actively and looks promising [72–78], but detailed description of such modification-specific proteomics is beyond the scope of this review.

Conversely, in 2-DE it is difficult to determine the total amount of proteins. Since many proteins appear as multi spots, it is necessary to sum all the spot intensities from a protein to know the total amount of the protein. Therefore, spots which show no change in intensity must be identified in addition to up- or down-regulated spots, which would be very time-consuming and labor-intensive. In contrast, most differences determined by shotgun peptide sequencing naturally indicate changes in the total protein amount as described above.

Thus, combination of these complementary two methods provides us with more information to understand biological systems. Cathepsin B is a cysteine protease, which is translated as 38 kDa of preprocathepsin B, secreted as 35 kDa procathepsin B with cleavage of signal peptide, and finally processed to 28 kDa active cathepsin B (Fig. 3) [79]. When we compared the proteome of secreted proteins from RAW264.7 cells during osteoclast differentiation by 2-DE, we observed down-regulation of 40 kDa spots (spots 1 and 2 in Fig. 4) and up-regulation of a 35 kDa spot (spot 3) during osteoclast differentiation [68]. PMF identified all three spots as cathepsin B. Observed relative molecular mass on the gels and close inspection of the peptide coverage map of these identifications suggested that spots 1 and 2 were procathepsin B whereas spot 3 was active cathepsin B. We also analyzed the same samples by ICAT analysis [68], in which cathepsin B was identified by using five ICAT-tagged peptides: three peptides were in the sequence of active cathepsin B and two peptides were in the sequence of the N-terminal propeptide. The average fold change of the three peptides in the active cathepsin B represents the change in the total amount of cathepsin B and the change of the two N-terminal peptides represents the change in the amount of procathepsin B. The observed changes in the peptides during osteoclast differentiation were 3.1- and 3.8-fold decreases, respectively. In summary, the total amount of cathepsin B decreased 3.1-fold, while at the same time, the ratio of active cathepsin B increased during osteoclast differentiation (Fig. 4). This is a good example to demonstrate the usefulness

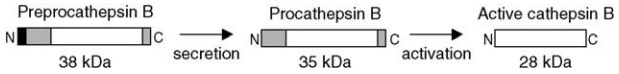

Fig. 3. Cathepsin B maturation. Cathepsin B is translated as preprocathepsin B of 38 kDa. The N-terminal signal sequence of preprocathepsin B was removed during secretion to form procathepsin B of 35 kDa. Finally, the N- and C-terminal sequences of procathepsin B were processed to form cathepsin B of 28 kDa as an active enzyme.

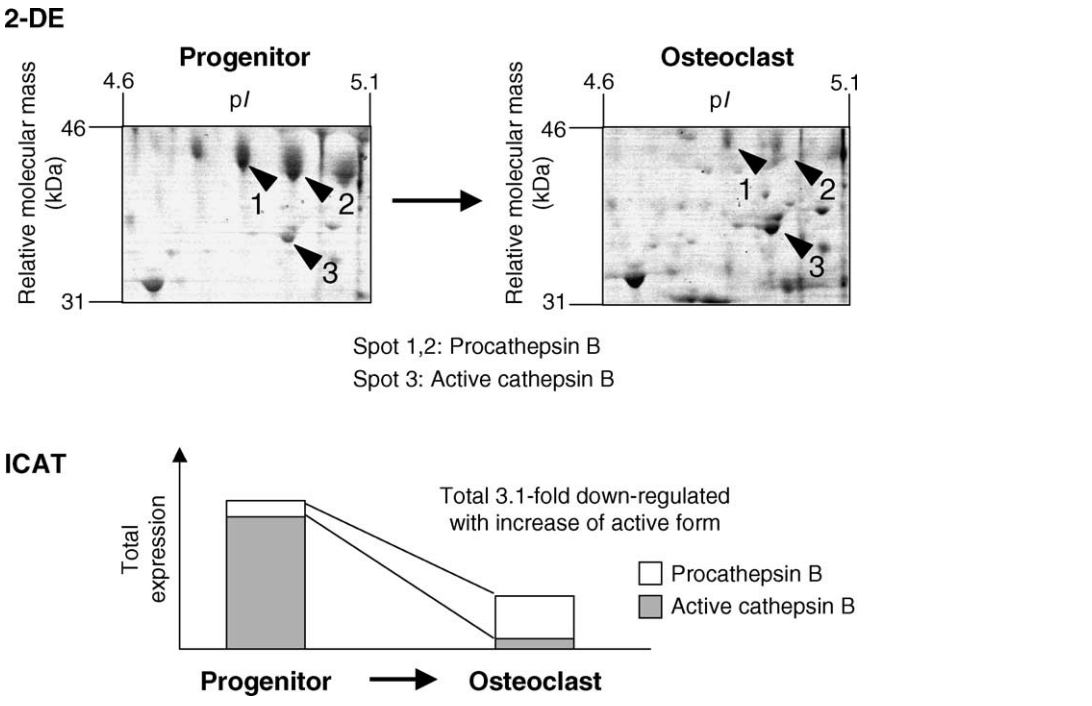

Fig. 4. Combination of 2-DE and ICAT analysis: cathepsin B during osteoclast differentiation. During differentiation from osteoclast progenitor to mature osteoclast, procathepsin B (spots 1 and 2 in 2-DE) decreased and active cathepsin B (spot 3) increased, whereas the total amount of cathepsin B decreased, as determined by ICAT analysis.

Table 1
Comparison of two-dimensional electrophoresis vs. shotgun peptide sequencing

Category	2-DE	Shotgun
Comprehensiveness		
Low abundant	–	++
Hydrophobic	+	++
Basic	+	++
Information		
Total amount	+	++
p*I*	++	–
Molecular weight	++	–
Post-translational modifications	+	–
Identification		
Peptide mass fingerprinting	++	–
MS/MS	++	++
Throughput		
Parallelism	++	+
Automation	+	++

(–) very difficult; (+) possible; (++) favourable.

of the combination of information from 2-DE and shotgun peptide sequencing analysis in comparative proteomics.

5. Conclusion

Proteins consist of 20 amino acids and some derivatives, and this diversity brings a broad range of characteristics to proteins. Therefore, regrettably, there is no single way to analyze comparative proteomics. The strengths and weaknesses of the 2-DE and shotgun peptide sequencing methods are summarized in Table 1. Even though combination of the two methods is insufficient to meet all needs, the 2-DE and shotgun peptide sequencing approaches are complementary methods at present and their combination is beneficial for better comparative proteomics, especially in comprehensiveness of coverage of the proteome and protein information.

6. Nomenclature

2-DE two-dimensional electrophoresis
DIGE difference gel electrophoresis
ICAT isotope-coded affinity tags
IEF isoelectric focusing
IPG immobilized pH gradient
LC/MS/MS liquid chromatography tandem mass spectrometry
MALDI matrix-assisted laser desorption/ionization
MS mass spectrometry
MS/MS tandem mass spectrometry
p*I* isoelectric point
PMF peptide mass fingerprinting
PTM post-translational modification
TOF time-of-flight

Acknowledgement

We thank M. Kubota for encouragement and helpful discussion.

References

[1] V.C. Wasinger, S.J. Cordwell, A. Cerpa-Poljak, J.X. Yan, A.A. Gooley, M.R. Wilkins, M.W. Duncan, R. Harris, K.L. Williams, I. Humphery-Smith, Electrophoresis 16 (1995) 1090.
[2] M.R. Wilkins, J.C. Sanchez, A.A. Gooley, R.D. Appel, I. Humphery-Smith, D.F. Hochstrasser, K.L. Williams, Biotechnol. Genet. Eng. Rev. 13 (1996) 19.
[3] A. Pandey, M. Mann, Nature 405 (2000) 837.
[4] R. Aebersold, M. Mann, Nature 422 (2003) 198.
[5] L.A. Huber, Nat. Rev. Mol. Cell Biol. 4 (2003) 74.
[6] J. Klose, C. Nock, M. Herrmann, K. Stuhler, K. Marcus, M. Bluggel, E. Krause, L.C. Schalkwyk, S. Rastan, S.D. Brown, K. Bussow, H. Himmelbauer, H. Lehrach, Nat. Genet. 30 (2002) 385.
[7] E. Lasonder, Y. Ishihama, J.S. Andersen, A.M. Vermunt, A. Pain, R.W. Sauerwein, W.M. Eling, N. Hall, A.P. Waters, H.G. Stunnenberg, M. Mann, Nature 419 (2002) 537.
[8] L. Florens, M.P. Washburn, J.D. Raine, R.M. Anthony, M. Grainger, J.D. Haynes, J.K. Moch, N. Muster, J.B. Sacci, D.L. Tabb, A.A. Witney, D. Wolters, Y. Wu, M.J. Gardner, A.A. Holder, R.E. Sinden, J.R. Yates, D.J. Carucci, Nature 419 (2002) 520.
[9] P. Uetz, L. Giot, G. Cagney, T.A. Mansfield, R.S. Judson, J.R. Knight, D. Lockshon, V. Narayan, M. Srinivasan, P. Pochart, A. Qureshi-Emili, Y. Li, B. Godwin, D. Conover, T. Kalbfleisch, G. Vijayadamodar, M. Yang, M. Johnston, S. Fields, J.M. Rothberg, Nature 403 (2000) 623.
[10] T. Ito, T. Chiba, R. Ozawa, M. Yoshida, M. Hattori, Y. Sakaki, Proc. Natl. Acad. Sci. U.S.A. 98 (2001) 4569.
[11] J.C. Rain, L. Selig, H. De Reuse, V. Battaglia, C. Reverdy, S. Simon, G. Lenzen, F. Petel, J. Wojcik, V. Schachter, Y. Chemama, A. Labigne, P. Legrain, Nature 409 (2001) 211.
[12] L. Giot, J.S. Bader, C. Brouwer, A. Chaudhuri, B. Kuang, Y. Li, Y.L. Hao, C.E. Ooi, B. Godwin, E. Vitols, G. Vijayadamodar, P. Pochart, H. Machineni, M. Welsh, Y. Kong, B. Zerhusen, R. Malcolm, Z. Varrone, A. Collis, M. Minto, S. Burgess, L. McDaniel, E. Stimpson, F. Spriggs, J. Williams, K. Neurath, N. Ioime, M. Agee, E. Voss, K. Furtak, R. Renzulli, N. Aanensen, S. Carrolla, E. Bickelhaupt, Y. Lazovatsky, A. DaSilva, J. Zhong, C.A. Stanyon, R.L. Finley Jr., K.P. White, M. Braverman, T. Jarvie, S. Gold, M. Leach, J. Knight, R.A. Shimkets, M.P. McKenna, J. Chant, J.M. Rothberg, Science 302 (2003) 1727.
[13] S. Li, C.M. Armstrong, N. Bertin, H. Ge, S. Milstein, M. Boxem, P.O. Vidalain, J.D. Han, A. Chesneau, T. Hao, D.S. Goldberg, N. Li, M. Martinez, J.F. Rual, P. Lamesch, L. Xu, M. Tewari, S.L. Wong, L.V. Zhang, G.F. Berriz, L. Jacotot, P. Vaglio, J. Reboul, T. Hirozane-Kishikawa, Q. Li, H.W. Gabel, A. Elewa, B. Baumgartner, D.J. Rose, H. Yu, S. Bosak, R. Sequerra, A. Fraser, S.E. Mango, W.M. Saxton, S. Strome, S. Van Den Heuvel, F. Piano, J. Vandenhaute, C. Sardet, M. Gerstein, L. Doucette-Stamm, K.C. Gunsalus, J.W. Harper, M.E. Cusick, F.P. Roth, D.E. Hill, M. Vidal, Science 303 (2004) 540.
[14] G. Rigaut, A. Shevchenko, B. Rutz, M. Wilm, M. Mann, B. Seraphin, Nat. Biotechnol. 17 (1999) 1030.
[15] A.C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J.M. Rick, A.M. Michon, C.M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M.A. Heurtier, R.R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T.

Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, Nature 415 (2002) 141.
[16] Y. Ho, A. Gruhler, A. Heilbut, G.D. Bader, L. Moore, S.L. Adams, A. Millar, P. Taylor, K. Bennett, K. Boutilier, L. Yang, C. Wolting, I. Donaldson, S. Schandorff, J. Shewnarane, M. Vo, J. Taggart, M. Goudreault, B. Muskat, C. Alfarano, D. Dewar, Z. Lin, K. Michalickova, A.R. Willems, H. Sassi, P.A. Nielsen, K.J. Rasmussen, J.R. Andersen, L.E. Johansen, L.H. Hansen, H. Jespersen, A. Podtelejnikov, E. Nielsen, J. Crawford, V. Poulsen, B.D. Sorensen, J. Matthiesen, R.C. Hendrickson, F. Gleeson, T. Pawson, M.F. Moran, D. Durocher, M. Mann, C.W. Hogue, D. Figeys, M. Tyers, Nature 415 (2002) 180.
[17] W.K. Huh, J.V. Falvo, L.C. Gerke, A.S. Carroll, R.W. Howson, J.S. Weissman, E.K. O'Shea, Nature 425 (2003) 686.
[18] M. Norin, M. Sundstrom, Trends Biotechnol. 20 (2002) 79.
[19] A. Sali, R. Glaeser, T. Earnest, W. Baumeister, Nature 422 (2003) 216.
[20] R.J. Simpson, L.M. Connolly, J.S. Eddes, J.J. Pereira, R.L. Moritz, G.E. Reid, Electrophoresis 21 (2000) 1707.
[21] L. Zhang, W. Yu, T. He, J. Yu, R.E. Caffrey, E.A. Dalmasso, S. Fu, T. Pham, J. Mei, J.J. Ho, W. Zhang, P. Lopez, D.D. Ho, Science 298 (2002) 995.
[22] H. Wang, S. Hanash, J. Chromatogr. B. Anal. Technol. Biomed. Life Sci. 787 (2003) 11.
[23] D.B. Wall, M.T. Kachman, S. Gong, R. Hinderer, S. Parus, D.E. Misek, S.M. Hanash, D.M. Lubman, Anal. Chem. 72 (2000) 1099.
[24] P. Hoffmann, H. Ji, R.L. Moritz, L.M. Connolly, D.F. Frecklington, M.J. Layton, J.S. Eddes, R.J. Simpson, Proteomics 1 (2001) 807.
[25] H. Zischka, G. Weber, P.J. Weber, A. Posch, R.J. Braun, D. Buhringer, U. Schneider, M. Nissum, T. Meitinger, M. Ueffing, C. Eckerskorn, Proteomics 3 (2003) 906.
[26] A.J. Link, J. Eng, D.M. Schieltz, E. Carmack, G.J. Mize, D.R. Morris, B.M. Garvik, J.R. Yates III, Nat. Biotechnol. 17 (1999) 676.
[27] M.B. Goshe, R.D. Smith, Curr. Opin. Biotechnol. 14 (2003) 101.
[28] W.A. Tao, R. Aebersold, Curr. Opin. Biotechnol. 14 (2003) 110.
[29] G. MacBeath, S.L. Schreiber, Science 289 (2000) 1760.
[30] H. Zhu, M. Bilgin, R. Bangham, D. Hall, A. Casamayor, P. Bertone, N. Lan, R. Jansen, S. Bidlingmaier, T. Houfek, T. Mitchell, P. Miller, R.A. Dean, M. Gerstein, M. Snyder, Science 293 (2001) 2101.
[31] P.H. O'Farrell, J. Biol. Chem. 250 (1975) 4007.
[32] J. Klose, Humangenetik 26 (1975) 231.
[33] P.G. Righetti, C. Macelloni, J. Biochem. Biophys. Methods 6 (1982) 1.
[34] A. Görg, W. Postel, S. Günther, J. Weser, J.R. Strahler, S.M. Hanash, L. Somerlot, R. Kuick, Electrophoresis 9 (1988) 37.
[35] K. Berggren, E. Chernokalskaya, T.H. Steinberg, C. Kemper, M.F. Lopez, Z. Diwu, R.P. Haugland, W.F. Patton, Electrophoresis 21 (2000) 2509.
[36] J.A. Mackintosh, H.Y. Choi, S.H. Bae, D.A. Veal, P.J. Bell, B.C. Ferrari, D.D. Van Dyk, N.M. Verrills, Y.K. Paik, P. Karuso, Proteomics 3 (2003) 2273.
[37] M. Unlu, M.E. Morgan, J.S. Minden, Electrophoresis 18 (1997) 2071.
[38] T. Rabilloud, Proteomics 2 (2002) 3.
[39] J. Klose, U. Kobalz, Electrophoresis 16 (1995) 1034.
[40] P. Jungblut, B. Thiede, Mass Spectrom. Rev. 16 (1997) 145.
[41] W.P. Blackstock, M.P. Weir, Trends. Biotechnol. 17 (1999) 121.
[42] J.R. Yates III, J. Mass Spectrom. 33 (1998) 1.
[43] S.E. Ong, A. Pandey, Biomol. Eng. 18 (2001) 195.
[44] M.R. Wilkins, E. Gasteiger, J.C. Sanchez, A. Bairoch, D.F. Hochstrasser, Electrophoresis 19 (1998) 1501.
[45] S.P. Gygi, G.L. Corthals, Y. Zhang, Y. Rochon, R. Aebersold, Proc. Natl. Acad. Sci. U.S.A. 97 (2000) 9390.
[46] V. Santoni, M. Molloy, T. Rabilloud, Electrophoresis 21 (2000) 1054.
[47] M.P. Molloy, Anal. Biochem. 280 (2000) 1.
[48] K. Pennington, E. McGregor, C.L. Beasley, I. Everall, D. Cotter, M.J. Dunn, Proteomics 4 (2004) 27.
[49] S. Luche, H. Diemer, C. Tastet, M. Chevallet, A. Van Dorsselaer, E. Leize-Wagner, T. Rabilloud, Proteomics 4 (2004) 551.
[50] M.P. Molloy, B.R. Herbert, B.J. Walsh, M.I. Tyler, M. Traini, J.C. Sanchez, D.F. Hochstrasser, K.L. Williams, A.A. Gooley, Electrophoresis 19 (1998) 837.
[51] X. Zuo, L. Echan, P. Hembach, H.Y. Tang, K.D. Speicher, D. Santoli, D.W. Speicher, Electrophoresis 22 (2001) 1603.
[52] B. Herbert, Electrophoresis 20 (1999) 660.
[53] D.F. Hunt, R.A. Henderson, J. Shabanowitz, K. Sakaguchi, H. Michel, N. Sevilir, A.L. Cox, E. Appella, V.H. Engelhard, Science 255 (1992) 1261.
[54] A.L. McCormack, D.M. Schieltz, B. Goode, S. Yang, G. Barnes, D. Drubin, J.R. Yates III, Anal. Chem. 69 (1997) 767.
[55] A.R. Dongre, J.K. Eng, J.R. Yates III, Trends. Biotechnol. 15 (1997) 418.
[56] M.P. Washburn, D. Wolters, J.R. Yates III, Nat. Biotechnol. 19 (2001) 242.
[57] C.C. Wu, M.J. MacCoss, K.E. Howell, J.R. Yates III, Nat. Biotechnol. 21 (2003) 532.
[58] J.S. Andersen, C.J. Wilkinson, T. Mayor, P. Mortensen, E.A. Nigg, M. Mann, Nature 426 (2003) 570.
[59] M. Kikuchi, N. Hatano, S. Yokota, N. Shimozawa, T. Imanaka, H. Taniguchi, J. Biol. Chem. 279 (2004) 421.
[60] S.P. Gygi, B. Rist, S.A. Gerber, F. Turecek, M.H. Gelb, R. Aebersold, Nat. Biotechnol. 17 (1999) 994.
[61] Y. Oda, K. Huang, F.R. Cross, D. Cowburn, B.T. Chait, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 6591.
[62] T. Guina, M. Wu, S.I. Miller, S.O. Purvine, E.C. Yi, J. Eng, D.R. Goodlett, R. Aebersold, R.K. Ernst, K.A. Lee, J. Am. Soc. Mass Spectrom. 14 (2003) 742.
[63] J.A. Ranish, E.C. Yi, D.M. Leslie, S.O. Purvine, D.R. Goodlett, J. Eng, R. Aebersold, Nat. Genet. 33 (2003) 349.
[64] Y. Shiio, S. Donohoe, E.C. Yi, D.R. Goodlett, R. Aebersold, R.N. Eisenman, EMBO J. 21 (2002) 5088.
[65] L.R. Yu, M.D. Johnson, T.P. Conrads, R.D. Smith, R.S. Morrison, T.D. Veenstra, Electrophoresis 23 (2002) 1591.
[66] N.S. Baliga, M. Pan, Y.A. Goo, E.C. Yi, D.R. Goodlett, K. Dimitrov, P. Shannon, R. Aebersold, W.V. Ng, L. Hood, Proc. Natl. Acad. Sci. U.S.A. 99 (2002) 14913.
[67] Y. Oda, T. Owa, T. Sato, B. Boucher, S. Daniels, H. Yamanaka, Y. Shinohara, A. Yokoi, J. Kuromitsu, T. Nagasu, Anal. Chem. 75 (2003) 2159.
[68] K. Kubota, K. Wakabayashi, T. Matsuoka, Proteomics 3 (2003) 616.
[69] D.K. Han, J. Eng, H. Zhou, R. Aebersold, Nat. Biotechnol. 19 (2001) 946.
[70] H. Zhou, J.A. Ranish, J.D. Watts, R. Aebersold, Nat. Biotechnol. 20 (2002) 512.
[71] D. Baltimore, Nature 409 (2001) 814.
[72] M. Mann, O.N. Jensen, Nat. Biotechnol. 21 (2003) 255.
[73] H. Zhou, J.D. Watts, R. Aebersold, Nat. Biotechnol. 19 (2001) 375.
[74] Y. Oda, T. Nagasu, B.T. Chait, Nat. Biotechnol. 19 (2001) 379.
[75] M.B. Goshe, T.D. Veenstra, E.A. Panisko, T.P. Conrads, N.H. Angell, R.D. Smith, Anal. Chem. 74 (2002) 607.
[76] S.B. Ficarro, M.L. McCleland, P.T. Stukenberg, D.J. Burke, M.M. Ross, J. Shabanowitz, D.F. Hunt, F.M. White, Nat. Biotechnol. 20 (2002) 301.
[77] H. Kaji, H. Saito, Y. Yamauchi, T. Shinkawa, M. Taoka, J. Hirabayashi, K. Kasai, N. Takahashi, T. Isobe, Nat. Biotechnol. 21 (2003) 667.
[78] H. Zhang, X.J. Li, D.B. Martin, R. Aebersold, Nat. Biotechnol. 21 (2003) 660.
[79] S.J. Chan, B. San Segundo, M.B. McCormick, D.F. Steiner, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 7721.