Bioscience Reports, Vol. 25, Nos. 1/2, February/April 2005 (© 2005)
DOI: 10.1007/s10540-005-2847-z

Interaction Proteomics

Maria Monti,[1] Stefania Orrù,[1] Daniela Pagnozzi,[1] and Piero Pucci[1,2]

The term proteome is traditionally associated with the identification of a large number of proteins within complex mixtures originating from a given organelle, cell or even organism. Current proteome investigations are basically focused on two major areas, expression proteomics and functional proteomics. Both approaches rely on the fractionation of protein mixtures essentially by two-dimensional polyacrylamide gel electrophoresis (2D-gel) and the identification of individual protein bands by mass spectrometric techniques (2D-MS). Functional proteomics approaches are basically addressing two main targets, the elucidation of the biological function of unknown proteins and the definition of cellular mechanisms at the molecular level. In the cell many processes are governed not only by the relative abundance of proteins but also by rapid and transient regulation of activity, association and localization of proteins and protein complexes. The association of an unknown protein with partners belonging to a specific protein complex involved in a particular process would then be strongly suggestive of its biological function. The identification of interacting proteins in stable complexes in a cellular system is essentially achieved by affinity-based procedures. Different strategies relying on this simple concept have been developed and a brief overview of the main approaches presently used in functional proteomics studies is described.

KEY WORDS: Functional proteomics; mass spectrometry; protein–protein interactions; affinity-based strategies.

ABBREVIATIONS: 1D-gel: monodimensional gel electrophoresis; 2D-gel: two-dimensional gel electrophoresis; AldA-NRE: Aldolase A negative regulatory element; ES-LC-MS/MS: Electrospray Liquid Chromatography Tandem Mass Spectrometry; FCP1: TFIIF-associating component of CTD phosphatase; GST: Glutathione S-transferase; KRAB-ZFPs: Krüppel-like zinc-finger proteins; MALDI-MS: Matrix Assisted Laser Desorption Ionization- Mass Spectrometry; MEP50: Methylosome protein 50; RNAPII-CTD: RNA Polymerase II – Carboxy Terminal Domain; SDS-PAGE: sodium dodecylphosphate polyacrylamide gel electrophoresis; TAP: Tandem Affinity Purification; ZnF224: Zinc Finger Protein 224.

INTRODUCTION

With the increase in the number of genome sequencing projects, there is a concomitant exponential growth in the number of protein sequences whose function is still unknown. Biological sciences are currently experiencing a sort of paradoxal

[1]CEINGE Biotecnologie Avanzate s.c.a r.l. and Dipartimento di Chimica Organica e Biochimica, Università di Napoli Federico II, Complesso Universitario di Monte S. Angelo, via Cinthia 4, 80126 Napoli, Italy.

[2]To whom correspondence should be addressed. E-mail: pucci@unina.it

situation in which the protein sequence, the corresponding coding gene, its chromosomic localization or even the regulation mechanisms may have been elucidated but the biological role of the protein in the cell is still completely obscure.

The challenge has then shifted to identify and localize proteins within a given organelle, cell or even organism as well as to unravel protein pathways in cellular systems [1–8]. These new goals, however, cannot be easily achieved as intrinsic difficulties increase by several orders of magnitude when moving from genome to proteome research. The static nature of the genome, in fact, cannot be compared to the dynamic properties of the proteome; protein expressions profiles change several times during the cell cycle and are heavily affected by a number of intra- and extracellular stimuli (temperature, stress, apoptotic signals, etc.) [1]. Moreover, the occurrence of alternative splicing and post-translational modifications led to a complete re-thinking of the old paradigm ''one gene-one protein'' that does not reflect anymore the real nature of the cellular proteome.

Current proteome investigations are essentially focused on two major areas, the expression proteomics, which aims to measure up-and down-regulation of protein levels, and functional proteomics studies aimed at the characterisation of protein activities, multiprotein complexes and signaling pathways [9–12]. Typically, expression proteomics studies are investigating the expression protein patterns in abnormal cells (i.e. malignant, stimulated by drug treatment, etc...) in comparison to normal cells. In biomedical applications, this comparative approach is usually employed to identify proteins that are up- or down-regulated in a disease specific manner for use as diagnostic markers or therapeutic targets [13–16]. In these studies, a reliable analysis of quantitative changes in protein expression is crucial. Such changes are often obtained from the staining intensities of protein spots on gels, a labor-intensive method that is prone to error. Recently, better and more reliable results were achieved using stable isotope methodologies or dual fluorescent techniques [17–20].

Functional proteomics approaches are addressing two major topics, the elucidation of biological function of unknown proteins and the definition of cellular mechanisms at the molecular level. In the cells, many proteins display their biological functions through the rapid and transient association within large protein complexes [21]. Understanding protein functions as well as unraveling molecular mechanisms within the cell is then depending on the identification of the interacting protein partners. The association of an unknown protein with partners belonging to a specific protein complex involved in a particular process would in fact be strongly suggestive of its biological function [22, 23]. Furthermore, a detailed description of the cellular signalling pathways might greatly benefit from the elucidation of protein–protein interactions in the cell [24].

PROTEIN IDENTIFICATION BY MASS SPECTROMETRY METHODOLOGIES

The key step in any proteomic study consists in the identification of proteins that have been either fractionated by gel electrophoresis or digested by enzymatic procedures to generate peptide mixtures. Protein identification from 1D or 2D

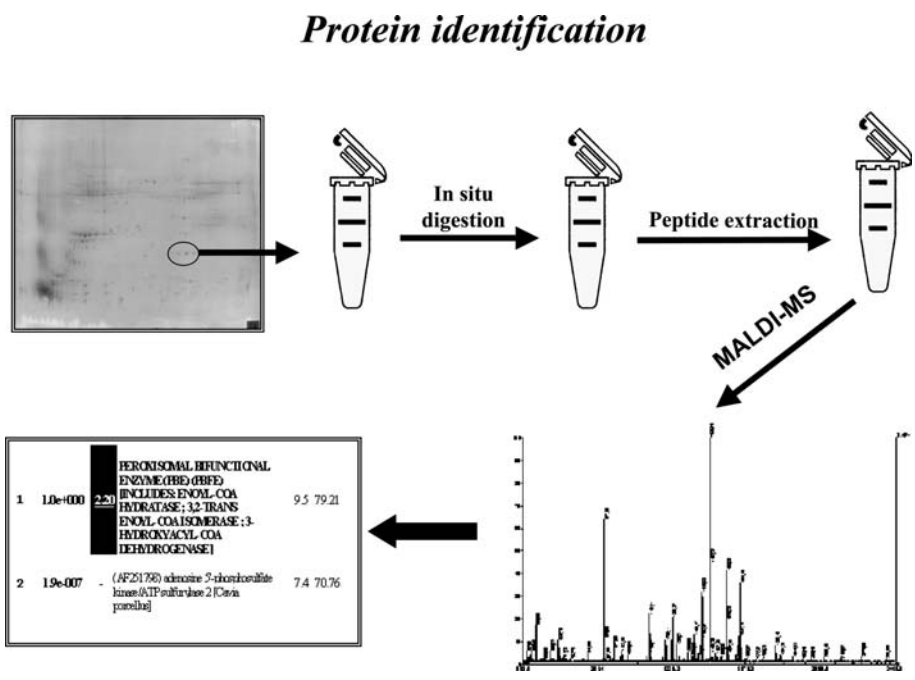

Fig. 1. Outline of protein identification strategy by peptide mass fingerprinting.

gel-electrophoresis is obtained through peptide mass fingerprinting essentially using MALDI-MS (Fig. 1). After fractionation by electrophoresis, in the majority of the cases, proteins are stained by colloidal Coomassie, excised from the gel and submitted to different cycles of swelling and shrinking by alternate washing with aqueous and organic solutions. Protein components are then reduced and alkylated with iodoacetamide to irreversibly block the cysteine residues and digested *in situ* with suitable amount of trypsin overnight. The resulting peptide mixture is extracted from the gel by repeating the swelling/shrinking procedure and directly analysed by MALDI-MS (using a reflectron instrument). Identification of the various proteins is carried out through the peptide mass fingerprinting procedure: the mass values together with other information, such as the protease used for the hydrolysis and the protein molecular mass roughly estimated from the SDS-PAGE gel, are introduced into different mass search programs (e.g. ProFound, Mascot, MS-Fit, etc) available on the net. The mass values are compared to those originating from the theoretical digestion of all the proteins occurring in the database, leading to the identification of the protein(s).

Alternatively, when the mass fingerprinting procedure is not sufficient to identify the proteins, electrospray ionization tandem mass spectrometry (ES-LC-MS/MS) methods can be employed (Fig. 2). Peptide mixtures produced by *in situ* digestions are fractionated by capillary HPLC analysis, the fractions eluted from the column are directly inserted into the ES mass spectrometry source and their mass values determined. Peptide ions will simultaneously be isolated and fragmented within the mass spectrometer, producing daughter ion spectra from which

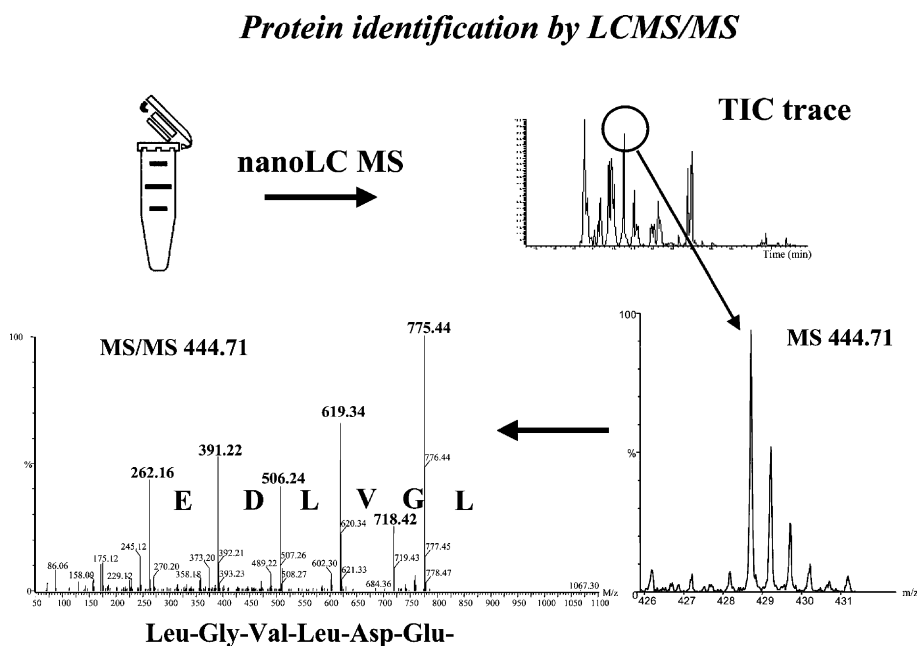

Fig. 2. Schematic description of protein identification performed by the LCMSMS approach.

sequence information on individual peptides can be obtained. This information together with the peptide mass values are then used to search for protein databases, leading to the identification of the protein components. It has been shown, in fact, that sequence information from just two peptides is sufficient to unambiguously identify a protein by searching protein and expressed sequence tag databases.

IDENTIFICATION OF PROTEIN PARTNERS BY FUNCTIONAL PROTEOMICS APPROACHES

It has become clear that a large number of proteins occur in protein complexes and that understanding the function of a given protein within the cell necessitates identification of its interacting partners [25]. A key contribution to the identification of interacting proteins in stable complexes in cellular systems is provided by affinity-based approaches. The basic idea is to express the protein of interest with a suitable tag to be used as a bait to fish its specific partners out from a cellular extract. Isolation of the entire multi-protein complex can then be accomplished by taking advantage of the availability of several anti-tag systems, immobilised on agarose–sepharose supports, and showing high binding efficiency, as illustrated in Table 1 [26]. Different strategies relying on this simple concept have been developed and a brief overview of the main approaches presently used in functional proteomics studies is described below.

Table 1. Affinity tags and ligands commonly used in the isolation of multiprotein complexes

Tag	Ligand
Poly-His	Ni++
Biotin	Streptavidin
Calmodulin-binding peptide	Calmodulin (Ca++)
GST	Glutathione
Specific Epitope (FLAG, c-myc, HA, etc.)	Monoclonal Ab

"Fishing for Partners" Strategies

Recently, a new specific strategy was developed for the identification of protein partners interacting, even transiently, with a specific target, leading to an effective alternative to molecular biology procedures based on the two-hybrid technique. Using commercially available protein expression systems, the protein bait can be produced as a hybrid protein fused to different tags, such as the Glutathione S-transferase (GST-fused Protein), or small peptide epitopes i.e. FLAG, HA or c-myc, or containing a poly-His tail or covalently modified with biotin [27]. In all cases, the tagged bait can be immobilised onto agarose beads derivatised with the appropriate anti-tag ligand (glutathione, anti-epitope antibodies, Nickel ions, Streptavidin, etc.). All these affinity tag systems provide a general applicability with a large number of proteins and a minimal effect on the tertiary structure and the biological activity of the bait, preventing instability of complexes.

The entire cellular extract and/or, when appropriate, extracts from specific organelles can then be incubated with the immobilised bait. The protein components specifically recognised by the bait are retained while the unbound proteins are removed by washing steps. The protein partners are successively eluted, fractionated by SDS-PAGE, stained and then submitted to the mass spectrometry procedures for protein identification. An outline of this approach is shown in Fig. 3.

This strategy was applied to the identification of the protein partners of ZnF224, a zinc-finger protein of about 82 kDa belonging to the "Krüppel-like" zinc-finger proteins family (KRAB-ZFPs), one of the largest classes of transcription factors. This protein contains the box A (45 aa) of a Krüppel-associated box (KRAB) domain at the N-terminus, which is an evolutionarily conserved regulatory domain and 19 Cys_2Hys_2 zinc-finger motifs at the C-terminus [28]. ZnF224 specifically binds to the negative regulatory element (AldA-NRE) located in the promoter region of the human aldolase A gene through its array of zinc-fingers and inhibits the transcription by the 45-amino-acid KRAB-A domain. The proteins interacting with ZnF224 and involved in the transcriptional inhibition as well as the molecular mechanisms of these negative regulation processes are still unknown. In order to elucidate these aspects, the full-length cDNAs of ZnF224 and its deletion mutant ZnF224-M1 containing only eight zinc-fingers were expressed as fused protein to the C-terminus of GST and purified on glutathione–sepharose resin. The purified chimeric bait was then linked to GSH-activated beads.

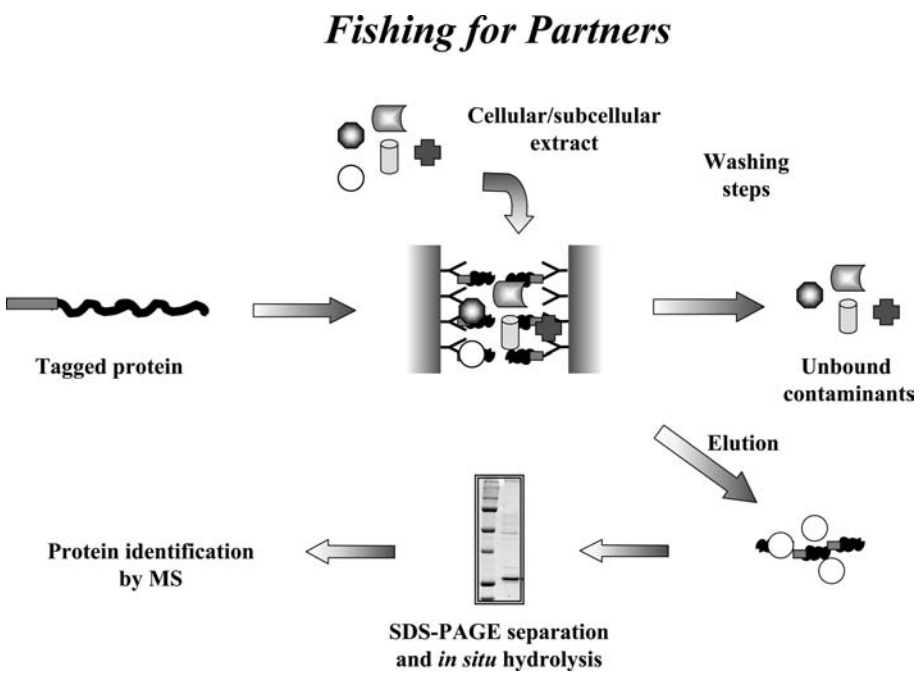

Fig. 3. Fishing for partners strategy.

Nuclear 293 cell extracts were pre-purified by incubation with GST-beads that retained all the proteins unspecifically interacting with both the matrix and the GST protein that would lead to false positives. The pre-cleaned extract was then incubated with the GST–ZnF224 bait. After several washings to remove unbound proteins, the complex components were eluted from the beads, separated by SDS-PAGE and stained with Colloidal Coomassie (Fig. 4). Protein bands indicated by arrows appeared to be present only in the sample and were then selected for identification by *in situ* digestion and LC-MS/MS analysis.

Although this approach has found large applications in the studies of protein–protein interactions, a conspicuous number of drawbacks exists. The pre-cleaning procedure allowed us to avoid the occurrence of an excessive background; however, the presence of several identical bands in both the sample and the control and the difficulties in identifying the proteins specifically interacting with the bait immediately underline the limitations of this procedure. Extensive pre-cleaning of the extract is needed and a number of control samples have to be prepared. Cellular lysis under hard conditions leads to the disruption of the architecture of subcellular compartments thus generating non physiological interactions among proteins that are normally segregated in different organelles. However, the major criticism to this approach is that the interactions among the bait and its protein partners take place *in vitro* on the derivatised beads and might not be indicative of functional interactions. The overall effect of these drawbacks is the possible occurrence of false positives that should always be considered when using these procedures.

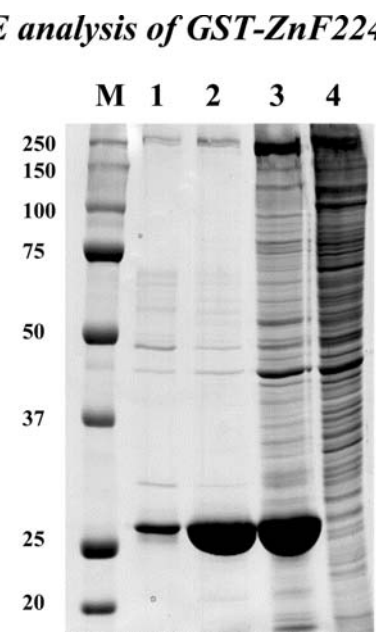

Fig. 4. SDS-PAGE analysis of GST-ZnF224-M1 partners: lane M: markers; lane 1 proteins bound specifically to the protein bait; lane 2: second pre-cleaning on the resin pre-incubated with GST alone; lane 3: first pre-cleaning on the resin pre-incubated with GST alone; lane 4: unbound proteins.

The success of an affinity-based approach then depends on the absence of excessive unspecific interactions that in turn is related to the specificity of the bait-partners recognition. When this binding specificity is extremely high, as in the case of DNA-binding proteins, a low levels of false positives is expected. In this particular variant of the fishing strategy, the bait consists of a specific oligonucleotide linked to an insoluble support. Nuclear proteins can then be incubated with the bait in search for specific partners, following the strategy outlined above [29]. Control experiments can easily be designed using randomised oligonucleotides. This strategy was applied to the identification of the transcriptional repressor that specifically binds to the human aldolase A (AldA) negative regulatory element (NRE). This factor was eventually identified as the ZnF224 protein [28].

It should be underlined that when a putative candidate is identified by proteomic approaches, its identity has to be confirmed by independent methodologies. In the case of ZnF224, both the specificity of DNA sequence recognition and the negative regulation of transcriptional activity of the AldolaseA gene had to be tested. Therefore, the recombinant forms of wild type ZnF224 and two deletion mutants were prepared and incubated with the AldANRE oligonucleotide region in a classical band shift experiment, demonstrating the ability of the wild type protein to specifically recognise the DNA binding site. Moreover, a CAT reporter gene assay was designed using the recombinant plasmid encoding ZNF224 and containing two AldA-NRE core elements upstream of a heterologous basal promoter. The expressed ZNF224 protein negatively modulated the reporter CAT gene transcription in a

dose-dependent manner, showing that ZnF224 was indeed able to repress the AldA-NRE-mediated transcription of a heterologous promoter [26].

Immunoprecipitation Strategies

Alternative strategies essentially relying on immunoprecipitation techniques have been introduced in order to overcome the major criticisms associated with the fishing procedure (Fig. 5) [30]. The gene coding for the bait tagged with an epitope against which good antibodies exists (FLAG, HA, c-myc, etc.), is transfected into the appropriate cell line and expressed in the cognate host. Protein complexes are allowed to form and the cell extracts are immunoprecipitated with anti-tag monoclonal antibodies. The immunoprecipitated material containing the protein bait and its interacting partners can then be fractionated by SDS-PAGE and the individual protein components identified by different mass spectrometric methodologies.

The immunoprecipitation strategy was employed to identify the protein partners of FCP1, a conserved phosphatase involved in the regulation of eukaryotic RNA polymerase II [31]. In order to elucidate the role of FCP1, identification of FCP1 associating factors using a 3 × FLAG-tagged FCP1 stably expressing cell line was performed [32]. Following complex formation, nuclear extracts were immunoprecipitated with anti-FLAG agarose-conjugated antibody. The protein bands separated on SDS-PAGE and stained by colloidal Coomassie were identified essentially by mass fingerprinting. Among several other specific FCP1 partners, the methylo-

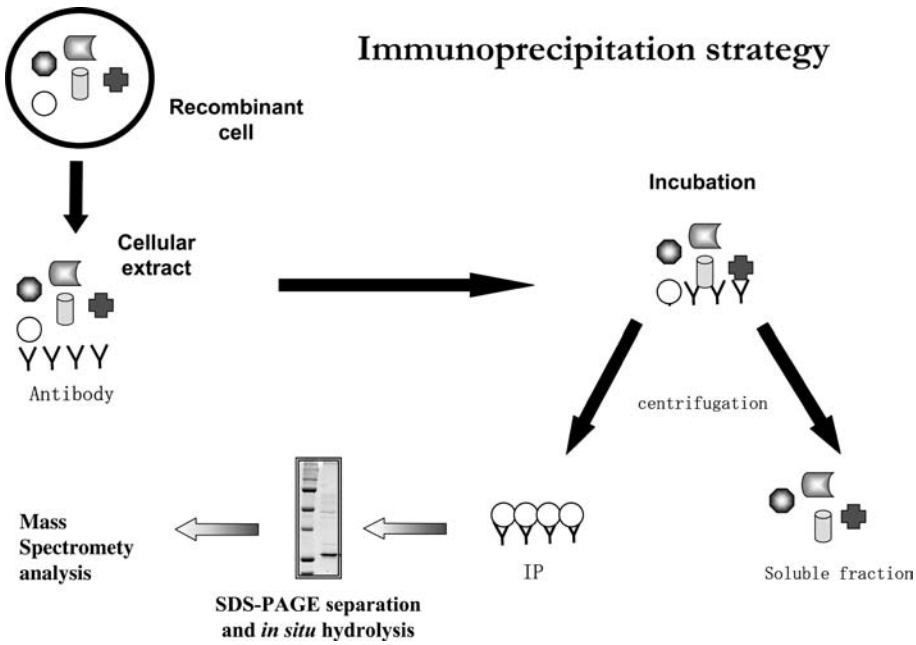

Fig. 5. Outline of the immunoprecipitation strategy.

some protein 50, MEP50, was identified. However, this protein belongs to a cytosolic complex whereas FCP1 was exclusively found in the nucleus, thus making this identification questionable. Independent verification experiments were then designed, including co-immunoprecipitation of FLAG-FCP1 and a recombinant form of MEP50 containing a c-myc-tag and sub-cellular localization by both sedimentation profiling and immunofluorescence techniques. The results obtained demonstrated that MEP50 and FCP1 associate into the nucleus in a complex of the same size distinct from the 20S methylosome complex and that FCP1 is able to interact with components of the pre-mRNA spliceosomal complex. These findings add further support to the concept that there is functional intercommunication between the transcription and splicing machineries, and the RNAPII-CTD appears to play a pivotal role in coordinating transcription and pre-mRNA processing [32, 33 and references therein].

Although the immunoprecipitation approach seems to provide significant data in most of the cases, some drawbacks need to be discussed. Antibodies used in immunostaining methods are not always suitable for immunoprecipitation protocols that require a more efficient (and quantitative) recognition of the proper antigen compared to Western blotting or ELISA applications. Cross-reaction with unspecific antigens or unspecific binding of proteins to the antibodies, to the peptide tags or to the insoluble support can lead to false positives. Pre-cleaning of the cellular extract with antibodies of the animal host not yet immunised against the specific antigen is then strongly suggested.

Recently a debated question arose concerning the use of antibodies specifically directed against the protein bait; this antibody might compete with the interacting proteins for binding to bait epitopes thus leading to destabilization of protein–protein interactions and dissociation of the complexes, at least partially. These problems have been overcome by using tagged proteins although the presence of the tag might affect protein conformation, altering or impairing complex formation. A first clue to solve this issue comes from preliminary experiments carried out on protein baits tagged at either the N- or the C-terminus; moreover, the three dimensional structure of the bait, when available, should carefully be considered to decide where the tag should be posed. Finally, overexpression of the tagged protein in the host cells should definitely be avoided since a high concentration of the bait alters the stoichiometric ratio with its natural partners often leading to the formation of nonspecific and/or nonnatural protein interactions with host proteins [34].

The Tap Tag System

The tandem affinity purification (TAP) tag system was developed for the purification of protein complexes in high yield under native conditions [35]. In this procedure, two different tags usually separated by an enzyme-cleavable linker sequence are inserted on the same protein and the protein complexes are purified by two affinity purification steps. The first proposed TAP tag system consisted of two IgG binding domains of *Staphylococcus aureus* protein A (ProtA) and a calmodulin binding peptide (CBP) separated by a TEV protease cleavage site (Fig. 6) [36]. This system is very flexible and variations to the original strategy, including application of the tag cassette to either the N- or the C-terminal end of the protein, the introduction

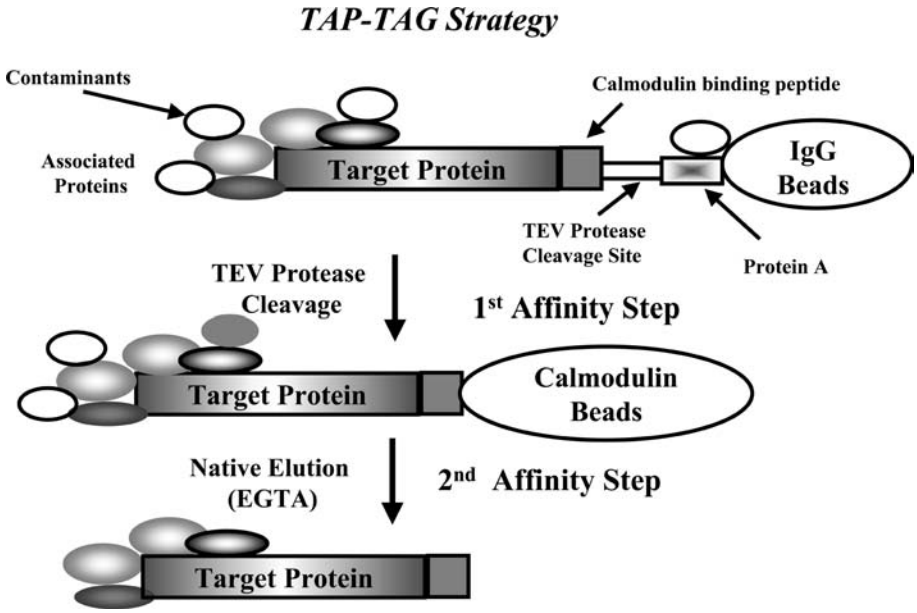

Fig. 6. Schematic representation of the TAP-Tag strategy.

of alternative tags and the tailoring of the system for various host organisms can easily be obtained.

The TAP tags are introduced in-frame with the coding region of the protein bait in an appropriate expression vector using standard DNA cloning procedures. The tagged construct can then be transiently or stably introduced into recipient cells or organisms. Optimally, the recombinant vector should replace the endogenous wild-type gene, although this condition might not always be possible. In all cases, overexpression of the protein bait is avoided since the TAP tag system was specifically designed for recovery of protein complexes expressed at their own natural level.

The first affinity purification step of the native complex consists in the binding of ProtA to an IgG matrix. Elution of the bound material under native conditions is achieved by using the TEV protease and the eluate of this first affinity step is then incubated with calmodulin-coated beads in the presence of calcium. Nonspecific protein contaminants and the excess of TEV protease are removed by repetitive washing and the bound material is released under mild conditions by elution with EGTA. Individual protein components of the eluted complex can then be fractionated by SDS-PAGE and identified by different mass spectrometric methodologies.

This purification procedure based on a double affinity step significantly reduces the possible occurrence of nonspecific protein contaminants, thus decreasing both the unspecific background noise on the SDS gel and the possible presence of false positives. This method was originally developed in yeast [36] and has been found widespread use for the description of multiprotein complexes in *Saccharomices cerevisiae* [22]. However, optimized conditions have been developed for the generic use of the TAP strategy. Recently the system has been successfully optimized even in

mammalian cells [37] by using alternative tags to further reduce unspecific interactions between tags and extract proteins [38].

CONCLUSIONS AND PERSPECTIVES

With the increasing number of genome sequencing projects coming to a successful end, understanding protein function and unraveling cellular mechanisms at the molecular level constitute today a major need in modern biology. These goals can be achieved by determining which macromolecules interact with a given protein in a specific manner. For the first time, we have the possibility to define the transient formation of functional protein complexes or to describe signal transduction pathways just as the scientists in the early '30s were able to describe "step by step" the metabolic pathways. The functional proteomic approaches described in this paper have proven to be useful tools for the detection of interacting partners of a target protein, although each of them highlighted the occurrence of possible drawbacks. Particular attention should be paid to false positives which might result in misleading interpretations; improvements and refining of the affinity-based procedures are needed to take care of this aspect. A further factor that should carefully be considered is the dynamics of the formation and dissociation of protein complexes. Proteins assemble at the right place and the right time to fulfill a specific function; complexes then dissociate and individual components can participate in the formation of other complexes, following the occurrence of specific signals.

Future aims of proteomic investigations will need to address the transfer of these approaches to an *in vivo* system by generating animal models bearing a tagged form of the given protein. If vital animals are obtained, homozygous embryos will then provide tissues and/or progenitor cells for immunoprecipitation assays. Proteomic analyses of the protein complexes occurring *in vivo* will disclose the identity of the individual components and whether they differ from a territory to another.

ACKNOWLEDGMENT

This work was supported in part by MIUR (PRIN 2003).

REFERENCES

1. Godovac-Zimmermann, J. and Brown, L. R. (2001) *Mass Spectrom. Rev.* **20:**1–57.
2. Panisko, E. A., Conrads, T. P., Goshe, M. B., and Veenstra, T. D. (2002) *Exp. Hematol.* **30:**97–107.
3. Michaud, G. A. and Snyder, M. (2002) *Biotechniques* **33:**1308–1316.
4. Souchelnytskyi, S. (2002) *J Mammary Gland. Biol. Neoplasia* **7:**359–371.
5. Taylor, S. W., Fahy, E., and Ghosh, S. S. (2003) *Trends Biotechnol.* **21:**82–88.
6. Patterson, S. D. and Aebersold, R. H. (2003) *Nat. Genet.* **33:**311–323.
7. Dreger, M. (2003) *Eur J. Biochem.* **270:**589–599.
8. Godovac-Zimmermann, J. and Brown, L. R. (2003) *Curr. Opin. Mol. Ther.* **5:**241–249.
9. Neubauer, G., Gottschalk, A., Fabrizio, P., Seraphin, B., Lurhmann, R., and Mann, M. (1997) *Proc. Natl. Acad. Sci. USA* **94:**385–390.

10. Shevchenko, A., Keller, P., Scheiffele, P., Mann, M., and Simons, K. (1997) *Electrophoresis* **18:**2591–2600.
11. Pandey, A., Fernandez, M. M., Stehen, H., Blagoev, B., Nielsen, M. M., Roche, S., Mann, M., and Lodish, H. F. (2000) *J. Biol. Chem.* **275:**38633–38639.
12. Hinsby, A. M., Olsen, J. V., Bennett, K. L., and Mann, M. (2003) *Mol. Cell Proteomics* **2:**29–36.
13. Soldes, O. S., Kuick, R. D., Thompson, II, , I. A., Hughes, S. J., Orringer, M. B., Iannettoni, M. D., Hanash, S. M., and Beer, D. G. (1999) *Br. J. Cancer.* **79:**595–603.
14. Banks, R. E., Dunn, M. J., Hochstrasser, D. F., Sanchez, J. C., Blackstock, W., Pappin, D. J., and Selby, P. J. (2000) *Lancet* **356:**1749–1756.
15. Dunn, M. J. (2000) *Drug Discov Today* **5:**76–84.
16. Jungblut, P. R., Zimny-Arndt, U., Zeindl-Eberhart, E., Stulik, J., Koupilova, K., Pleißner, K. P., Otto, A., Müller, E. C., Sokolowska-Köhler, W., Grabher, G., and Stöffler, G. (1999) *Electrophoresis* **20:**2100–2110.
17. Gygi, S. P., Rist, B., Gerber, S. A., Turecek, F., Gelb, M. H., and Aebersold, R. (1999) *Nat. Biotechnol.* **17:**994–999.
18. Zhou, H., Ranish, J. A., Watts, J. D., and Aebersold, R. (2002) *Nat. Biotechnol.* **20:**512–515.
19. Yan, J. X., Devenish, A. T., Wait, R., Stone, T., Lewis, S., and Fowler, S. (2002) *Proteomics* **2:**1682–1698.
20. Alban, A., David, S. O., Bjorkesten, L., Andersson, C., Sloge, E., Lewis, S., and Currie, I. (2003) *Proteomics* **3:**36–44.
21. Alberts, B. (1998) *Cell.* **92:**291–294.
22. Gavin, A. C., Bosche, M., and Krause, R. (2002) *Nature* **415:**141–147.
23. Ho, Y., Gruhler, A., and Heilbut, A. (2002) *Nature* **415:**180–183.
24. Lewis, T. S., Hunt, J. B., Aveline, L. D., Jonscher, K. R., Louie, D. F., Yeh, J. M., Nahreini, T. S., Resing, K. A., and Ahn, N. G. (2000) *Mol. Cell.* **6:**1343–1354.
25. Pawson, T. and Scott, J. D. (1997) *Science* **278:**2075–2080.
26. Terpe, K. (2003) *Appl Microbiol. Biotechnol.* **60:**523–533.
27. Orru', S., Caputo, I., D'Amato, A., Ruoppolo, M., and Esposito, C. (2003) *J. Biol. Chem.* **278:**31766–31773.
28. Medugno, L., Costanzo, P., Lupo, A., Monti, M., Florio, F., Pucci, P., and Izzo, P. (2003) *FEBS Lett.* **534:**93–100.
29. Kadonag, J. T. and Tijan, R. (1986) *Proc. Natl. Acad. Sci. USA.* **83:**5889–5893.
30. Cho, S., Park, S. G., Lee, D. H., and Park, B. C. (2004) *J. Biochem. Mol. Biol.* **37:**45–52.
31. Kobor, M. S., Archambault, J., Lester, W., Holstege, F. C., Gileadi, O., Jansma, D. B., Jennings, E. G., Kouyoumdjian, F., Davidson, A. R., Young, R. A., and Greenblatt, J. (1999) *Mol. Cell.* **4:**55–62.
32. Licciardo, P., Amente, S., Ruggiero, L., Monti, M., Pucci, P., Lania, L., and Macello, B. (2003) *Nucleic Acids Res.* **31:**999–1005.
33. Maniatis, T. and Reed, R. (2002) *Nature* **416:**499–506.
34. Swaffield, J. C., Melcher, K., and Johnston, S. A. (1995) *Nature* **374:**88–91.
35. Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M., and Seraphin, B. (2001) *Methods* **24:**218–229.
36. Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M., and Seraphin, B. (1999) *Nat. Biotechnol.* **17:**1030–1032.
37. Bouwmeester, T., Bauch, A., Ruffner, H., Angrand, P. O., Bergamini, G., Croughton, K., Cruciat, C., Eberhard, D., Gagneur, J., Ghidelli, S., Hopf, C., Huhse, B., Mangano, R., Michon, A. M., Schirle, M., Schlegl, J., Schwab, M., Stein, M. A., Bauer, A., Casari, G., Drewes, G., Gavin, A. C., Jackson, D. B., Joberty, G., Neubauer, G., Rick, J., Kuster, B., and Superti-Furga, G. (2004) *Nat. Cell Biol.* **6:**97–105.
38. Knuesel, M., Wan, Y., Xiao, Z., Holinger, E., Lowe, N., Wang, W., and Liu, X. (2003) *Mol. Cell Proteomics* **2:**1225–1233.