Minireview

Recent developments in the analysis of protein complexes[1]

Andrzej Dziembowski, Bertrand Séraphin*

Equipe Labelisée 'La Ligue', Centre de Génétique Moléculaire, CNRS UPR 2167 Associated with University Paris 6, Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France

Received 4 June 2003; accepted 1 July 2003

First published online 26 November 2003

Edited by Angus Lamond

Abstract The goal of this review is to analyse how recent technical developments contributed to the biochemical characterisation of protein complexes. Improvement of tags used for protein purification, including in our own laboratory, and the development of new strategies have allowed the use of generic procedures for the purification of a wide variety of protein complexes. Together with increased mass spectrometry sensitivity and automation, this made high throughput studies of protein complexes possible and allowed proteome-wide analyses of protein complexes. However, knowledge of protein complex composition, even at the cellular level, will not be sufficient to understand their function. We suggest that the next level of analysis in this area will be the definition of internal subunit arrangement in complexes as a first step toward more detailed structural analyses.

Key words: Protein interaction; Protein purification; Mass spectrometry; Tandem affinity purification; Yeast two-hybrid; Protein cross-linking

1. Introduction

Proteins, which have enzymatic, structural and regulatory functions, carry out the vast majority of all biological processes in cells. They most often do not work as monomeric entities but rather interact with each other forming stable or transient complexes. Interestingly, the sequences of higher eukaryotic genomes suggest that the complexity of the cognate organisms is not mediated by a dramatic increase in the number of genes but rather by a more complex pattern of protein–protein interactions [1]. Establishing the molecular interactions forming the basis of these regulatory networks is one of the major tasks of molecular biology in the post-genomic era. Because of the wide diversity in the properties of proteins, this task is technically more demanding than genome or transcriptome analyses. Indeed, interactions between proteins in cells cover a wide range of affinities and half-lives from stable complexes to transient interactions. Until recently, strategies allowing the deciphering of complex interaction networks have been lacking. However, data related to protein complex formation are now growing steadily in databases thanks to the development of new methodologies. In this review, we will give an overview of some technical advances that allow high throughput protein complex analyses and discuss the results of the first examples of such studies. We also address some of the future questions to be solved for a full understanding of protein complex function.

2. Developments in mass spectrometry and affinity purification allow the efficient purification of protein complexes

For decades, the bottleneck in protein analyses has resided in protein identification. Relatively large amounts of protein were required for their identification and cumbersome sequencing procedures allowed the processing of only a limited number of samples [2]. Nowadays the protein identification by mass spectrometry is very sensitive and relatively broadly available [3]. Furthermore, a large part of the process can be automated making high throughput studies possible. In this new context, the strategy that seems to be best adapted to characterise cellular protein complexes is their purification followed by subunit identification by mass spectrometry. Thus solving the protein identification problem created a new demand for methods making it possibly to rapidly and efficiently purify sufficient amounts of intact protein complexes of appropriate purity.

Originally, protein complexes were purified using classical biochemical methods [4]. This process was generally time-consuming, especially for low abundant complexes as very large amounts of starting material (hundreds of grams of cells or tissues) and numerous purification steps were needed. Yields were usually low and the procedure was so long that less stable complexes were not preserved. Most importantly, a new set of purification steps had to be empirically designed for each new complex. Nevertheless, such classical purification procedures have allowed the purification of relatively large complexes such as the human spliceosome [5–8].

The development of affinity purification and epitope tagging techniques greatly reduced the time and costs of purification [9]. Usually proteins targeted for purification with their associated partners are modified by addition of a peptide suitable for affinity purification (for example the His6 tag). Fu-

*Corresponding author. Fax: (33)-1-69 82 38 77.
E-mail address: seraphin@cgm.cnrs-gif.fr (B. Séraphin).

[1] This is the first instalment of our Special Review Series entitled 'Proteomics Meets Cell Biology'. Articles in the series will appear regularly throughout the coming year.

Abbreviations: TAP, tandem affinity purification; Y2H, yeast two-hybrid

0014-5793/03/$30.00 © 2003 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/S0014-5793(03)01357-7

sion of the target protein sequence and peptide tag coding sequence is done using standard DNA cloning techniques. The recombinant gene thus created is then introduced and expressed in the cognate host for which a transformation procedure must be available. For yeasts such as *Saccharomyces cerevisiae* or *Schizosaccharomyces pombe* this step is performed by introduction of a plasmid containing a tagged gene or more elegantly by gene replacement mediated by site-specific in vivo recombination [10]. For higher eukaryotes transient or stable transfections are performed. An alternative strategy to the use of peptides is to raise antibodies against one of the components and use of immunoaffinity chromatography [11]. However, production of antibodies is time-consuming and it remains uncertain whether reagents of sufficient quality will be obtained. Therefore this method cannot be used as a general purification strategy, especially for high throughput purposes.

There are many different affinity purification tags [12], but many of them have low affinity for their cognate ligands such that low or medium abundance proteins are recovered in low yield. It is difficult to directly compare different affinity purification tags since there are few comparative studies and only a few publications of high throughput protein complex purification data. In our laboratory, major progress in protein complex purification was made by development of the tandem affinity purification (TAP) method [13,14]. This method is based on two successive affinity chromatography steps. Originally it was developed for yeast and the tag was introduced by in vivo recombination. The tag fused to a target protein is composed of protein A having very high affinity for IgG, a TEV protease cleavage site and calmodulin binding peptide having high affinity for calmodulin. An extract containing the TAP-tagged target protein is mixed with IgG affinity resin before being incubated with TEV protease that will release the target protein by cleavage. This eluate is used for a second affinity step where the target protein will bind to calmodulin in the presence of calcium before release of the purified complex by EGTA chelating calcium ions essential for calmodulin binding (Fig. 1). The purification buffers have been optimised for highest yield, while generally maintaining protein complex integrity in an environment not too highly divergent from the intracellular conditions. Introduction of two different affinity purification steps greatly enhances the specificity of the purification procedure. In most cases, purification from 2 l of yeast culture (roughly 10 g of wet yeast cells) gives sufficient amounts of complex to visualise proteins by staining of sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) gels and identify subunits by mass spectrometry. The purification of yeast U1 snRNP and histone methylase complexes are concrete examples of the efficiency of the TAP method. In both cases, these complexes were purified independently by standard biochemical procedures. In the case of U1 snRNP, isolation from 2 l of yeast culture gave cleaner, more intact complexes and a higher yield than purification from 16 l of yeast culture using immunoaffinity chromatography (making use of a high affinity antibody) followed by His tag nickel affinity chromatography [13,15]. In the case of the histone methyltransferase complex the TAP method made it possible to reduce the yeast culture volume from 300 l to 5 l and to use only two standard and pre-optimised affinity steps instead of seven custom-developed purification steps [16].

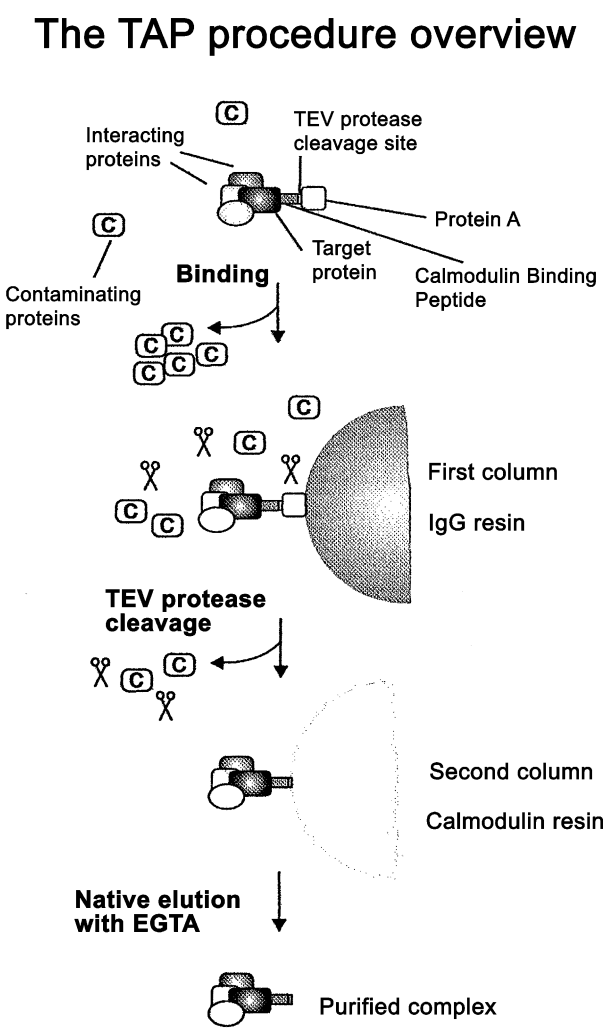

Fig. 1. Overview of the TAP strategy.

Due to the purification strategy, the module composing the TAP needs to be organised with protein A being at the protein extremity. Thus, TAP tag cassettes allowing introduction at both ends of proteins have been constructed and used successfully [14]. Additional variants of the TAP tag strategy have been described that are particularly useful in cases where some subunits belong to several different complexes, a rather common situation in cells. In the split TAP tag strategy, the two modules making the TAP tag (TEV cleavage site–protein A and calmodulin binding peptide respectively) can be independently fused to different targets to selectively recover complex(es) containing simultaneously the two target proteins [14,17]. Alternatively, undesired complexes may be selectively removed by fusing a specific subunit to a protein A module lacking a TEV protease cleavage site (subtraction strategy) [18]. The TAP strategy has been used to purify complexes from a wide variety of cellular compartments. Thus, with some modification of the standard conditions by addition of appropriate detergents, it has even been used to purify membrane protein complexes [19]. While the TAP strategy was originally developed using yeast protein complexes as a model system, it was designed to be easily adapted to other organisms. To our knowledge, it has currently been used in *S. ce-*

revisiae, *S. pombe*, trypanosomes, human, *Drosophila* and plant cells in culture as well as in *Xenopus* oocytes [20–23].

So far over 100 studies have reported the use of the TAP method to purify and identify subunits of the complexes in low throughput study. Interestingly, comparison of orthologous complexes purified from various sources has revealed that they share similar subunits [19,21,24]. Thus an alternative strategy based on phylogenetic conservation can easily be developed if the TAP strategy cannot be easily applied to a given system. In one case, a yeast homologue of a human target protein was purified using the TAP strategy and its partners were identified [25]. Database searches revealed the human homologues of these partners that were shown to associate with the original human target. Recently, an alternative strategy was proposed. It relied on the purification of TAP-tagged human protein expressed in *Drosophila* cells that had been depleted of the homologous protein by RNAi-mediated gene inactivation. Depletion of the endogenous homologous protein was implemented to make more partner subunits available for association with the target protein. This alternative strategy was thus reported to improve the number and level of partners recovered [20]. It remains to be shown, however, whether the extra RNAi step would have been truly required if the corresponding *Drosophila* protein had been TAP-tagged. Indeed, as it is likely that it would have had a higher affinity for its partners, similar results might have been obtained without the extra cost and work required by the RNAi step.

Comparison of the TAP purification results to the data about known protein–protein interactions suggests that this method has a low false negative and probably false positive error rate [26,27]. However, affinity purification methods all suffer from some limitations. First, the addition of a tag, large or small, to the protein may change its properties, causing changes in complex stability or composition. However, from our experience most genes can be tagged at one or the other extremity without major effects. If the addition of the tag is deleterious for a cell, alternative subunits may be targeted. Consistent with our data, a high throughput study (see below) revealed that 82% of the yeast essential proteins were still functional when fused to the TAP tag at their C-termini [19]. Some complexes are relatively unstable and may disassemble during purification. While the TAP method allowed the identification of transient interaction, further analyses will be required to evaluate the affinity required for such detection. Another problem that may be encountered is that the target protein belongs to several different complexes. While this is not problematic if partners need solely to be identified, this may create trouble if the activity of a given complex needs to be tested or if the various complexes are in very different stoichiometry. In the latter cases, the subtraction or the split TAP tag strategies described above may provide some solutions.

The major problem in all purification methods is the co-purification of 'contaminating' proteins. This includes abundant cellular proteins such as cytoskeletal proteins, translation factors (particularly for RNA–protein complexes) and molecular chaperones [19]. It is often difficult to conclude whether these 'contaminants' represent true endogenous partners or artificial associations induced by cell disruption. In this vein, the use of a triple tag to improve further purification has been suggested and such a construct prepared [28]. It has been noticed, however, that because of constraints on the buffers, the three different purification steps cannot be performed in a row. Furthermore, purification of the master cell cycle kinase Cdc28 using this triple tag but only two purification steps led to the identification of numerous proteins still likely to be contaminants [19,28,29]. Thus it remains to be demonstrated that a triple purification step and/or this specific construct are truly advantageous over the two-step TAP tag process in terms of yield and purity.

Additional tags have been used to purify protein complexes from various sources by affinity chromatography. Among those, the Flag tag has been quite popular; this small peptide tag is selectively recognised by a monoclonal antibody [30]. Major drawbacks with the utilisation of the Flag tag as a single-step purification tool come, as for any affinity tag, from its specificity and contaminant levels. However, in addition the cost of Flag tag-based purification is also a major concern given the price of a monoclonal antibody-based column to which must be added the price of the competing peptide used, in some cases, for elution. Glutathione *S*-transferase (GST), His6, biotinylation substrates and many other tags used for the purification of recombinant proteins overexpressed in *Escherichia coli* [12] have also been used for purification of complexes [15]. However, these have not been adopted extensively, probably owing to their limited affinity and/or high background levels [13]. Future work will certainly

Table 1
Comparison of the high throughput biochemical protein interaction studies

Method	TAP[a]	HMS-PCI (Flag)[b]
Number of genes processed	1739	726
Fraction of successful integration	1548 (89%)	–
Fraction of proteins expressed at detectable level	1167 (75%)	–
Fraction of successful purification	589 (874) (61%)	600 (83%)
Fractions of purifications with interacting proteins	460 (78%)	493 (82%)
Number of interacting proteins in raw data	~4000	8111
Number of interacting proteins in filtered data	3225 (80%)	3617 (45%)
Number of different proteins identified by screens	1440	1578
Suggested false negative rate[c]	15%	50%
Proposed number of different complexes	225	–
Internal reproducibility	70%	sometimes 20%
Validation by immunoprecipitation	–	74% (64/86)

[a]From Gavin et al. [19].
[b]From Ho et al. [29].
[c]From Edwards et al. [26].

be facilitated by development of better tags for protein complex purification as well by increases in mass spectrometry sensitivity and throughput.

3. Global look at protein interaction data obtained from yeast

Due to the recent technical progress, high throughput biochemical studies of protein complexes are now possible. So far such large-scale analyses together with global analysis of low throughput data on protein complexes and interactions are far from saturation. This means that direct comparison of all available data cannot yet reveal the complete picture of cellular protein interaction networks or allow the evaluation of methods efficiency. Even for yeast, for which the greatest numbers of protein interaction studies at the proteomic level have been performed, the overlap between different approaches is still relatively low.

Two large-scale biochemical protein interaction studies have been performed (Table 1). One of them followed the TAP purification method (TAP data) [19] mentioned above, while the other used a single-step affinity purification with Flag tag (HMS-PCI data) [29]. Another major difference between the two studies resides in the strategy used to express the target proteins. While for TAP purification tagged proteins were expressed at their natural level from endogenous promoters, the HMS-PCI analysis used proteins overexpressed from plasmids using inducible promoters. In both cases, purification was followed by SDS–PAGE and mass spectrometry. In addition, the data obtained by these biochemical approaches may be compared with two global yeast two-hybrid (Y2H) screens that identified binary protein interactions [31,32].

As mentioned before, the overlap between these different approaches is relatively low, so it is difficult to assess directly the quality of the data. The purification methods assay only the composition of the complex and do not give any information about interactions between particular subunits. The two-hybrid system assays only for interactions between two proteins and cannot distinguish between stable and transient interactions, making the comparison more difficult.

Two global Y2H screens surprisingly show slightly less than 10% overlap [33] as only 159 interactions among the 1648 found in total by Ito et al. and Uetz et al. are shared. In addition to some slight differences in the experimental design, the internal error rate of the Y2H assay probably contributes to this problem. This conclusion is supported by a very striking example presented in a study by Matthews et al. [34]. This group re-evaluated previously published two-hybrid data using their own two-hybrid assay. From the 72 previously reported Y2H interactions, only 19 were recapitulated. This suggests a very high dependence of interaction detection upon the exact experimental conditions used for Y2H, for example expression vector and reporter types.

Comparison of the biochemical protein interaction studies (TAP and HMS-PCI data) for which 115 targets were shared also showed only about 10% overlap in the proteins recovered [33]. Even though this cannot be directly compared to interaction results obtained with Y2H, this could suggest that purification methods have a similar error rate as Y2H. However, this may also result from differences in the two methods due to the two strategies to express target proteins. For example, protein overexpression and the single-step purification in HMS-PCI may have generated a higher background than the low level expression and two-step purification used in the TAP strategy. A further complication in the comparison of the results resides in the fact that both studies used filtering criteria to remove contaminating proteins from the raw data. After this process, more than 50% of the potentially interacting proteins identified by HMS-PCI were removed while for TAP this represented only about 20%. The high throughput TAP purification study estimated that internal reproducibility of identified proteins was above 70%, thus a maximum of 30% of data should be treated with caution. For the HMS-PCI approach, a similar value is not available even though it is indicated that for a limited number of samples reproducibility was only 20%. Re-evaluation of 80 randomly chosen interactions by immunoprecipitation confirmed 74% of them.

These limitations in the comparison of the efficiency of the various strategies will be resolved with time and the accumulation of more complete data sets. Nevertheless, comparison of all four high throughput studies with data about known yeast complexes deposited in the MIPS database suggest that the TAP method has the lowest false negative rate (15%) followed by the HMS-PCI method (50%) and Y2H (45–75%) [26]. Comparison of common targets analysed by the TAP and the HMS-PCI studies to published data about binary interaction reveals that the TAP method is 30% better in finding previously known interactions [35]. This result is consistent with the previous comparison. It is much more difficult to estimate false positive error rates than false negative. Even if the crystal structure of a complex is known, some interactions not present in the crystal may be biologically relevant, for example during assembly of the complex. One method that can give some estimation of false positive error rates is evaluating protein interaction data using mRNA co-expression profiles optioned from microarray experiments. The rationale behind this analysis is that interacting proteins are usually similarly regulated. Again interactions identified by the TAP method gave the highest correlation for co-expression trends suggesting a rather low false positive error rate [27]. Accumulation of the data about protein interactions and their cross-validation by different methods will in the future allow more accurate estimations of the positive error rate of each strategy.

Overall, the current studies have demonstrated that technical conditions for high throughput biochemical analyses of protein interactions are now available. However, because such studies are still in their infancy, further technical advances will certainly arise before a definitive optimum strategy is found.

4. What is next: analysis of internal structure of protein complexes?

Identification of the protein complex subunits does not provide the data about its internal structure and interactions of these components. Without such information it is very difficult to understand the molecular mechanism of their action, especially when the complex does not have any obvious enzymatic activity. For example, numerous data related to the composition of the spliceosome and protein complexes involved in splicing have accumulated for years but there is still only limited structural information and in most cases, it is not known how these factors contribute to splicing catalysis [36].

There is no general way to obtain structural information about protein complexes. Obviously crystal structures show all stable interactions and architecture of the complex but this method requires very large amounts of pure homogeneous complex to succeed, a goal that is often difficult and time-consuming to reach. An additional problem encountered during the crystallisation of large complexes is that they cannot usually be reconstituted from recombinant components. As an example, the structure of ribosomes was solved only because these large assemblies can be isolated in high amounts from a natural source [37].

Thus, because crystallisation studies are difficult, alternative strategies leading to lower structural resolution are often attractive. Electron microscopy is one such method [38]. Unfortunately in most cases resolution is too low (more than 10 Å) to determine the precise location of specific subunits, even if immunolocalisation is used. Phylogenetic data may, however, be useful in this case. Indeed, if the crystal structure of a related complex is known and/or if additional data about protein interactions in the complex are available, electron microscopy and homology modelling may lead to the generation of useful models. As an example, Alloy et al. published the structural model of the yeast exosome based on electron microscopy analysis of TAP-purified exosome, structural similarity with the related bacterial RNA-degrading enzyme PNPase and additional protein interaction data [39] (see also [40]). Similarly, in a very elegant study, Lutzmann et al. presented reconstitution and the rough structure of seven nucleoporins [41]. Their data were derived from the electron microscopy structure of different subcomplexes overexpressed in bacteria. A major advantage with the use of electron microscopy is that a limited amount of material is required and the sample does not need to be extremely homogeneous.

At an even lower scale the two-hybrid assay may be used to identify interacting subunits inside a complex of interest. Obviously, the high false positive error rate encountered in screens and, to a lesser extent, the high false negative error rate become less important when pairwise interactions are analysed. Similar information can be derived from biochemical approaches that assay binary interactions in artificial environments such as GST pull-down or far-Western. Obviously, combination of these methods gives more accurate and credible data, which make it possible to derive a relatively good picture of complex architectures. An example can be taken from the analysis of the bacterial RNA degradation complex called degradosome [42]. It is composed of the above-mentioned PNPase, endoribonuclease E, RNA helicase RhlB and the Krebs cycle enzyme enolase. Using each approach (two-hybrid, far-Western and immunoprecipitation) only partial data about subunit interaction were obtained. Combination of these results suggests, however, that the complex is arranged on RNase E dimers to which all other components bind. In the near future, these studies may also be combined with the probing of protein microarrays to detect binary interaction information. While this strategy is still in its infancy, the first available data suggest that useful information may be obtained with this technique [43].

Another approach, which potentially should give very accurate data about interaction sites of the complex, is chemical cross-linking [44]. The purified complex is usually treated with cross-linking chemicals before fractionation of the reaction products by SDS–PAGE. The cross-linked species are then identified by their size, immunoreactivity and/or direct sequence determination. The strategy seems to be simple, but identification of cross-linked proteins especially for large complexes is always a problem. Another limitation often encountered is that various cross-linkers have to be tested and conditions optimised for each complex without a definitive guarantee that an existing contact will ever be detected. Given the limited number of publications reporting the use of cross-linking with mass spectrometry to assay the complex architecture, it is currently difficult to assess which method will prove to be optimal. However, reliable results have clearly been obtained with this strategy. For example, the nucleoporin complex discussed above was analysed by cross-linking beside electron microscopy [45]. The purified native complex from yeast was treated with cross-linking reagent before fractionation by SDS–PAGE. Proteins present in cross-linked species were identified by matrix-assisted laser desorption/ionization time of flight mass spectrometry. The resulting interactions were not as detailed as those obtained by electron microscopy but cross-links were in most cases consistent with the structural model. A major problem in cross-linking is a resolution of cross-linked species. For the six-subunit nucleoporin complex some bands contained more than two proteins leaving some ambiguities on the actual cross-linked partners. For larger complexes this problem will obviously become even more important.

One of the strategies to overcome these difficulties is to identify cross-linked peptides rather than cross-linked proteins. Such analyses identify the sites of interactions between proteins while simultaneously bypassing the need to resolve cross-linked species. Bennet et al. used thio-cleavable cross-linkers [46]. Comparison of the peptide maps obtained from trypsin-digested cross-linked dimers in the presence and absence of a thiol reagent made it possible to identify cross-linked species [46]. Unfortunately, in most cases the concentration of such peptides is relatively low, so for more complex samples it is difficult to analyse the mass spectrum. Another way to identify cross-linked peptides is using isotope-tagged cross-linking reagents. It is easy to see cross-linked species in a mass spectrum by mixing 1/1 deuterium-labelled and unlabelled cross-linker [47].

New mass spectrometry methods, which allow the transfer of whole protein complexes to the gas phase, should also be very useful in analysing the architecture of protein complexes [48]. These methods can very accurately show masses of the complexes and heterogeneity of the samples. Dissection of the complex by tandem mass spectrometry to sub-particles can give information about interactions between subunits. Combination of these methods with chemical cross-linking could be extremely useful in structural analysis of protein complexes.

5. Conclusion

Overall, while major developments in protein complex purification and mass spectrometry are likely to facilitate analysis of protein interactions in the future, the current technology is ripe to make large-scale analysis of protein complexes possible. We are thus likely to obtain soon an accurate description of cellular complexes. Proteomic studies will reveal in parallel the organisation of these proteins in the even larger structure constituted by cellular compartments such as the nucleolus or mitochondria [49–51]. Understanding the dynam-

ics and function of such protein interaction networks will be a challenge for the years to come.

Acknowledgements: We thank the various members of the Séraphin laboratory for suggestions and encouragement. This work was supported by La Ligue contre le Cancer: Equipe Labelisée, the Human Frontier Science Program and the CNRS. A.D. is supported by a fellowship from the Foundation for Polish Science.

References

[1] Rubin, G.M. (2001) Nature 409, 820–821.
[2] Matsudaira, P. (1990) Methods Enzymol. 182, 602–613.
[3] Mann, M., Hendrickson, R.C. and Pandey, A. (2001) Annu. Rev. Biochem. 70, 437–473.
[4] Deutscher, M. (1990) Guide to Protein Purification, Academic Press, San Diego, CA.
[5] Neubauer, G., King, A., Rappsilber, J., Calvio, C., Watson, M., Ajuh, P., Sleeman, J., Lamond, A. and Mann, M. (1998) Nat. Genet. 20, 46–50.
[6] Jurica, M.S., Licklider, L.J., Gygi, S.R., Grigorieff, N. and Moore, M.J. (2002) RNA 8, 426–439.
[7] Rappsilber, J., Ryder, U., Lamond, A.I. and Mann, M. (2002) Genome Res. 12, 1231–1245.
[8] Makarov, E.M., Makarova, O.V., Urlaub, H., Gentzel, M., Will, C.L., Wilm, M. and Luhrmann, R. (2002) Science 298, 2205–2208.
[9] Fritze, C.E. and Anderson, T.R. (2000) Methods Enzymol. 327, 3–16.
[10] Petracek, M.E. and Longtine, M.S. (2002) Methods Enzymol. 350, 445–469.
[11] Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[12] Terpe, K. (2003) Appl. Microbiol. Biotechnol. 60, 523–533.
[13] Rigaut, G., Shevchenko, A., Rutz, B., Wilm, M., Mann, M. and Seraphin, B. (1999) Nat. Biotechnol. 17, 1030–1032.
[14] Puig, O., Caspary, F., Rigaut, G., Rutz, B., Bouveret, E., Bragado-Nilsson, E., Wilm, M. and Seraphin, B. (2001) Methods 24, 218–229.
[15] Gottschalk, A., Tang, J., Puig, O., Salgado, J., Neubauer, G., Colot, H.V., Mann, M., Seraphin, B., Rosbash, M., Luhrmann, R. and Fabrizio, P. (1998) RNA 4, 374–393.
[16] Miller, T., Krogan, N.J., Dover, J., Erdjument-Bromage, H., Tempst, P., Johnston, M., Greenblatt, J.F. and Shilatifard, A. (2001) Proc. Natl. Acad. Sci. USA 98, 12902–12907.
[17] Caspary, F., Shevchenko, A., Wilm, M. and Seraphin, B. (1999) EMBO J. 18, 3463–3474.
[18] Bouveret, E., Rigaut, G., Shevchenko, A., Wilm, M. and Seraphin, B. (2000) EMBO J. 19, 1661–1671.
[19] Gavin, A.C., Bosche, M., Krause, R., Grandi, P., Marzioch, M., Bauer, A., Schultz, J., Rick, J.M., Michon, A.M., Cruciat, C.M., Remor, M., Hofert, C., Schelder, M., Brajenovic, M., Ruffner, H., Merino, A., Klein, K., Hudak, M., Dickson, D., Rudi, T., Gnau, V., Bauch, A., Bastuck, S., Huhse, B., Leutwein, C., Heurtier, M.A., Copley, R.R., Edelmann, A., Querfurth, E., Rybin, V., Drewes, G., Raida, M., Bouwmeester, T., Bork, P., Seraphin, B., Kuster, B., Neubauer, G. and Superti-Furga, G. (2002) Nature 415, 141–147.
[20] Forler, D., Kocher, T., Rode, M., Gentzel, M., Izaurralde, E. and Wilm, M. (2003) Nat. Biotechnol. 21, 89–92.
[21] Estevez, A.M., Kempf, T. and Clayton, C. (2001) EMBO J. 20, 3831–3839.
[22] Tasto, J.J., Carnahan, R.H., McDonald, W.H. and Gould, K.L. (2001) Yeast 18, 657–662.
[23] Westermarck, J., Weiss, C., Saffrich, R., Kast, J., Musti, A.M., Wessely, M., Ansorge, W., Seraphin, B., Wilm, M., Valdez, B.C. and Bohmann, D. (2002) EMBO J. 21, 451–460.
[24] Chen, C.Y., Gherzi, R., Ong, S.E., Chan, E.L., Raijmakers, R., Pruijn, G.J., Stoecklin, G., Moroni, C., Mann, M. and Karin, M. (2001) Cell 107, 451–464.
[25] Schmitt, C., von Kobbe, C., Bachi, A., Pante, N., Rodrigues, J.P., Boscheron, C., Rigaut, G., Wilm, M., Seraphin, B., Carmo-Fonseca, M. and Izaurralde, E. (1999) EMBO J. 18, 4332–4347.
[26] Edwards, A.M., Kus, B., Jansen, R., Greenbaum, D., Greenblatt, J. and Gerstein, M. (2002) Trends Genet. 18, 529–536.
[27] Kemmeren, P., van Berkum, N.L., Vilo, J., Bijma, T., Donders, R., Brazma, A. and Holstege, F.C. (2002) Mol. Cell 9, 1133–1143.
[28] Honey, S., Schneider, B.L., Schieltz, D.M., Yates, J.R. and Futcher, B. (2001) Nucleic Acids Res. 29, E24.
[29] Ho, Y., Gruhler, A., Heilbut, A., Bader, G.D., Moore, L., Adams, S.L., Millar, A., Taylor, P., Bennett, K., Boutilier, K., Yang, L., Wolting, C., Donaldson, I., Schandorff, S., Shewnarane, J., Vo, M., Taggart, J., Goudreault, M., Muskat, B., Alfarano, C., Dewar, D., Lin, Z., Michalickova, K., Willems, A.R., Sassi, H., Nielsen, P.A., Rasmussen, K.J., Andersen, J.R., Johansen, L.E., Hansen, L.H., Jespersen, H., Podtelejnikov, A., Nielsen, E., Crawford, J., Poulsen, V., Sorensen, B.D., Matthiesen, J., Hendrickson, R.C., Gleeson, F., Pawson, T., Moran, M.F., Durocher, D., Mann, M., Hogue, C.W., Figeys, D. and Tyers, M. (2002) Nature 415, 180–183.
[30] Einhauer, A. and Jungbauer, A. (2001) J. Biochem. Biophys. Methods 49, 455–465.
[31] Uetz, P., Giot, L., Cagney, G., Mansfield, T.A., Judson, R.S., Knight, J.R., Lockshon, D., Narayan, V., Srinivasan, M., Pochart, P., Qureshi-Emili, A., Li, Y., Godwin, B., Conover, D., Kalbfleisch, T., Vijayadamodar, G., Yang, M., Johnston, M., Fields, S. and Rothberg, J.M. (2000) Nature 403, 623–627.
[32] Ito, T., Chiba, T., Ozawa, R., Yoshida, M., Hattori, M. and Sakaki, Y. (2001) Proc. Natl. Acad. Sci. USA 98, 4569–4574.
[33] Ito, T., Ota, K., Kubota, H., Yamaguchi, Y., Chiba, T., Sakuraba, K. and Yoshida, M. (2002) Mol. Cell. Proteomics 1, 561–566.
[34] Matthews, L.R., Vaglio, P., Reboul, J., Ge, H., Davis, B.P., Garrels, J., Vincent, S. and Vidal, M. (2001) Genome Res. 11, 2120–2126.
[35] Bader, G.D. and Hogue, C.W. (2002) Nat. Biotechnol. 20, 991–997.
[36] Brow, D.A. (2002) Annu. Rev. Genet. 36, 333–360.
[37] Ramakrishnan, V. and Moore, P.B. (2001) Curr. Opin. Struct. Biol. 11, 144–154.
[38] Frank, J. (2002) Annu. Rev. Biophys. Biomol. Struct. 31, 303–319.
[39] Aloy, P., Ciccarelli, F.D., Leutwein, C., Gavin, A.C., Superti-Furga, G., Bork, P., Bottcher, B. and Russell, R.B. (2002) EMBO Rep. 3, 628–635.
[40] Symmons, M.F., Williams, M.G., Luisi, B.F., Jones, G.H. and Carpousis, A.J. (2002) Trends Biochem. Sci. 27, 11–18.
[41] Lutzmann, M., Kunze, R., Buerer, A., Aebi, U. and Hurt, E. (2002) EMBO J. 21, 387–397.
[42] Vanzo, N.F., Li, Y.S., Py, B., Blum, E., Higgins, C.F., Raynal, L.C., Krisch, H.M. and Carpousis, A.J. (1998) Genes Dev. 12, 2770–2781.
[43] 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. and Snyder, M. (2001) Science 293, 2101–2105.
[44] Mattson, G., Conklin, E., Desai, S., Nielander, G., Savage, M.D. and Morgensen, S. (1993) Mol. Biol. Rep. 17, 167–183.
[45] Rappsilber, J., Siniossoglou, S., Hurt, E.C. and Mann, M. (2000) Anal. Chem. 72, 267–275.
[46] Bennett, K.L., Kussmann, M., Bjork, P., Godzwon, M., Mikkelsen, M., Sorensen, P. and Roepstorff, P. (2000) Protein Sci. 9, 1503–1518.
[47] Muller, D.R., Schindler, P., Towbin, H., Wirth, U., Voshol, H., Hoving, S. and Steinmetz, M.O. (2001) Anal. Chem. 73, 1927–1934.
[48] Sobott, F. and Robinson, C.V. (2002) Curr. Opin. Struct. Biol. 12, 729–734.
[49] Andersen, J.S., Lyon, C.E., Fox, A.H., Leung, A.K., Lam, Y.W., Steen, H., Mann, M. and Lamond, A.I. (2002) Curr. Biol. 12, 1–11.
[50] Fox, A.H., Lam, Y.W., Leung, A.K., Lyon, C.E., Andersen, J., Mann, M. and Lamond, A.I. (2002) Curr. Biol. 12, 13–25.
[51] Pflieger, D., Le Caer, J.P., Lemaire, C., Bernard, B.A., Dujardin, G. and Rossier, J. (2002) Anal. Chem. 74, 2400–2406.