Proteomics to study genes and genomes

Akhilesh Pandey* & Matthias Mann†

*Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, Massachusetts 02142, and Department of Pathology, Brigham and Women's Hospital, Boston, Massachusetts 02115, USA
†Protein Interaction Laboratory (PIL), University of Southern Denmark, Campusvej 55, DK-5230 Odense M, and Denmark and Protana A/S, Staermosegaardsvej 16, DK-5230 Odense M, Denmark (e-mail: mann@cebi.sdu.dk)

Proteomics, the large-scale analysis of proteins, will contribute greatly to our understanding of gene function in the post-genomic era. Proteomics can be divided into three main areas: (1) protein micro-characterization for large-scale identification of proteins and their post-translational modifications; (2) ‘differential display’ proteomics for comparison of protein levels with potential application in a wide range of diseases; and (3) studies of protein–protein interactions using techniques such as mass spectrometry or the yeast two-hybrid system. Because it is often difficult to predict the function of a protein based on homology to other proteins or even their three-dimensional structure, determination of components of a protein complex or of a cellular structure is central in functional analysis. This aspect of proteomic studies is perhaps the area of greatest promise. After the revolution in molecular biology exemplified by the ease of cloning by DNA methods, proteomics will add to our understanding of the biochemistry of proteins, processes and pathways for years to come.

Large-scale DNA sequencing has transformed biomedical research in a short span of time. With the discovery of most human genes, it is now apparent that a ‘factory approach’ to address biological problems is desirable if we are to gain a comprehensive understanding of complex biological processes. In this article we will review how proteomics is similarly making a crucial contribution to our understanding of biology and medicine through the global analysis of gene products.

Defining proteomics
Proteomics is the large-scale study of proteins, usually by biochemical methods. The word proteomics has been associated traditionally with displaying a large number of proteins from a given cell line or organism on two-dimensional polyacrylamide gels. In this sense, proteomics already dates back to the late 1970s when researchers started to build databases of proteins using the then newly developed technique of two-dimensional gel electrophoresis (Box 1). This resulted in extensive cataloguing of spots from two-dimensional gels to create databases of all expressed proteins. However, even when such gels could be run reproducibly between laboratories, determining the identity of the proteins was difficult because of a lack of sensitive and rapid analytical methods for protein characterization (such as the polymerase chain reaction and the automated sequencer for DNA analysis). In the 1990s, biological mass spectrometry emerged as a powerful analytical method that removed most of the limitations of protein analysis. This development, coupled with the availability of the entire human coding sequence in public databases, marks the beginning of a new era. Today, the term proteomics covers much of the functional analysis of gene products or ‘functional genomics’, including large-scale identification or localization studies of proteins and interaction studies using the yeast two-hybrid system. The more focused large-scale study of protein structure, however, is usually not included and designated ‘structural genomics’ instead. Likewise, strategies that target only genes or messenger RNA, such as large-scale mutagenesis or antisense experiments, should not be considered part of proteomics.

Why is proteomics necessary?
With the accumulation of vast amounts of DNA sequences in databases, researchers are realizing that merely having complete sequences of genomes is not sufficient to elucidate biological function. A cell is normally dependent upon a multitude of metabolic and regulatory pathways for its survival. There is no strict linear relationship between genes and the protein complement or ‘proteome’ of a cell. Proteomics is complementary to genomics because it focuses on the gene products, which are the active agents in cells. For this reason, proteomics directly contributes to drug development as almost all drugs are directed against proteins.

The existence of an open reading frame (ORF) in genomic data does not necessarily imply the existence of a functional gene. Despite the advances in bioinformatics, it is still difficult to predict genes accurately from genomic data (see review in this issue by Eisenberg et al., pages 823–826, and refs 7, 8). Although the sequencing of related organisms will ease the problem of gene prediction through comparative genomics, the success rate for correct prediction of the primary structure is still low. This is particularly true in the case of small genes (which can be missed entirely) or genes with little or no homology to other known genes. A recent study concluded that the error rate was as least 8% in the annotations for 340 genes from the Mycoplasma genitalium genome. If such error rates are extrapolated to the human genome, the outcome and consequences can easily be imagined. Therefore, verification of a gene product by proteomic methods is an important first step in ‘annotating the genome’. Modifications of the proteins that are not apparent from the DNA sequence, such as isoforms and...
post-translational modifications, can be determined only by proteomic methodologies. Furthermore, it may be necessary to determine the protein expression level directly as mRNA levels may or may not correlate with the protein level\(^{12,13}\). The localization of gene products, which is often difficult to predict from the sequence, can be determined experimentally. Mechanisms such as regulation of protein function by proteolysis, recycling and sequestration in cell compartments affect gene products and not genes. Finally, protein–protein interactions and the molecular composition of cellular structures such as organelles can be determined only at the protein level.

**Identification and analysis of proteins**

**Protein preparation methods**

One of the most crucial steps in proteomics is obtaining and handling the protein sample. Out of the entire complement of the genome of about 100,000 genes, a given cell line may express about 10,000 genes and an even higher number is expressed in tissues. Furthermore, the dynamic range of abundance of proteins in biological samples can be as high as 10\(^7\). Because even the best two-dimensional gels can routinely resolve no more than 1,000 proteins, it is obvious that only the most abundant proteins can be visualized by gel electrophoresis if a crude protein mixture is used. The ideal solution to reduce complexity and differences in abundance is to use affinity-based protein purification strategies using the whole protein complement. For example, the erythropoietin receptor is of medium abundance, occurring in about 1,000 copies per cell, or less than two picomoles (100 ng) in one litre of cell culture. This protein would not be visualized from whole-cell extracts but can be enriched easily by antibody-based affinity purification to yield a silver-stained band. This fact has to be borne in mind if signalling and other regulatory molecules are being studied.

After obtaining the protein fraction, the method of choice for proteomic studies is one- or two-dimensional gel electrophoresis. The advantages of one-dimensional electrophoresis as a preparation method are that virtually all proteins are soluble in SDS, the range of relative molecular mass from 10,000 to 300,000 is readily covered, and extremely acidic and basic proteins are easily visualized.

**Mass spectrometric identification of proteins**

The most significant breakthrough in proteomics has been the mass spectrometric identification of gel-separated proteins, which extends analysis far beyond the mere display of proteins. Mass spectrometry has essentially replaced the classical technique of Edman degradation even in traditional protein chemistry, because it is much more sensitive, can deal with protein mixtures and offers much higher throughput. It relies on digestion of gel-separated proteins into peptides by a sequence-specific protease such as trypsin. The reason for analysing peptides rather than proteins is that gel-separated proteins are difficult to elute and to analyse by mass spectrometry, and that the molecular weight of proteins is not usually sufficient for database identification. In contrast, peptides are easily eluted from gels and even a small set of peptides from a protein provides sufficient information for identification. The steps typically involved in the mass spectrometric analysis of a protein are illustrated by an example that shows analysis of a molecule involved in platelet-derived growth factor (PDGF) signalling (Fig. 1). A detailed protocol describing methods and strategies for the mass spectrometric identification of signalling molecules can be found in ref. 14.

There are two main approaches to mass spectrometric protein identification. In the ‘peptide-mass fingerprint’ approach, initially suggested by Henzel and co-workers\(^{15}\), the mass spectrum of the eluted peptide mixture is acquired, which results in a ‘peptide-mass fingerprint’ of the protein being studied. This mass spectrum is obtained by a relatively simple mass spectrometric method — matrix-assisted laser desorption/ionization (MALDI) — which results in a time-of-flight distribution of the peptides comprising the mixture (Box 2 and Fig. 1b). Advances have been made in automation of the MALDI identification procedure whereby hundreds of protein spots can be excised, digested enzymatically, their mass spectra obtained and automatically searched against databases\(^{16,17}\). As more full-length human genes are represented in the database, the success rate of identification by MALDI will increase further.

In a two-step procedure for rapid and unambiguous protein identification, MALDI fingerprinting is the first step\(^6\). The second method for protein identification relies on fragmentation of individual peptides in the mixture to gain sequence information. In this method, the peptides are ionized by ‘electrospray ionization’ directly from the liquid phase. The peptide ions are sprayed into a ‘tandem mass spectrometer’ which has the ability to resolve peptides in a mixture, isolate one species at a time and dissociate it into amino- or carboxy-terminal-containing fragments (Fig. 1c). The tandem mass spectrometric method is technically more complex and less scalable than MALDI fingerprinting. Its main advantage is that sequence information derived from several peptides is much more specific for the identification of a protein than a list of peptide masses. The fragmentation data can not only be used to search protein sequence databases but also nucleotide databases such as expressed sequence...
tag (EST) databases and more recently even raw genomic sequence databases (B. Küster, P. Mortensen, J. S. Andersen and M. Mann, unpublished data).

New developments in mass spectrometry

Biological mass spectrometry is still evolving rapidly owing to continued technological advances in various areas. For instance, a new type of mass spectrometer that combines a MALDI ion source with a highly efficient tandem mass spectrometer unit that can fragment the individual peptides has recently been developed. If this ‘MALDI quadruple time of flight’ instrument proves to be sufficiently sensitive, it would combine the high throughput of the peptide mapping method with the specificity of the peptide sequencing method, allowing a one-step instead of a two-step mass spectrometric analysis strategy. In our experience, this instrument already significantly improves the analysis of small proteins and improves the throughput when analysing simple protein mixtures. There are also efforts at miniaturizing protein preparation using microfabricated ‘chips’, which have obtained promising results. However, these methods have not yet yielded the sensitivity or robustness of preparations using standard tube or microtitre plate formats. There are also longstanding efforts to scan one- or two-dimensional gels directly by MALDI mass spectrometry. A recent variation uses an intercalating membrane containing immobilized trypsin for digestion of proteins during electrophoretic transfer onto a collecting membrane. The membrane is then rasterized and analysed by MALDI yielding a peptide map for each position of the gel. In the future, it would be desirable to analyse a protein sample directly by mass spectrometry, without gel separation or enzymatic digestion. Smith et al. have loaded crude protein extracts into capillaries and performed capillary electrophoresis to separate the proteins.
The separated proteins were then infused directly into a specialized Fourier-transformed ion cyclotron resonance (FTICR) mass spectrometer (Fig. 2), and the precise molecular masses of hundreds of proteins were acquired during a single run. In this experiment, the mass distribution was biased towards small proteins and only the masses, not the identity of the proteins, were determined. But in the future it may become possible to use this strategy to identify proteins by on-line fragmentation of the proteins. This would enable researchers to perform the whole proteomic analysis in a single automated experiment at least for a subset of soluble proteins of medium abundance.

**Post-translational modifications**

One of the unique features of proteomics studies is the ability to analyze the post-translational modifications of proteins. Phosphorylation, glycosylation and sulphation as well as many other modifications are extremely important for protein function as they can determine activity, stability, localization and turnover. These modifications are not generally apparent from genomic sequence or mRNA expression data. Whereas mass spectrometry is the proteomic method of choice to determine protein modifications, this task is much more difficult than the mere determination of protein identity. Minimal data is sufficient to identify the protein in sequence databases — often as few as one or two peptides need to be fragmented. However, for obtaining the nature and location of post-translational modifications, all the peptides that do not have the expected molecular mass need to be analysed further. Because of this and other reasons, much more material is needed to study post-translational modifications than is required for protein identification, glycosylation and sulphation as well as many other modifications are extremely important for protein function as they can determine activity, stability, localization and turnover. These modifications are not generally apparent from genomic sequence or mRNA expression data. Whereas mass spectrometry is the proteomic method of choice to determine protein modifications, this task is much more difficult than the mere determination of protein identity. Minimal data is sufficient to identify the protein in sequence databases — often as few as one or two peptides need to be fragmented. However, for obtaining the nature and location of post-translational modifications, all the peptides that do not have the expected molecular mass need to be analysed further. Because of this and other reasons, much more material is needed to study post-translational modifications than is required for protein identification, glycosylation and sulphation.
Identification. Continuing progress is being made in this field, especially in the case of phosphorylation. Phosphorylation events can be studied by generic strategies, because phosphopeptides are 80 Da heavier than their unmodified counterparts, give rise to a specific fragment (PO4, mass 79), bind to metal resins, are recognized by specific antibodies and the phosphate groups can be removed by phosphatases. As an example, Fig 1d shows the detection of phosphopeptides following metal resin-based affinity micropurification and phosphatase treatment.

Phosphorylation and signalling pathways

Several receptor-mediated signalling pathways result in tyrosine phosphorylation of a large set of substrates. To identify these substrates, the lysates from unstimulated and growth factor-stimulated cells can be prepared and resolved by two-dimensional gels. The proteins of interest can be detected by 32P labelling or by western blotting with antibodies that recognize only the activated state of molecules (such as phosphotyrosine- or phosphoserine-specific antibodies). These spots can then be identified by mass spectrometry as demonstrated recently. A better alternative, however, is to first enrich for these substrates by using anti-phosphotyrosine antibodies in an immunoprecipitation step followed by mass spectrometric identification. Several known and new components were recently reported in one such study on the epidermal growth factor (EGF)-receptor pathway.

Differential-display proteomics

The two-dimensional gel approach

Until recently, proteomics was almost synonymous with two-dimensional gel electrophoresis (Fig. 3). In biomedical applications of the comparative two-dimensional gel approach, the objective is usually to identify proteins that are up- or downregulated in a disease-specific manner for use as diagnostic markers or therapeutic targets. There are several technical challenges in such experiments. First, hydrophobic and large proteins usually do not enter the second dimension of the gel. Second, the issue of dynamic range makes it difficult to visualize all but the most abundant proteins. Particularly in body fluids such as serum and cerebrospinal fluid, more than 99% of the protein complement consists of serum albumin and globulins. Third, because of the biological variation inherent in these samples, it is difficult to define normal protein-expression patterns that can be compared with the disease state. For several of these applications, methods of array-based mRNA expression profiling can not only be more comprehensive (as they provide data on all the genes applied to the chip), but also faster and more convenient, as shown by a number of studies (see review in this issue by Lockhart and Winzeler, pages 827–836, and refs 37–40).

In spite of these difficulties of comparing two-dimensional gel patterns, several applications have appeared in the literature. For example, Celis and co-workers have found a putative urinary marker, psoriasin, which can be used for the follow-up of patients with bladder squamous cell carcinomas. This marker was identified when they compared the profile of secreted proteins from normal tissue with that from cancerous tissue. A similar study compared the proteome of normal human luminal and myoepithelial breast cells using immunopurified cell populations. It detected 170 protein spots that were twofold differentially expressed, of which 51 were identified. However, almost all of these proteins were abundant cytoskeletal proteins such as actin and keratin. A recent study compared the protein complement from different fractions of brain extracts from two different strains of mice, finding over 1,000 genetically variant protein spots. Such studies may be useful in other situations as well, for example, in comparing the proteome of wild-type with that of knockout mice. Toxicology studies frequently use proteomic analysis to understand the mechanism of action of a drug or to identify its targets. Aicher and colleagues discovered an association between decreased levels of a calcium-binding protein, calbindin-D 28K, and cyclosporine A-induced nephrotoxicity when kidney samples were compared from species that were either

---

**Figure 4** A schematic showing use of arrays for proteomic analysis. Recombinant proteins can be expressed and purified in a large-scale format. These proteins are pooled into wells as shown and assayed for functions such as enzymatic activity. This approach has been termed biochemical genomics. A protein chip can be prepared in several ways. The surface can be immobilized with recombinant proteins or their domains (such as bacterially expressed GST–fusion proteins) and then cell lysates containing putative interaction partners are applied to the chip followed by washing to remove unbound material. The bound proteins can then be eluted and identified by mass spectrometry. Alternatively, instead of cell lysates, a phage cDNA display library can be applied to the chip followed by washing and amplification steps to isolate individual interacting phage particles. The inserts in these phage particles can then be sequenced to determine the identity of the interacting partners. The yeast two-hybrid system is also amenable to an array-based analysis. First, yeast cells can be transformed with individual ORF–activation domain fusions. These cells can be grown in an array format on plates or filters such that each element of the array contains a yeast clone with a unique ORF. Such an array can be probed in a mating assay with yeast cells containing a single ORF–DNA-binding domain fusion, one at a time. The nutritional selection ensures that only the yeast cells containing interacting partners survive. These interacting clones can be re-screened to reduce false positives or be sequenced directly.
sustainable or resistant to nephrotoxicity.

When two-dimensional gels are used as a method of separating a qualitative subset of proteins, as opposed to comparing whole-cell preparations, or when immunological methods are used to highlight a subset of proteins, biologically relevant answers can be more readily obtained. For example, many secreted proteins can be identified by two-dimensional gel analysis of supernatants of cell lines and explants from tumour tissues. Several groups have probed two-dimensional gels of proteins from allergy-causing organisms using antibodies derived from allergic patients. Identification of the responsible allergen by mass spectrometry can be exploited in the rational design of preventive and therapeutic strategies.

We predict that protein expression analysis will be most useful in well-defined areas such as (1) analysis of samples that do not contain mRNA such as some body fluids; (2) cases where the protein abundance does not correlate with the mRNA abundance; (3) cases where the critical changes involve post-translational modifications of proteins such as glycosylation or phosphorylation, rather than changes in protein abundance; (4) cases where an overview of the proteome such as that of a microbe. These predicted peptide masses are compared with those obtained experimentally by MALDI analysis. The protein can be identified correctly if there are a sufficient number of peptide matches with a protein in the database, resulting in a high score. High mass accuracy is critical for unambiguous identification and serves mainly to eliminate the false positives. MALDI identification by peptide-mass fingerprints requires that the full-length gene be present in the databases. Therefore, the success rate of this method will receive an additional boost with the availability of all predicted genes in sequence databases.

**MalDI and peptide-mass mapping**

In this approach, a portion of the tryptic peptide mixture is analysed by MALDI mass spectrometry. Because trypsin cleaves the protein backbone at the amino acids arginine and lysine, the masses of tryptic peptides can be predicted theoretically for any entry in a protein sequence database. These predicted peptide masses are compared with those obtained experimentally by MALDI analysis. The protein can be identified correctly if there are a sufficient number of peptide matches with a protein in the database, resulting in a high score. High mass accuracy is critical for unambiguous identification and serves mainly to eliminate the false positives. MALDI identification by peptide-mass fingerprints requires that the full-length gene be present in the databases. Therefore, the success rate of this method will receive an additional boost with the availability of all predicted genes in sequence databases.

Electrospray and tandem mass spectrometry

There are two major mass spectrometric strategies that use electrospray ionization. In one of them the unseparated mixture of peptides is applied to a low-flow device called nanoelectrospray. The peptide mixture is then electrosprayed from a very fine needle (tip internal diameter of 1 μm) into the mass spectrometer. Individual peptides from the mixture are isolated in the first step and fragmented during the second step to sequence the peptide (hence tandem mass spectrometry). The fragments obtained by this method are derived from the N or C terminus of the protein and are designated ‘b’ or ‘y’ ions, respectively. The other strategy uses liquid chromatography for initial separation of peptides followed by sequencing as they elute into the electrospray ion source. This method can also be used without gel electrophoresis where a mixture of proteins is digested in solution and the ‘scrambled’ set of peptides are sequenced, ideally resulting in several peptide hits for each of the proteins that was initially present in the mixture. A great deal of data can be obtained from a single run in an automated fashion.

**Using mass spectrometry data to search databases**

In tandem mass spectrometry, peptides are fragmented by collision with gas molecules in the mass spectrometer. Spacing of these fragments by the molecular mass of one amino acid reveals the identity and location of that amino acid in the peptide. Only two such amino acids, combined with the knowledge of their location in the peptide — a ‘peptide sequence tag’ — is sufficient to locate the peptide in large sequence databases. Alternatively, the theoretical fragmentation spectra of all possible peptides can be compared with the experimental spectrum to find the sequence that most likely gave rise to it. As a result, more complex mixtures of proteins can be analysed and the corresponding peptides found in EST databases or even directly in genomic databases. Routine sensitivities achieved by many laboratories are in the low picomole range (50–100 ng for most proteins), but specialized laboratories achieve higher sensitivities down to the low femtomole range of protein applied to the gel. The overall sensitivity of detection is determined mainly by the protein preparation methods as the mass spectrometer itself is capable of detecting sub-femtomole amounts of peptides under optimized conditions.
other modifications, peptides, protein fragments or proteins may also be immobilized onto chips and samples (for example, phage library or patient serum) applied onto the chip followed by detection of binding. One approach using protein chips couples the above techniques with a direct MALDI readout of the bound material.\(^{49,50}\)

**Quantification by mass spectrometry**

In addition to the above methods, differential-display proteomics can also be done using limited or no protein separation followed by mass spectrometric quantification. Because the intensity of a peptide peak in the mass spectrum cannot be predicted, quantification is achieved by labelling one of the two states by stable isotopes. Such methods have been used traditionally in mass spectrometry of small molecules but have only recently been applied to proteomics. Microbes can, for example, be grown in one state in normal medium and in another state in medium containing only N\(^{15}\) instead of N\(^{14}\). Protein preparations from the two states are then mixed, separated and analysed by mass spectrometry. Two versions of any peptide can now be detected where one is greater in mass by its number of nitrogen atoms and the ratio of peak heights accurately quantifies the relative amounts of the corresponding proteins. As an alternative, Aebersold and colleagues introduced an isotopic non-radioactive label on cysteines after cell lysis before quantifying the samples by mass spectrometry.\(^{51}\) This strategy enables quantification of peptides from the most abundant components of very crude protein mixtures without gel electrophoresis.

**Protein–protein interactions**

A key question about a protein, in addition to when and where it is expressed, is with which other proteins does it interact. Interaction partners are an immediate lead into biological function and can potentially be exploited for therapeutic purposes. Creation of a protein–protein interaction map of the cell would be of immense value to understanding the biology of the cell.

**Purification of protein complexes**

Proteomics can make a key contribution to the study of protein–protein interactions.\(^{32-35}\) An attractive way to study protein–protein interactions is to purify the entire multi-protein complex by affinity-based methods. This can be achieved in a variety of ways such as by using glutathione S-transferase (GST)–fusion proteins, antibodies, peptides, DNA, RNA or a small molecule binding specifically to a cellular target. One of the generic ways of identifying the interaction partners of a new protein is to tag it with an epitope. This protein can then be overexpressed in cells and — together with its interaction partners — immunoprecipitated by an antibody against the epitope.
This requires only the full-length complementary DNA clone of the gene and no time is spent in generating a precipitating antibody against the gene of interest. Because full-length cDNAs may soon be available for most human genes\(^6\), large-scale interaction studies will become possible. Making fusion proteins such as GST–fusions is another generic way to obtain interaction partners (Fig. 5). The multi-protein complex associates with the ‘bait’, which is immobilized on a solid support. After washing away the proteins that interact nonspecifically, the protein complex is eluted, separated by gel electrophoresis and analysed by mass spectrometry. Thus, in a single experiment, the components of an entire multi-protein complex can be identified. As an example, the human spliceosome has been purified using biotinylated RNA as the ‘bait’ on which the complex assembled\(^7\). Its protein components were then displayed by two-dimensional gel electrophoresis (Fig. 6a). From a single two-dimensional gel, 19 new factors were obtained (mostly in EST databases) and several of them were cloned and analysed further. Co-localization using immunofluorescence of the new protein with other members of the complex served to establish that they are bona fide members of the complex (Fig. 6b). Several of the new factors identified from this study were cloned and GST–fusion proteins generated. Using the strategy shown in Fig. 5, one of these proteins, designated S14, precipitated a subset of the spliceosome proteins (Fig. 6c), which, together with other experiments and bioinformatics analysis of the sequence, indicated a function of this protein. Many protein complexes have now been characterized using the strategy outlined above. Some of these complexes include the yeast Arp2/3 complex\(^8\), proteins found in the yeast nuclear-pore complex\(^9\) and proteins bound to the chaperonin GroEL\(^10\).

These studies provide insight into mechanisms and open up new lines of investigations. Because no assumptions are made about the complex, unsuspected connections between cellular processes routinely emerge. For example, a study of profilin-I and -II binding proteins in mouse brain resulted in the discovery of two sets of proteins, one consisted of signalling molecules that regulate actin cytoskeleton and the other was involved in endocytosis. This indicated a link between signal transduction pathways and microfilament assembly involving profilin\(^11\).

Once members of a multi–protein complex have been identified by mass spectrometry, their function is studied by pertinent assays. At this stage, proteomics can be used in an iterative fashion to define either direct interaction partners of a new protein in the complex and/or to connect to other complexes in the cell\(^12\).

The success of the above-mentioned strategies relies on sufficient affinity of the protein complex to the bait and on optimized conditions for purification steps. For example, use of a double-tagging strategy improves complex recovery and reduces nonspecific protein binding\(^13\). Lower-affinity interactions can potentially be captured by chemically crosslinking the protein complex before affinity purification because it relies on spatial proximity rather than affinity. Crosslinking can also help in elucidating the topological structure of a protein complex by the determination of nearest neighbours\(^14\).

Components of specific organelles have also begun to be analysed. The yeast Golgi apparatus has been catalogued and the components of the chloroplast of garden pea have been similarly investigated to identify proteins involved in the processing, targeting, insertion and assembly of photosynthetic complexes\(^15\). The interchromatin granules have been examined by the analysis of the crude peptide mixture obtained after digestion in solution of the entire sample\(^16\).

Components of specific organelles have also begun to be analysed. The yeast Golgi apparatus has been catalogued and the components of the chloroplast of garden pea have been similarly investigated to identify proteins involved in the processing, targeting, insertion and assembly of photosynthetic complexes\(^15\). The interchromatin granules have been examined by the analysis of the crude peptide mixture obtained after digestion in solution of the entire sample\(^16\).

The yeast two-hybrid system has emerged as a powerful tool to study protein–protein interactions\(^17\). It is a genetic method based on the modular structure of transcription factors wherein close proximity of the DNA-binding domain to the activation domain induces increased transcription of a set of genes. The yeast hybrid system uses ORFs fused to the DNA-binding or –activation domain of GAL4 such that increased transcription of a reporter gene results when the proteins bound to the chaperonin GroEL\(^10\).

Components of specific organelles have also begun to be analysed. The yeast Golgi apparatus has been catalogued and the components of the chloroplast of garden pea have been similarly investigated to identify proteins involved in the processing, targeting, insertion and assembly of photosynthetic complexes\(^15\). The interchromatin granules have been examined by the analysis of the crude peptide mixture obtained after digestion in solution of the entire sample\(^16\).

The yeast two-hybrid system has emerged as a powerful tool to study protein–protein interactions\(^17\). It is a genetic method based on the modular structure of transcription factors wherein close proximity of the DNA-binding domain to the activation domain induces increased transcription of a set of genes. The yeast hybrid system uses ORFs fused to the DNA-binding or –activation domain of GAL4 such that increased transcription of a reporter gene results when the proteins bound to the chaperonin GroEL\(^10\).

Components of specific organelles have also begun to be analysed. The yeast Golgi apparatus has been catalogued and the components of the chloroplast of garden pea have been similarly investigated to identify proteins involved in the processing, targeting, insertion and assembly of photosynthetic complexes\(^15\). The interchromatin granules have been examined by the analysis of the crude peptide mixture obtained after digestion in solution of the entire sample\(^16\).

The yeast two-hybrid system has emerged as a powerful tool to study protein–protein interactions\(^17\). It is a genetic method based on the modular structure of transcription factors wherein close proximity of the DNA-binding domain to the activation domain induces increased transcription of a set of genes. The yeast hybrid system uses ORFs fused to the DNA-binding or –activation domain of GAL4 such that increased transcription of a reporter gene results when the proteins bound to the chaperonin GroEL\(^10\).

Components of specific organelles have also begun to be analysed. The yeast Golgi apparatus has been catalogued and the components of the chloroplast of garden pea have been similarly investigated to identify proteins involved in the processing, targeting, insertion and assembly of photosynthetic complexes\(^15\). The interchromatin granules have been examined by the analysis of the crude peptide mixture obtained after digestion in solution of the entire sample\(^16\).

The yeast two-hybrid system has emerged as a powerful tool to study protein–protein interactions\(^17\). It is a genetic method based on the modular structure of transcription factors wherein close proximity of the DNA-binding domain to the activation domain induces increased transcription of a set of genes. The yeast hybrid system uses ORFs fused to the DNA-binding or –activation domain of GAL4 such that increased transcription of a reporter gene results when the proteins bound to the chaperonin GroEL\(^10\).

Components of specific organelles have also begun to be analysed. The yeast Golgi apparatus has been catalogued and the components of the chloroplast of garden pea have been similarly investigated to identify proteins involved in the processing, targeting, insertion and assembly of photosynthetic complexes\(^15\). The interchromatin granules have been examined by the analysis of the crude peptide mixture obtained after digestion in solution of the entire sample\(^16\).

The yeast two-hybrid system has emerged as a powerful tool to study protein–protein interactions\(^17\). It is a genetic method based on the modular structure of transcription factors wherein close proximity of the DNA-binding domain to the activation domain induces increased transcription of a set of genes. The yeast hybrid system uses ORFs fused to the DNA-binding or –activation domain of GAL4 such that increased transcription of a reporter gene results when the proteins bound to the chaperonin GroEL\(^10\).

Components of specific organelles have also begun to be analysed. The yeast Golgi apparatus has been catalogued and the components of the chloroplast of garden pea have been similarly investigated to identify proteins involved in the processing, targeting, insertion and assembly of photosynthetic complexes\(^15\). The interchromatin granules have been examined by the analysis of the crude peptide mixture obtained after digestion in solution of the entire sample\(^16\).

The yeast two-hybrid system has emerged as a powerful tool to study protein–protein interactions\(^17\). It is a genetic method based on the modular structure of transcription factors wherein close proximity of the DNA-binding domain to the activation domain induces increased transcription of a set of genes. The yeast hybrid system uses ORFs fused to the DNA-binding or –activation domain of GAL4 such that increased transcription of a reporter gene results when the proteins bound to the chaperonin GroEL\(^10\).

Components of specific organelles have also begun to be analysed. The yeast Golgi apparatus has been catalogued and the components of the chloroplast of garden pea have been similarly investigated to identify proteins involved in the processing, targeting, insertion and assembly of photosynthetic complexes\(^15\). The interchromatin granules have been examined by the analysis of the crude peptide mixture obtained after digestion in solution of the entire sample\(^16\).

The yeast two-hybrid system has emerged as a powerful tool to study protein–protein interactions\(^17\). It is a genetic method based on the modular structure of transcription factors wherein close proximity of the DNA-binding domain to the activation domain induces increased transcription of a set of genes. The yeast hybrid system uses ORFs fused to the DNA-binding or –activation domain of GAL4 such that increased transcription of a reporter gene results when the proteins bound to the chaperonin GroEL\(^10\).
proteins encoded by two ORF’s interact in the nucleus of the yeast cell (Fig. 7a, b). One of the main consequences of this is that once a positive interaction is detected, the ORF is identified simply by sequencing the relevant clones. For these reasons it is a generic method that is simple and amenable to high-throughput screening of protein–protein interactions.

On a large scale, this strategy has been used in two formats. In the array method, yeast clones containing ORFs as fusions to DNA or activation domains are arrayed onto a grid and the ORFs to be tested (as reciprocal fusions) are screened against the entire grid to identify interacting clones (Fig. 4). In the library screening method, one set of ORFs is first pooled to generate a library and then the reciprocal ORF–fusions are mated with the library one by one or several at a time (Fig. 7c).

Such analyses on a genome-wide scale have already been reported in Saccharomyces cerevisiae and to a more limited extent in Caenorhabditis elegans12–15. In yeast, the array method was performed on 192 ORFs and the library screening method for 87% of the yeast genome. Together, this experiment resulted in 957 putative interactions (192 ORFs and the library screening method for 87% of the yeast genome) (Fig. 7c).

ORF–fusions are mated with the library one by one or several at a time. ORFs are first pooled to generate a library and then the reciprocal (as reciprocal fusions) are screened against the entire grid to identify activation domains are arrayed onto a grid and the ORFs to be tested for interaction.

Conclusions
Proteomics provides a powerful set of tools for the large-scale study of gene function directly at the protein level. In particular, the mass spectrometric study of gel-separated proteins is leading to a renaissance in biochemical approaches to protein function. Protein characterization will continue to improve in throughput, sensitivity and completeness. Post-translational modifications cannot currently be studied at high throughput but certain categories such as phosphorylation are beginning to be amenable to generic approaches. We predict that proteomics will move away from the monitoring of protein expression using two-dimensional gels. Mass spectrometry-based methods that use affinity purification followed by only one-dimensional electrophoresis will continue to gain in importance. In the near future, proteomics will provide a wealth of protein–protein interaction data, which will probably be its most important and immediate impact on biology. Because proteins are one step closer to function than are genes, these studies will frequently lead directly to biological discoveries or hypotheses. The ready availability of many human genes as full-length clones is itself an extremely important extension of the genome projects that will make possible several proteomic strategies. Assays to determine protein function using purified proteins will be automated and performed in miniaturized grid formats in parallel for thousands of proteins. Finally, advances in genomics will directly fuel large-scale protein assays that use genetics as a readout, such as the two-hybrid screen.


32. Neubauer, G. & Mann, M. Mapping of phosphorylation sites of gel-isolated proteins by nanoelectrospray