PROTEOMICS OF ORGANELLES AND LARGE CELLULAR STRUCTURES

John R. Yates III[*], *Annalyn Gilchrist*[‡], *Kathryn E. Howell*[§] *and John J. M. Bergeron*[‡]

Abstract | The mass-spectrometry-based identification of proteins has created opportunities for the study of organelles, transport intermediates and large subcellular structures. Traditional cell-biology techniques are used to enrich these structures for proteomics analyses, and such analyses provide insights into the biology and functions of these structures. Here, we review the state-of-the-art proteomics techniques for the analysis of subcellular structures and discuss the biological insights that have been derived from such studies.

[*]*Department of Cell Biology, 10550 North Torrey Pines Road, The Scripps Research Institute, La Jolla, California 92037, USA.*
[‡]*Department of Anatomy and Cell Biology, Strathcona Anatomy and Dentistry Building, McGill University, Montreal, Quebec H3A 2B2, Canada.*
[§]*Department of Cell and Developmental Biology, Mail Stop 8108, University of Colorado School of Medicine at Fitzsimons, P.O. BOX 6511, Aurora, Colarado 80045, USA.*
Correspondence to J.R.Y.
e-mail: jyates@scripps.edu
doi:10.1038/nrm1711

Eukaryotic cells segregate and organize specific molecules that carry out defined functions in organelles. Organelles — for example, the nucleus, endoplasmic reticulum (ER) and Golgi complex — are dynamic membrane-bounded compartments that have distinct structures and functions. Vesicles and tubules transport proteins and lipids between organelles, which results in organelles having a constantly changing molecular composition. Lipid bilayers form a barrier between organelle lumens and the cytoplasm, and these bilayers contain many transmembrane proteins that can interact with components of the cytoskeleton and signalling pathways. Organelles are composed of molecules that are present almost all of the time ('resident' molecules), molecules that are in transit and molecules that transiently interact with the organelle to carry out distinct functions. Approaches to identify and delineate all of these molecules will provide the data necessary to understand organelle function.

In the post-genomic era, large-scale proteomics technologies and tools have made organelle-scale studies feasible, and have become powerful methods for studying organelles, their components and dynamics[1–5]. Historically, the presence of lipid bilayers limited the protein chemistry technologies that could be applied, but new strategies are emerging to identify and characterize integral membrane proteins[6–8]. These methods and strategies have created opportunities for the complete characterization of the different types of transmembrane protein, including obtaining information on protein domains and post-translational modifications[7]. Combining large-scale proteomics studies with traditional cell-biology techniques is providing a strategy for the functional characterization of organelles and the molecules they interact with in the cytoplasm, for example, those in signalling pathways[9].

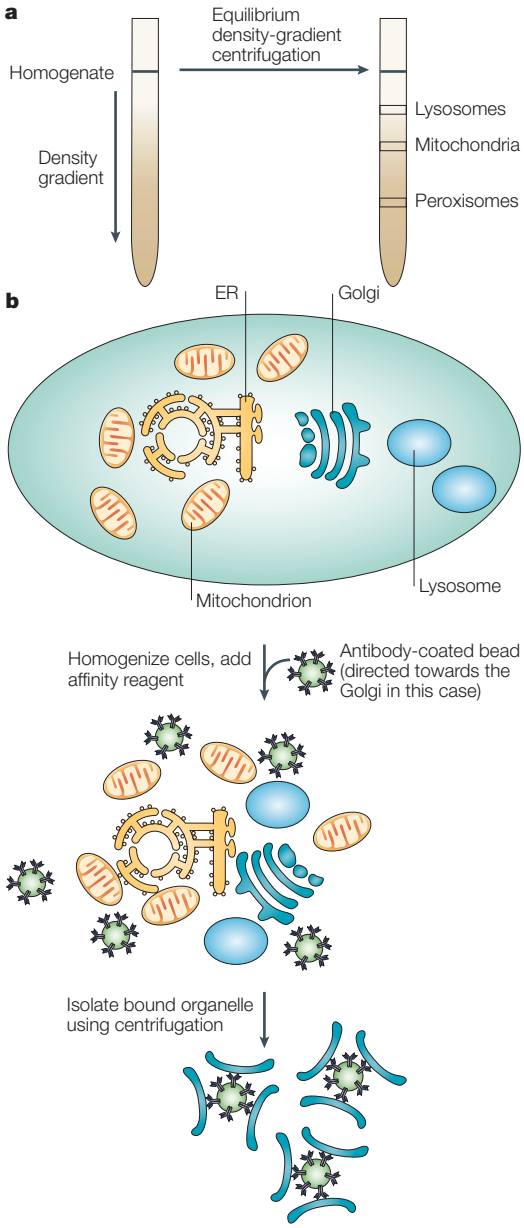
Organelle proteomics studies begin with the isolation, by classic cell-biology techniques (BOX 1), of an enriched fraction that contains the organelle of interest. This allows the proteins that are isolated with the organelle to be identified using proteomics techniques (BOXES 2,3). There are five important caveats to this analysis. First, the extent of the enrichment will vary and molecules that are not bona fide components of the organelle will be present in the fraction and identified as being a part of the proteome (BOX 4). Second, as mentioned above, the composition of organelles is always changing, with molecules being delivered to and transported from organelles by vesicles and tubules. This trafficking is mediated by components of the cytoskeleton — actin, tubulin and intermediate filaments — as well as by the motors and accessory molecules that function with each of the cytoskeletal components. These components might or might not be isolated with the organelle, which results in varying, but seldom logical, data that are used to classify these

Box 1 | **Organelle fractionation methods**

The reductionist approaches that are used to further our understanding of the roles of organelles and the proteins that are needed for organelle function require cellular fractionation methods. The most common strategy is to homogenize cells in the absence of detergents and to fractionate the components using combinations of centrifugation approaches. Centrifugation separates components on the basis of their size and density by using different time periods and velocities of centrifugation (see figure, part a). Another fractionation technique that can be used with centrifugation is affinity-mediated isolation using antibodies against the cytoplasmic domain of an organelle transmembrane protein or against a molecular tag[74,75] (see figure, part b). Using these techniques, high enrichments can be achieved, although usually at the expense of yield. In addition, the methods that are used to elute the organelle from the affinity reagent, typically in the form of a vesicle, can dissociate peripheral proteins that are bona fide proteins of the organelle.

Latex beads and silica can be used to isolate components of the endocytic and phagocytic pathways[76]. Cells phagocytose latex beads, and the phagocytic membranes remain adherent to the bead and can be isolated by floating the bead. Some selectivity can be achieved by coating the latex beads with specific molecules or antigens, and cationic silica microbeads can be used to isolate parts of the plasma membrane or structures such as CAVEOLAE[56,77].

Although all of these methods have provided a way to fractionate cells and enrich organelles, each technique results in the isolation of a different group of proteins, which produces varying results for proteomics analyses on a particular organelle. Separation methods that give a higher resolution would facilitate the analysis of all cellular components using proteomics technologies, so the development of new methods is a challenge for the field.



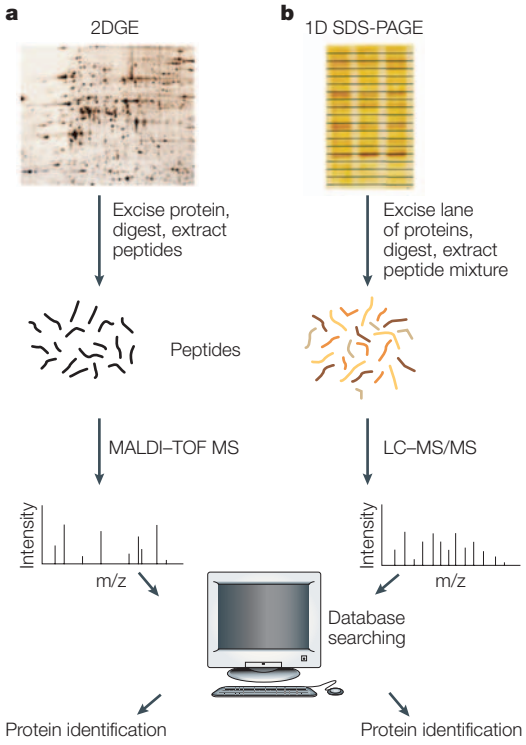
molecules when they are identified in proteomics studies. Third, many molecules in the cytoplasm interact with organelles to carry out defined functions, especially signalling molecules that are specifically activated by hormones, growth factors and other signals. In this case, whether these molecules will be associated with a specific organelle depends on the previous history of the cells that are being analysed. Fourth, the composition of an organelle from different cells and different tissues will vary. Last, many proteins in organelles, as well as the transmembrane proteins and the cytoplasmic proteins that are associated with organelles, are post-translationally modified. Understanding these post-translational modifications is paramount to dissecting function. Although there are now many methods to study post-translational modifications, comprehensive methods that uncover all post-translational modifications are time consuming and probably never carried out in a comprehensive way.

CAVEOLAE
A plasma-membrane invagination that is enriched in caveolin, cholesterol and glycosphingolipids.

Insights from organelle proteomics analyses

The analysis of organelles using proteomics methods is an active field of research, and significant progress is being made in defining the proteomes of organelles. So far, a sufficient number of proteomics studies have been published so that we can ask if the methods and technologies are suitable for organelle studies and, after the initial 'shock and awe' that surrounded proteomics technologies, if new functional or biological insights are being derived from these experiments.

Box 2 | **Large-scale proteomics analyses of organelles**



Our understanding of organelles has been facilitated by the sequencing of the genomes of many species. An almost complete set of genes is now available for human, mouse, rat and other model organisms[78–85]. This information has driven the development of mass spectrometry (MS)-based methods to identify, rather than sequence, proteins using the data that are present in sequence databases[1,86–91]. MS data for peptides and proteins provide information that can be used to identify proteins using these databases[92].

One of the most highly resolving methods for soluble protein separation is two-dimensional (2D) gel electrophoresis[93] (2DGE; see figure, part a). In 2DGE, proteins are separated in the first dimension on the basis of their isoelectric point and in the second by their molecular weight. A protein can then be excised from the gel, digested with a protease and the peptides extracted. These peptides can then be analysed using MS techniques such as matrix-assisted laser desorption/ionization–time-of-flight (MALDI–TOF). However, 2DGE has significant limitations for the separation of transmembrane proteins[94,95]. An important advantage of 2DGE is that it provides information on the size and the charged forms of a protein, which can give insights into post-translational modifications and protein processing.

One-dimensional (1D) SDS-PAGE is a preferred method for fractionating proteins on the basis of their molecular weight. The charged detergent SDS has two purposes — it effectively solubilizes membrane proteins and coats the solublized proteins such that the separation reflects their molecular weight. SDS-PAGE provides an effective means to fractionate complex protein mixtures, not to homogeneity, but sufficiently to allow a method such as liquid-chromatography–tandem MS (LC–MS/MS) to identify the proteins that are present in discrete sections of the gel (see figure, part b). This MS strategy for protein-mixture analysis, which was first described by Eng *et al.*[96], makes SDS-PAGE more effective for the analysis of membrane proteins then 2DGE[97,98]. m/z, mass-to-charge ratio.

Organelles and other large cellular structures have been analysed using proteomics techniques (BOX 5). However, no systematic or organized effort is underway to characterize the subcellular proteome of a particular cell. Rather than facilitating a comprehensive analysis of a single cell type, cell types and organisms are chosen more on the basis of the interest of the investigator, the convenience of sample preparation, and their relevance to disease. In the following sections, we review recent proteomics analyses and discuss the biological insights that have been derived.

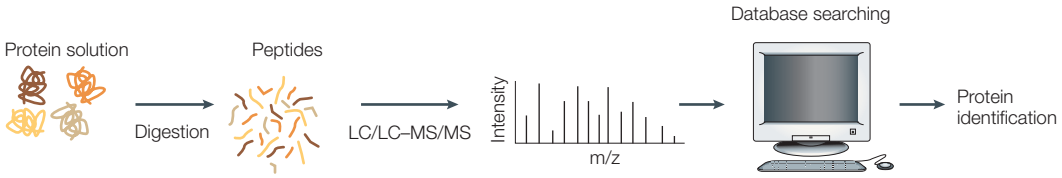
Exosomes. Many of the vesicles that transport lipids and proteins in cells have been studied using different proteomics approaches. EXOSOMES (BOX 5) are vesicles that are discharged into the extracellular milieu when multivesicular endosomes fuse with the plasma membrane. Recent studies have investigated the role of exosomes in the immune response[10–13], which has led to the hypothesis that these secreted vesicles have a role in intercellular signalling[14]. To analyse this, exosomes were purified from the supernatants obtained from melanoma cell lines and analysed using two-dimensional (2D) gel electrophoresis (2DGE) followed by tandem mass spectrometry (MS/MS)[15]. The analysis of 49 protein spots that were not obvious in cell-lysate controls resulted in the identification of 41 different proteins, some of which had been previously identified in exosomes, but some of which were novel to exosomes — for example, p120 catenin, radixin and immunoglobulin-superfamily member-8. In another study, urinary epithelial-cell-derived exosomes from six normal male volunteers were analysed by one-dimensional (1D) SDS-PAGE followed by nanospray liquid chromatography (LC)–MS/MS, which identified 295 unique proteins[16] (BOX 5). Of these, 73 were known membrane-trafficking proteins, mainly endosomal, which further confirmed the proposed endosomal origin of exosomes. In addition, a number of proteins that are known to be involved in specific kidney diseases were identified, which indicates that the analysis of exosomes that are excreted in the urine might provide a method for the early detection of renal disease.

Phagosomes. Phagocytosis is used by cells to internalize large macromolecular complexes, especially pathogens, for degradation. The complex or pathogen binds to cell-surface receptors, which results in the invagination of the plasma membrane and the internalization of the complex or pathogen. As mentioned in BOX 1,

EXOSOMES
Small vesicles of endocytic origin that are secreted by various cell types

LC–MS/MS
(liquid-chromatography–tandem mass spectrometry). This process uses an instrument in which high-performance liquid chromatography is linked directly to a tandem mass spectrometer through electrospray ionization.

Box 3 | **Multidimensional liquid-chromatography–tandem mass spectrometry**



An alternative strategy to those discussed in BOX 2 to identify soluble and membrane proteins in mixtures involves the use of liquid-chromatography–tandem MS (LC–MS/MS). In this strategy, proteins are proteolytically digested before they are separated[96,99] (see figure). Eng *et al.* showed that tandem mass spectra of peptides can be used to search sequence databases to identify proteins by matching amino-acid sequences to each spectrum[96]. Tandem mass spectrometers can select peptide ions for the process of COLLISION-INDUCED DISSOCIATION**, which can be used to provide information about the peptide sequence. Under computer control, the process is both fast and efficient, but not selective for specific peptide ions because any ion of sufficient abundance is selected for analysis. This situation results in a semi-random sampling process when complex mixtures are analysed[100].**

The proteolysis of protein mixtures creates complex mixtures of peptides. To fractionate complex peptide mixtures, Link *et al.*[101] integrated a two-dimensional liquid-chromatography separation technique (LC/LC) with a tandem mass spectrometer to create a multidimensional protein-identification technology (MUDPIT**), and this was subsequently optimized and improved by Washburn *et al.*[6] and Wolters *et al.*[102] The method produces a serial separation on the basis of charge interactions with a** STRONG CATION-EXCHANGE RESIN **(SCX) followed by hydrophobic interactions with a** REVERSED-PHASE SUPPORT**. Peptides are eluted from the SCX material using increasing salt concentrations, and they are subsequently trapped on the reversed-phase support. The salt is washed from the system and then the peptides are eluted into a tandem mass spectrometer using an increasing gradient of non-polar organic solvent. Alternatively, fractions can be collected 'off-line' from the SCX separation and then each fraction can be subjected to MS/MS. The use of this approach for protein identification is heavily dependent on informatics techniques for the analysis of the data obtained.**

COLLISION-INDUCED DISSOCIATION
A process that is used in tandem mass spectrometry to cause ions to fragment and reveal information about their molecular structure. Peptides fragment at their amide bonds to produce a ladder of sequence ions that are representative of the amino-acid sequence of the peptide.

MUDPIT
(multidimensional protein-identification technology). A method to identify the proteins that were digested to form peptide mixtures. The method uses multidimensional liquid chromatography together with tandem mass spectrometry and database searching.

STRONG CATION-EXCHANGE RESIN
An ion-exchange resin with a negatively charged surface that binds positively charged ions.

REVERSED-PHASE SUPPORT
A chromatographic resin that binds molecules on the basis of their hydrophobicity. It is often used for the separation of peptides.

PHAGOSOME
A membrane-bounded intracellular vesicle that arises from the ingestion of particulate material by phagocytosis.

PHAGOLYSOSOME
A phagocytic vesicle that has fused with a lysosome to become a digestive vacuole.

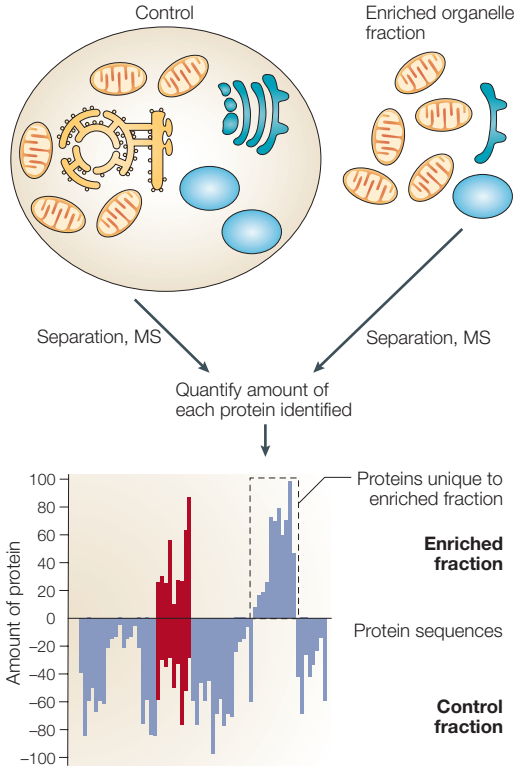
QUADRUPOLE TIME-OF-FLIGHT–MS/MS
(quadrupole time-of-flight–tandem mass spectrometry). A hybrid tandem-mass-spectrometry technique that combines a quadrupole mass analyser (which uses oscillating electric fields on four metal rods to separate ions on the basis of their mass-to-charge ratio) with a time-of-flight mass analyser (which converts the time it takes an ion to travel a specific distance into a mass-to-charge ratio).

latex-bead internalization, which is a model system for phagocytosis, has allowed the phagosomal compartment to be significantly enriched for proteomics analysis. Using this strategy, a total of 520 PHAGOSOME proteins have been identified[9]. As phagosomes fuse with organelles of the endocytic and biosynthetic pathways, separating the stages of maturation is difficult, and the proteins that are identified probably represent several stages of the phagocytic process. The interesting and novel finding of this proteomics analysis was the presence of many ER proteins in the PHAGOLYSOSOME proteome, because no obvious phagosome–ER link was known. However, studies by Desjardins and colleagues proposed that a portion of the phagosome membrane originates from the ER[17,18] and, by using a proteomics strategy, they showed that phagosomes have a full cross-presentation machinery for exogenous antigens[19] (BOX 5). Further proteomics studies investigated the role of protein kinase Cα (PKCα) in the regulation of phagolysosome biogenesis. Phagosomes isolated from macrophages overexpressing a dominant-negative kinase-dead mutant of PKCα showed inhibition of the recruitment of the phagosome components Rab7, cathepsin-D and cathepsin-S when compared to phagosomes isolated from control mouse macrophages. These data indicate that PKCα has a role in phagolysosome biogenesis[20] and show how functional insights can be obtained from proteomics studies.

Clathrin-coated vesicles. Clathrin-coated vesicles (CCVs) (BOX 5) that form using adaptor protein-1 (AP1) are involved in transport from the *trans*-Golgi network (TGN) to the endosomal system. Adaptor protein-2 (AP2)-positive CCVs function in a range of endocytic processes that start at the plasma membrane. These include the uptake of signalling receptors, plasma-membrane pumps and nutrients.

A proteomics analysis of a CCV fraction from rat brain, which used 1D SDS-PAGE and LC–QUADRUPOLE TIME-OF-FLIGHT–MS/MS, identified 209 proteins including 8 novel proteins[21,22]. These proteins were ranked according to their abundance by counting the number of fragmented peptides that were assigned to each protein. Using this method, the expected 1:1 stoichiometry was observed for the clathrin heavy chain and clathrin light chain. Interestingly, a 3:1 stoichiometry was found for AP2 to AP1, which indicates that most CCVs in the brain function in endocytosis rather than in budding from the TGN[22]. These data are consistent with the identification of known protein components of synaptic vesicles in the CCV proteome. As an important function of brain CCVs is to recycle synaptic vesicles, the proteomics data support a full-fusion model for synaptic vesicle exocytosis[22]. Furthermore, enthoprotin/epsinR and adaptin ear-binding coat-associated protein-2 (NECAP2) were identified and shown to be new components of CCVs that form at the TGN and plasma membrane, respectively[21,23]. Other studies showed that these proteins interact with AP1 and AP2, respectively, through previously unrecognized novel peptide motifs that are based around sequences with a θXXθ core (θ represents a bulky hydrophobic residue and X represents any amino acid)[24]. This increases the known complexity of the molecular sorting that

Box 4 | **Mass spectrometry data analysis**



Mass spectra contain information that can be used in powerful ways to study biology. Two types of mass spectrometry (MS) data are often collected for proteins. The first type is a map or fingerprint of the molecular weights of the peptides that were derived from the site-specific proteolysis of a protein or proteins[103]. Alternatively, tandem mass spectra of the peptides that were derived from a digested protein can be acquired, and these spectra contain fragmentation data that represent the amino-acid sequences of the peptides[92,96]. Software tools to analyse both forms of data have been developed.

Comparing different states of cells or organelles can often determine the protein changes that relate to the functional changes. However, given the difficulty of obtaining subcellular structures at high levels of purity, subtractive or differential analyses can be used to identify the proteins that are enriched compared to a control. Differential display in two-dimensional (2D) gel electrophoresis (2DGE) has been used for many years and the software to carry out these analyses is readily available. A new alternative for 2DGE separations is fluorescence difference 2D gel electrophoresis (DIGE)[104]. Furthermore, an alternative approach for subtractive or differential analyses uses two-dimensional liquid-chromatography–tandem mass spectrometry (LC/LC–MS/MS) to analyse the fractions and contaminants that are obtained from different cellular states and then compares or subtracts the protein differences[51,71] (FIG. 1). For such studies, an enriched set of proteins must be obtained.

Another strategy for the analysis of enriched organelle fractions uses a relative quantification method (see figure). In this method, an enriched organelle fraction is compared to a control fraction using the relative abundance of proteins present rather than the plus or minus method of subtractive analysis. Relative abundance measurements such as sequence coverage (see figure), spectral count and stable-isotope labelling have been used to identify proteins that are more abundant in an organelle fraction than in a control fraction[22,32,105,106]. Given the difficult nature of producing highly purified preparations of organelles and of vesicles that move molecules between organelles, these methods provide information on the proteins that are potentially enriched in, and specific to, a particular structure, so that they can then be targeted for functional studies.

DIGE
(fluorescence difference two-dimensional (2D) gel electrophoresis). A form of 2D gel electrophoresis that uses different colour fluorescent dyes to label proteins from different states that are then mixed and separated. The intensity difference of the different fluorophores reveals abundance differences.

'SHOTGUN' PROTEOMICS STRATEGY
A proteomics strategy that is based on the proteolysis of proteins, in particular, protein mixtures. The resulting peptide mixtures are usually analysed by liquid-chromatography–tandem mass spectrometry (LC–MS/MS) or multidimensional protein-identification technology (MudPIT).

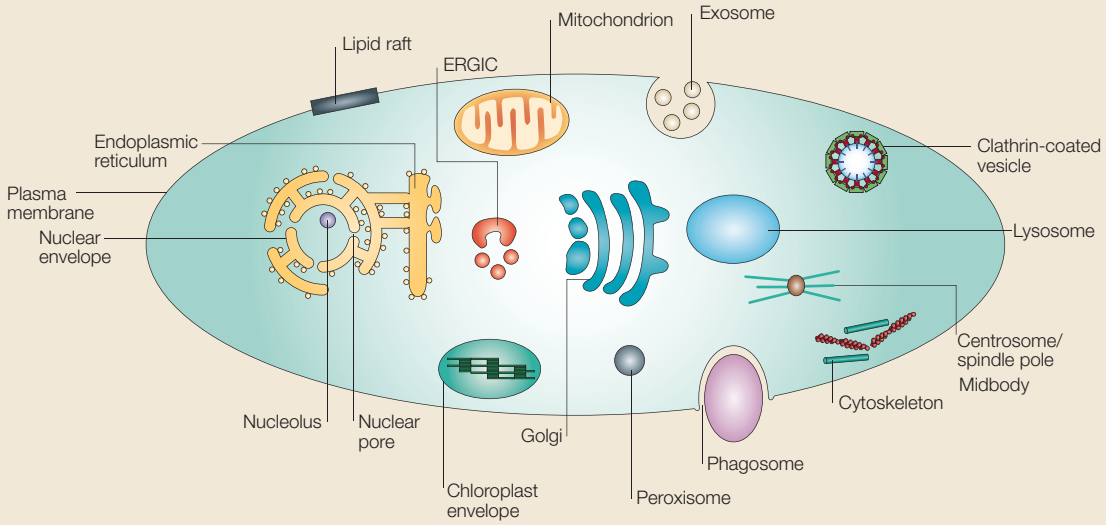
is carried out by CCVs. Finally, as mentioned above, this proteomics analysis discovered the protein entho-protin, which is probably responsible for susceptibility to a subtype of schizophrenia[25].

Mitochondria. Mitochondria function in oxidative phosphorylation and ATP production in all cells, but their protein composition varies depending on cell type, condition and disease state. Proteomics analyses can provide clues regarding how mitochondria physiologically adapt.

Four proteomics analyses of mitochondria that were isolated from three different species have identified 416–750 mitochondrion-associated proteins[26–30]. A study of the proteome of human-heart mitochondria identified 615 proteins using a 1D SDS-PAGE approach for protein fractionation[26], and the authors of this study subsequently used multidimensional-liquid-chromatography–MS/MS to obtain an expanded coverage of this proteome[27]. Sickmann *et al.*[28] used a combination of 2DGE and two-dimensional liquid chromatography (LC/LC)–MS/MS to identify 750 proteins in the mitochondria of *Saccharomyces cerevisiae*, whereas a study by Prokisch *et al.*[30] used a 'SHOT GUN' PROTEOMICS STRATEGY to identify 546 *S. cerevisiae* mitochondrial proteins. Of the 546 proteins identified by Prokisch *et al.*[30], 337 overlapped with the Sickmann *et al.*[28] study, and most of the remaining proteins could be classified as low abundance proteins[28,30]. Mootha *et al.*[29] carried out a proteomics study of mouse mitochondria, and produced a list of 591 mitochondrial proteins.

Both Mootha *et al.*[29] and Prokisch *et al.*[30] integrated data from expression analyses, deletion phenotype screening, protein-interaction analyses, computational predictions and subcellular localization studies to achieve a comprehensive view of mitochondria. Mootha *et al.*[29] isolated mitochondria from mouse brain, heart, kidney and liver, analysed the digested proteins using LC–MS/MS, and compared the proteomics data to RNA-expression profiles for the same tissues. These data provided insights into

Box 5 | **Selected proteomics studies of cellular structures**



Plasma membrane[56–60]
- 862 proteins identified (mouse brain cortex)
- 1,685 proteins identified (mouse hippocampal plasma membrane)
- 2,000 proteins identified, 2 validated (rat lung epithelium plasma membrane)
- Annexin A1 identified as a novel cancer target

Endoplasmic reticulum[33]
- 141 proteins identified, including 2 novel protein disulphide isomerases

Lipid raft[64–66]
- 241 proteins identified, most of which were signalling proteins

ERGIC[34]
- 24 proteins identified
- ERGIC-32 associated with ERV46 identified as a novel cycling complex
- SURF4 (Surfeit locus protein-4) implicated as a novel cargo receptor

Mitochondrion[26,28–30]
- 416–750 proteins identified
- Proteomics-based identification of a candidate gene for Leigh syndrome

Exosome[15,16]
- 295 proteins identified (including 73 known membrane-trafficking proteins, mainly endosomal, confirming the endosomal origin of exosomes)
- Eludcidation of potential biomarkers for renal and systemic diseases

Clathrin-coated vesicle[22]
- Quantitative elucidation of 209 proteins (32 derived from synaptic vesicles)
- 8 novel proteins, including enthoprotin, validated as novel regulators of clathrin-coated-vesicle assembly and disassembly
- Discovery of a new motif (WXXF) that interacts with adaptor protein-2 (AP2) through the identification of the novel protein NECAP2 (adaptin ear-binding coat-associated protein-2)
- Identification of a new target for schizophrenia (enthoprotin)

Lysosome[47]
- 215 proteins identified including 20 novel proteins in 'tritosomes' (Triton-WR1339-filled lysosomes)

Centrosome[71]**/spindle pole**[72]
- 114 centrosome proteins identified, including 23 novel proteins
- 795 spindle pole proteins identified, including 6 novel proteins

Midbody[73]
- 160 candidate proteins identified
- 85 linked to cytokinesis using RNA interference in *Caenorhabditis elegans*
- 10 novel proteins
- Unexpected role for the endoplasmic reticulum (ER) in chromosome segregation

Cytoskeleton[67,69,70]
- 122 proteins identified, including cell-wall-modification enzymes in *Arabidopsis thaliana* using tubulin-affinity chromatography
- 14-3-3 complexes analysed by FLAG-tagging established a functional link to the cytoskeleton

Phagosome[17,19,20,107]
- 520 proteins identified, including ER proteins
- ER proposed to add membrane and enable cross-presentation of antigens

Peroxisome[32]
- 70 proteins identified, including a link to actin through Rho1

Golgi[37,39–41]
- >400 proteins identified
- Arginine dimethylation identified as a novel Golgi post-translational modification

Chloroplast envelope[46]
- 89 proteins identified
- 70% assigned to transport or metabolic functions
- 30% have unknown functions

Nuclear pore[49,50]
- 30 proteins validated
- Proteomics-based Brownian-affinity-gating model proposed for nuclear transport

Nucleolus[52–54]
- 692 proteins identified, including 489 that traffic in and out of the nucleolus

Nuclear envelope[51]
- 80 membrane proteins predicted
- 8 novel proteins validated
- 23 potentially linked to disease by chromosome mapping

ERGIC, endoplasmic-reticulum–Golgi intermediate compartment. Display item modified with permission from REF. 9 © (2003) Elsevier Science.

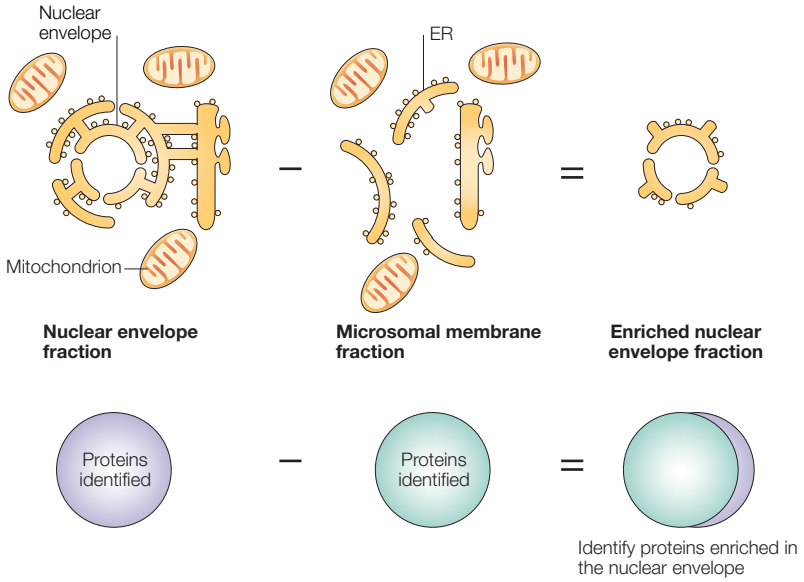


Figure 1 | **Subtractive proteomics methods to enrich proteins in organelles.** The analysis of proteins in the nuclear envelope was helped by identifying proteins that are present in microsomal membranes[51]. Microsomal membranes and nuclear envelopes were separately enriched by centrifugation techniques. Proteins in both fractions were digested using a protease such as trypsin and analysed using multidimensional liquid-chromatography–tandem mass spectrometry. Protein sequence databases were searched, using the data from the tandem mass spectra of the peptides that were identified in each fraction, to identify the proteins present. The proteins that were identified in the microsomal membranes were then subtracted from the list of proteins that were identified in the nuclear envelope fraction to produce an enriched set of protein identifications for the nuclear envelope.

both the tissue-specific differences in mitochondrial composition and the correlation between protein and RNA levels[29]. They identified 591 mitochondrial proteins, including 163 proteins that had not been previously linked to this organelle[29]. An important aspect of this study was the integration of proteomics and RNA-expression data with genotype data from patients with French-Canadian-type LEIGH SYNDROME, as this allowed the identification of a single candidate gene for this disease, *LRPPRC*[31] (BOX 5).

Such proteomics studies have provided important insights into mitochondrial function — for example, they have provided a greater understanding of the proteins that are imported into this organelle and have allowed the identity of DNA-repair enzymes for the small mitochondrial genome to be predicted. The broader value of these datasets is that they enable researchers to compare protein orthologues between species to gain a better understanding of the processes that occur in mitochondria.

Peroxisomes. These ubiquitous organelles are specialized for oxidative reactions that produce hydrogen peroxide and are linked to numerous functions including fatty acid metabolism and PLASMALOGEN synthesis. A proteomics strategy has been used to discriminate contaminants from bona fide peroxisomal proteins in *S. cerevisiae*, and 70 proteins were thought to be peroxisomal[32]. An important insight was provided by the discovery of Rho1 on peroxisome membranes. This provided the first evidence of a link between peroxisomes and the actin cytoskeleton, which indicates how peroxisome location and movement is regulated in the cell (BOX 5).

Endoplasmic reticulum. The mRNAs of transmembrane and secretory proteins are translated at the ER and the newly synthesized proteins subsequently enter the protein-folding 'factory' of the ER lumen. A proteomics analysis of the lumenal content of the ER that was fractionated from the livers of Balb/C mice identified 141 proteins[33] (BOX 5). Lumenal proteins were separated by 2DGE, and matrix-assisted laser desorption/ionization (MALDI) MS and MALDI MS/MS were used to analyse the excised spots. Of the 141 proteins, six novel proteins were identified, including ERp19 and ERp46. These proteins contain thioredoxin motifs, which functionally substituted for protein disulphide isomerase in *S. cerevisiae* complementation studies. This indicates that ERp19 and ERp46 are protein disulphide isomerases. This family of protein-folding enzymes catalyses productive protein folding by reducing and oxidizing disulphide bonds in substrate proteins until their correct tertiary structure is attained. The discovery of two new enzymes is indicative of the substrate specificity that exists in the molecular-chaperone function of these protein-folding enzymes.

ERGIC. Transport in the exocytic pathway from the ER to the Golgi is mediated by an intermediate compartment that is composed of tubular–vesicular structures and is known as the endoplasmic-reticululm–Golgi intermediate compartment (ERGIC). This compartment was isolated from the human hepatoma cell line HepG2 for proteomics analysis using an affinity technique[34]. The cells were treated with BREFELDIN A to induce the Golgi complex to fuse with the ER, leaving behind the brefeldin-A-resistant ERGIC compartment. ERGIC was isolated by subcellular fractionation using NYCODENZ gradients and immunopurification using magnetic beads that were coupled to a cytoplasmically exposed epitope of KDEL RECEPTOR. Following the tryptic digestion of its component proteins, 1D SDS-PAGE gels were used for peptide separation and MALDI–time-of-flight MS and nanospray MS/MS were used for peptide analysis.

Twenty-four proteins were identified including two that were previously uncharacterized — SURF4 (Surfeit locus protein-4) and ERGIC-32 (BOX 5). ERGIC-32 has sequence homology to the *S. cerevisiae* proteins ER vesicle-41 (Erv41) and Erv46, and was found to be a novel membrane protein that cycles between the ER and Golgi. Erv proteins are constituents of vesicles that were derived from the ER of *S. cerevisiae* using a cell-free assay that selects vesicles carrying newly synthesized cargo to the Golgi. These proteins are candidate receptors for newly synthesized cargo and they were also characterized by proteomics techniques[35]. In the study of Breuza *et al.*[34], ERGIC-32 was found to be localized to ERGIC by

LEIGH SYNDROME
An early-onset progressive neurodegenerative disorder with a characteristic neuropathology that consists of focal, bilateral lesions in one or more areas of the central nervous system.

PLASMALOGEN
Any glycerophospholipid in which a fatty acid group is replaced by a fatty aldehyde group.

MALDI MS
(matrix-assisted laser desorption/ionization mass spectrometry). MALDI is a technique that uses a laser to desorb and ionize molecules that are co-crystallized into an organic matrix that absorbs strongly at the wavelength of the laser. MALDI MS is carried out using a mass spectrometer that uses laser-based ionization to create ions. MALDI is most often combined with a time-of-flight mass spectrometer.

immunofluorescence microscopy. RNA interference (RNAi) of ERGIC-32 increased the turnover of human ERV46, which indicated that these proteins might form a complex, the stability of which requires the presence of both proteins. Remarkably, the novel protein SURF4 has sequence homology to Erv29, which has also been implicated as a cargo receptor[36]. The total complement of molecules that are required to move cargo between the ER and Golgi has not been defined, and it is not clear where in the dynamic ERGIC–Golgi compartments retrograde transport is initiated. Future studies, such as the one on Erv29 (REF. 36), in 'well-timed' systems will allow these questions to be addressed.

Golgi complex. The Golgi complex is the central organelle of the exocytic pathway. It is responsible for many of the post-translational modifications of newly synthesized proteins and lipids, as well as for the sorting of these molecules to their site of function. Four proteomics studies of the Golgi complex from rat-liver or mammary-epithelial cells have been reported, two using 2DGE[37,38], one using 1D SDS-PAGE[39], and another using multidimensional protein-identification technology (MudPIT)[40]. The studies by Taylor, Wu and colleagues[37,38,40] are the result of a collaboration between two groups, and represent an increased refinement of the proteomics technology. The starting material — a Golgi fraction — for the studies by Bell *et al.*[39] and by Taylor, Wu and colleagues[37,38,40] was isolated by different methods, but represented Golgi fractions that were enriched in stacked flattened cisternae in both cases. Bell *et al.*[39] first extracted the Golgi fraction in Triton-X 114 to enrich for transmembrane proteins and then separated the detergent-phase proteins using 1D SDS-PAGE. Both traditional EDMAN SEQUENCING and MALDI MS identified 81 proteins, 45 of which were considered to be Golgi proteins and 24 of which were contaminants, mostly from the ER and mitochondria. Many (32) of the 81 proteins were not transmembrane proteins but were probably proteins that interact with transmembrane proteins and that did not dissociate and partition into the detergent phase. Importantly, several novel proteins were identified, and the group focused on one called Golgi peripheral protein of 34 kDa (GPP34). The same protein was also identified by Taylor, Wu and colleagues. It was named Golgi matrix protein of 33 kDa (GMx33), was studied extensively[37,41] and was proposed to be a member of a novel family of peripheral *trans*-Golgi proteins.

Wu *et al.*[40] used LC/LC–MS/MS to obtain a more comprehensive Golgi proteome. They identified 421 proteins, 41 of which had no known function. Almost as many known ER proteins (96) were identified as known Golgi proteins (110). The remaining 215 proteins were sorted into categories on the basis of their reported localization and functions. Many of these proteins might functionally interact with the Golgi, but verification of their functional significance requires further localization and functional studies.

These proteomics studies identified novel proteins that can be targeted by future functional studies, and perhaps the most enlightening finding was that a large number of the proteins identified were ER proteins. Morphological studies have shown that the ER is adherent to numerous *trans*-Golgi cisternae[42–44], and the proteomics data indicate that the ER is truly adherent and isolates with stacked Golgi fractions. The literature indicates that this interaction could be mediated by CERT (ceramide ER transfer protein), which transfers ceramide from the ER to sphingomyelin synthetase in the Golgi[45].

Other new biological information came from mining the MudPIT data for post-translational modifications[40]. A novel cytoplasmic post-translational modification — arginine dimethylation — was identified on ten Golgi and five ER proteins, and one of the novel Golgi-localized proteins was predicted to be a methyltransferase that is itself dimethylated on an arginine residue (BOX 5). As this post-translational modification is known to be important in the nucleus, its presence in the cytoplasm indicates functional significance. In the future, the MudPIT data will be further analysed and will hopefully reveal more information on post-translational modifications. The Golgi proteome will vary in different cells and tissues, and in different physiological states. The data from these studies will therefore function as a baseline for comparison with the results of future studies.

Chloroplast envelope. In plants and algae, the chloroplast is the organelle in which photosynthesis occurs. Two membranes surround chloroplasts and form the chloroplast envelope. A specialized procedure was devised to obtain highly purified chloroplast envelopes from *Arabidopsis thaliana*, and these membranes were subjected to three different extraction procedures to maximize the number of membrane proteins that could be identified by LC–MS/MS[46]. From these three extractions, 112 proteins were identified, of which 89 were predicted to be genuine envelope proteins. These predictions were made using the literature or rigorous bioinformatics prediction methods to determine protein hydrophobicity, the presence of TRANSIT PEPTIDES, and homology to previously identified chloroplast proteins (BOX 5). Of the 89 proteins, 32% were involved — or were predicted to be involved — in transport functions, and even bioinformatics prediction methods could not propose a function for 30% of these proteins. Several of the transport proteins are involved in phosphate transport, which is particularly relevant to the regulation of stromal phosphate levels and the initiation of the CALVIN CYCLE. The final 38% were classified as metabolic proteins or as having other functions that remain to be elucidated[46]. These studies confirmed an important role for chloroplast envelope membranes in lipid metabolism, including fatty acid desaturation and in the synthesis of lipid growth regulators and defence molecules.

Lysosomes. Lysosomes are the main degradative compartment of the cell and represent the end point of the endocytic pathway. They contain many different hydrolytic enzymes that degrade cellular macromolecules.

BREFELDIN A
An inhibitor of intracellular protein transport, which prevents GTP binding to the nucleotide-exchange factor ARF (ADP-ribosylation factor) and thereby inhibits protein transport through the Golgi apparatus.

NYCODENZ
The trade name for a non-ionic iodinated chemical that is used to create density gradients.

KDEL RECEPTOR
A Golgi-located receptor for the C-terminal KDEL sequence that is present on endoplasmic-reticulum-resident lumenal proteins.

EDMAN SEQUENCING
A technique in which the N-terminal amino acids of a peptide are sequentially removed and identified in order to determine its amino-acid sequence.

TRANSIT PEPTIDES
Protein localization to chloroplasts is mediated by cleavable N-terminal transit peptides, which are sequences of 30–100 amino acids that direct proteins to the chloroplast for translocation across chloroplast membranes.

CALVIN CYCLE
The Calvin cycle is a metabolic pathway that occurs in the stroma of chloroplasts, in which carbon enters in the form of CO_2 and leaves in the form of sugar. The cycle uses ATP as an energy source and NADPH as a reducing agent.

A proteomics analysis of lysosomes — specifically of Triton-WR1339-filled lysosomes, which are known as 'tritosomes' — identified 215 proteins[47] (BOX 5). The enrichment of membrane proteins using alkaline sodium carbonate treatment followed by further fractionation enabled the identification of presumed cargo proteins (for example, Golgi glycosyltransferases as well as trafficking proteins, fusion proteins and transporters). An abundance of lipid-raft proteins in the lysosomal membrane confirmed the presence of these domains, which were previously identified in phagosomes[48]. An abundance of ER proteins was also found. This most recent study extends earlier lysosomal proteomics efforts (reviewed in REF. 9), which had identified signalling proteins such as CREG (cellular repressor of E1A-stimulated genes), the GTPase LRG-47 and the mitogen-activated-protein-kinase kinase (MEK)-binding protein MP1. Continuing efforts to extend the characterization of lysosomal membrane proteins, the lumenal proteins and the proteins that are bound to the cytoplasmic surface of this organelle might help to identify the proteins that regulate lysosomal abundance and location.

Nucleus. Proteins of the nuclear pore complex and the nuclear envelope in *S. cerevisiae* and mammalian cells have been identified by proteomics studies[49–51] (BOX 5). The study of the nuclear pore complex in *S. cerevisiae* elucidated a proteomics-based Brownian-affinity-gating model for nuclear transport[49]. The analysis of the nuclear envelope is complicated by the contiguous nature of the outer nuclear membrane and the ER. Schirmer *et al.*[51] derived a SUBTRACTION STRATEGY for analysing the nuclear envelope (FIG. 1). A comprehensive proteomics analysis of a MICROSOMAL MEMBRANE FRACTION was carried out and the ER proteins that were identified were subtracted from the nuclear-envelope proteome[51]. All of the previously identified nuclear-envelope proteins (13 in total) were identified in this study, as were 67 novel proteins. Eight of these were tagged and their nuclear-membrane localization was confirmed by immunolocalization. On the basis of the chromosomal location of the human homologues of these proteins, Schirmer *et al.*[51] suggested that some of these new nuclear-envelope proteins might be involved in the DYSTROPHIES that have not yet been linked to a disease gene (a number of dystrophies are thought to be linked to mutations in nuclear-envelope proteins). Twenty-three of the human homologues mapped to regions of the genome that have been linked to various dystrophies. These data therefore provide an excellent starting point for understanding what is a highly complicated inner-nuclear-membrane structure and its relationship to disease. The evaluation of the localization of the remaining 59 novel proteins is eagerly awaited in order to delineate the total complement and function of the nuclear-envelope-specific proteins.

A study of the nucleolus was recently reported by Andersen and colleagues[52]. The nucleolus is a ribosome-generating region of the nucleus, where transcription is coordinated with processing of ribosomal RNA and ribosome biogenesis. Nucleoli were isolated from HeLa cells using a standard protocol, and protein mixtures from individual 1D gel slices were in-gel digested. The resulting peptide mixtures were analysed using LC–MS/MS, as well as ion-trap–Fourier-transform mass spectrometry. Over 11,000 unique peptides were identified, which led to the identification of 692 proteins that were considered to be nucleolar. Remarkably, 90% of the previously characterized nucleolar proteins of *S. cerevisiae*[53] were matched with those found in this study (an increase compared to the percentage that matched a previous study[54]), which highlights the high degree of conservation of the fundamental nature of this compartment. In a remarkable demonstration of quantitative temporal proteomics, 489 proteins were identified that were recruited to or lost from the nucleolus following transcription inhibition or proteasome inhibition (BOX 5). Significant changes were found in the number of ribosomal proteins, which decreased following transcription inhibition and increased following proteasome inhibition. The roles of all of these proteins in nucleolar morphology and function can now be addressed.

Post-translational modifications are an important way to regulate cellular processes, and proteomics methods are beginning to focus on the identification of the different forms of post-translational modification. Most studies have focused on phosphorylation, and the large-scale characterization of phosphoproteins can provide information about the regulation of processes that are specific to an organelle. Beausoleil *et al.*[55] developed a strategy for the large-scale identification of phosphopeptides and applied it to the analysis of the nucleus of HeLa cells. By using strong cation-exchange chromatography to enrich phosphopeptides, 2,002 phosphorylation sites were identified in 967 proteins[55]. This enabled the specificities of the kinases that are responsible for these phosphorylation events to be determined, and showed that proline-directed kinases (for example, extracellular signal-regulated kinase-1) and acidophilic kinases (for example, casein kinase-I and -II) are responsible for 77% of the observed phosphorylation events.

Plasma membrane. An important challenge for proteomics, and for subcellular fractionation methods, is the characterization of cell-surface proteins, in particular, in the context of disease. A important advance was made by Oh *et al.*[56], who characterized the proteins in the plasma membranes of rat lung endothelial cells, which were isolated using an established silica-coated-bead methodology[56]. They identified 2,000 proteins, and showed that 2 were specific to lung-endothelial cells and were exposed on the cell surface. Comparing these data with information on the plasma-membrane proteins of lung endothelial cells from rats with breast adenocarcinomas highlighted 12 proteins that are enriched in tumour endothelium plasma membranes. One of these 12 proteins was annexin A1, and injecting antibodies against annexin A1 induced tumour remission. This application of plasma-membrane proteomics to cancer therapy adds to the work of Shin *et al.*[57] on the cell-surface proteome of cancer cells, and also adds to

SUBTRACTION STRATEGY
A process to subtract proteins that are found in a control state from an experimental or organelle-enriched state.

MICROSOMAL MEMBRANE FRACTION
An enriched fraction of microsomal membranes, which includes the endoplasmic reticulum, mitochondria and ribosomes.

DYSTROPHY
A degenerative disorder that mostly affects muscles.

DIGITONIN SHIFT
Digitonin is used to perturb the density of membraneous compartments that contain cholesterol. When it is added in molar excess to cholesterol in membrane fractions, the density of the membranes is shifted in proportion to the cholesterol content, and the largest shift is seen for plasma membranes.

LIPID RAFT
A type of lipid microdomain that is characterized by a relatively high content of cholesterol, sphingomyelin and glycosphingolipids. These microdomains are resistant to solubilization by non-ionic detergent because of their tight packing. They resemble liquid-ordered domains that are found in model membranes, which are characterized by tight packing and the high lateral mobility of lipids within the bilayer.

SILAC
(stable-isotope labelling by amino acids in cell culture). Stable-isotope-labelled amino acids are added to a cell-culture media in order to label proteins.

CENTROSOME
A structure that occurs close to the nucleus in eukaryotic cells during interphase. It comprises a pair of centrioles, satellite bodies and a cytoplasmic zone and, in animal cells, it serves as the main microtubule-organizing centre.

14-3-3 COMPLEXES
Discovered and named on the basis of their coordinates on two-dimensional gels, these cytosolic proteins bind to specific target proteins in a manner that is often regulated by the phosphorylation of their targets.

FLAG TAGGED
A peptide-sequence epitope from bacteriophage T7 is fused to a protein to allow it to be recognized by a monoclonal antibody directed towards the epitope. This enables protein purification and localization.

INTERPHASE
The period of the cell cycle between two mitotic divisions.

SPINDLE POLES
The region of the mitotic spindle where microtubule minus ends are focused.

the studies by Tam *et al.*[58], Zhang *et al.*[59] and Nuhse *et al.*[60] on various plasma membranes. Recently, Nielsen *et al.*[61] used a variation of the DIGITONIN SHIFT protocol[62] to characterize 862 plasma-membrane proteins of the mouse brain cortex and 1,685 plasma-membrane proteins of the mouse hippocampus (BOX 5). Collectively, these studies give insights into the proteins that are important for communication between the cell surface, the extracellular matrix and the intracellular milieu in the context of signalling[59,60] and disease[56–58].

Membrane domains and lipid rafts. Specific functional domains are found in the membranes of all organelles. LIPID RAFTS are the focus of much recent work and surrounding controversy[63]. These domains are extremely dynamic and it has been proposed that this allows them to function as sorting and signalling platforms[63]. They are commonly extracted from cells with TritonX-100 at 4°C, and the extracted domains only partially reflect the *in vivo* structures, which means that interpreting the results of proteomics studies on these structures is not straightforward.

Using the SILAC (stable-isotope labelling by amino acids in cell culture) method, a large-scale proteomics analysis of lipid rafts was carried out[64]. The SILAC method represents a quantitative strategy in which, in this case, leucine residues that each contained three deuterium atoms were incorporated into proteins. Peptides that were derived from these labelled proteins were easily identified, because they were offset by three mass units for each leucine residue. All the proteins in one of two cell populations were metabolically labelled with deuterium-substituted leucine, and one population was treated with a cholesterol-disrupting drug to destabilize lipid rafts. MS/MS was then used to identify the proteins that were depleted from lipid rafts. This approach identified 241 lipid-raft-specific proteins, which were mostly signalling proteins (BOX 5).

The problem of identifying lipid-raft proteins, which are extremely hydrophobic, was addressed by the use of an SDS-aided high-performance-LC–MALDI–MS/MS proteomics approach[65], which bypasses 1D gels and the in-gel digestion of proteins. This technique enabled the identification of 71 hydrophobic raft proteins, 45 of which had not been detected in previous experiments using in-gel digestion[65]. In another study, the dynamic nature of the lipid-raft proteome was assessed following T-cell receptor (TCR) triggering using 2DGE and MALDI–time-of-flight MS[66]. Although limited by the sampling problems of 2D gels, this study was remarkable and showed that TCR triggering promotes the temporally regulated recruitment of proteins that participate in TCR signalling to lipid rafts.

Large cellular structures

In addition to the study of organelles and transport intermediates, proteomics technologies have been successfully used to study large cellular structures such as the cytoskeleton and CENTROSOME. The cytoskeleton interacts with all organelles, and many cytoskeletal proteins have been identified in organelle proteomes. Studies that specifically focus on the cytoskeleton and on cytoskeletal organizing centres are important to identify regulatory molecules and molecules that function at the interface between the cytoskeleton and organelles.

Cytoskeleton. The cytoskeleton is a complex network of filaments, associated motor proteins and regulatory molecules. Novel proteomics approaches have been applied to the study of the cytoskeleton. Tubulin-affinity chromatography was used to isolate tubulin-binding proteins from *A. thaliana*[67]. The bound proteins were resolved using preparative 2DGE and identified using LC–MS/MS. In total, 122 proteins were identified from 86 spots on the original 2D gel and were divided into six functional categories: microtubule-associated proteins, translation factors, RNA-binding proteins, signalling proteins, metabolic enzymes and proteins with other functions. Half of the proteins identified had been previously shown to interact with microtubules. An interesting finding was the identification of cell-wall modification enzymes, such as endo-1,4-glucanase and endoxyloglucan glycosyl transferase, which modify cell-wall architecture and are needed for cell elongation and fruit ripening (BOX 5).

Another focus has been the identification of cytoskeleton-associated functional complexes — for example, human 14-3-3 COMPLEXES[68]. FLAG-TAGGED human 14-3-3 was stably expressed at a level equivalent to endogenous 14-3-3 isoforms in human embryonic kidney (HEK)293 cells, and its associated proteins were isolated using immobilized anti-Flag antibodies. LC–MS/MS analysis revealed 170 proteins that associated with 14-3-3 , including the seven endogenous 14-3-3 isoforms and other proteins with a broad range of functions, including control of the cytoskeleton[69]. Blocking the ability of 14-3-3 complexes to bind to phosphorylated proteins *in vivo* modified membrane dynamics and cell shape[69]. These data indicate the importance of phosphodependant 14-3-3 interactions for membrane dynamics and cytoskeletal function (BOX 5).

A study of detergent-resistant membrane fragments from bovine neutrophils highlighted a potential interaction of a cytoskeletal submembrane complex with transmembrane signalling molecules. MALDI–time-of-flight MS analysis identified 19 important proteins in this complex[70]. These proteins included the cytoskeletal proteins fodrin, myosin-IIA, myosin-IG, α-actinin, vimentin and the filamentous (F)-actin-binding-protein supervillin. The lipid-raft proteins stomatin, flotillin-1 and flotillin-2 were also identified. Together with data from immunofluorescence microscopy, these results indicate the existence of a lipid-raft-associated membrane skeleton in neutrophil plasma membranes[70].

Centrosome. Human centrosomes function as microtubule-organizing centres in INTERPHASE cells and as SPINDLE POLES during mitosis. Centrosomes from interphase cells were quantitatively analysed using PROTEIN-CORRELATION PROFILING[71]. Each fraction from a density separation was proteolytically digested and the

PROTEIN-CORRELATION PROFILING
A consensus fractionation profile for peptides that can be assigned to known organelle proteins is established, and peptides that deviate from this profile are considered nonspecific. This technique allows the analysis of any organelle or cellular structure that can be enriched but not purified to homogeneity.

KINETOCHORE
A multisubunit protein complex that is located at the pericentric region of DNA and that provides an attachment point for the spindle microtubules.

CYTOKINESIS
The process by which a mother cell divides to form two daughter cells.

MIDBODY
A structure that forms before a mother cell has completely divided to form two daughter cells. It is a dense proteinaceous structure that is associated with microtubules and is found at the site of division in animal cells.

resulting peptides were analysed using LC–MS/MS and database searching. Centrosome-specific proteins were distinguished from nonspecific proteins by tracking their abundance in a fractionation profile of peptides that were identified in five sucrose fractions of the centrosome preparation. A consensus profile for peptides that could be assigned to known centrosomal proteins was established, and peptides that deviated from this profile were considered nonspecific. When the abundance of the peptides correlated with the abundance of the peptides from proteins that are known to localize to the centrosome, the corresponding proteins were presumed to be localized to the centrosome. In this analysis 50 known centrosome proteins, 41 candidate centrosome proteins and 23 novel centrosome-localized components were identified (BOX 5). Localizing the last group of proteins to the centrosome provided new insights into possible centrosome functions.

Mitotic spindle. The mitotic spindle encompasses microtubule spindle poles, centrosomes and the KINETOCHORE. Mitotic spindle poles that were enriched from HeLa cells were analysed using MS/MS after protein separation on 1D SDS-PAGE gels[72]. A total of 151 proteins that were previously known to be associated with the spindle apparatus, centrosomes or kinetochores were identified. However, most of the other proteins that were identified (644) had not been previously shown to be centrosome associated, and 154 of these proteins were uncharacterized. 17 of the uncharacterized proteins were tagged and transfected into mitotic cells, which led to the identification of 6 new spindle components[72] (BOX 5). This emphasizes the point that not all proteins that are identified in cellular fractions are bona fide components of the compartment under investigation.

Midbody. During CYTOKINESIS, when membrane cleavage is almost complete, the plasma membrane of the cleavage furrow tapers to form the MIDBODY. The midbody is derived from the central spindle and persists as an attachment between the two daughter cells before final separation. A clear function has never been ascribed to this structure, but it is assumed that it will contain proteins that are involved in cytokinesis. The mammalian midbody was isolated from Chinese hamster ovary cells and analysed using MudPIT[73]. A total of 160 candidate mammalian midbody proteins were identified, and these were functionally characterized in *Caenorhabditis elegans* using RNAi. Of the 160 candidate proteins, 147 had obvious homologues in *C. elegans*, and the RNAi-mediated suppression of protein expression resulted in scorable defects for 141 of these proteins. RNAi against 85 of the 147 homologues caused defects in cytokinesis, and 10 novel proteins were localized to the midbody in HeLa cells using immunofluorescence. Unexpectedly, a role for the abundant ER molecular chaperone GRP94 was uncovered in chromosome segregation (BOX 5). These identifications mean that many new molecules can now be tested for their involvement in the process of cytokinesis. The confirmation of proteomics results using techniques such as RNAi is exactly what is needed to exploit the power of proteomics.

Conclusions and future directions

The use of proteomics technologies to characterize organelles and large cellular structures has provided functional insights, although the data become more complete and convincing when the proteomics analyses are combined with protein-localization and protein-knockdown techniques. Identifying the phenotype that is produced by disrupting a protein that is localized to a specific organelle can clarify how the organelle functions and indicate the role of the individual protein. It is clear that MS-based proteomics methods are being used to study organelle function and will be used increasingly to understand diseases and to identify disease specific biomarkers. Recent advances in characterizing membrane proteins and post-translational modifications and in bioinformatics methods are essential for the future of organelle studies, and we anticipate continued advances in proteomics technologies. In our opinion, the time has come for an organized effort to characterize the subcellular proteomes of several different types of human cell comprehensively. Such an effort would allow us to improve technologies to enrich subcellular structures and to carry out high-throughput, follow-up studies such as immunofluorescence-localization and RNAi studies. The real power of proteomics will be realized when comprehensive and comparative analyses of organelles are carried out in different cell types, under different physiological states, and in normal and diseased cells.

1. Yates, J. R. 3rd. Mass spectral analysis in proteomics. *Annu. Rev. Biophys. Biomol. Struct.* **33**, 297–316 (2004).
2. Yates, J. R. 3rd. Mass spectrometry as an emerging tool for systems biology. *Biotechniques* **36**, 917–919 (2004).
3. Gygi, S. P. *et al.* Quantitative analysis of complex protein mixtures using isotope-coded affinity tags. *Nature Biotechnol.* **17**, 994–999 (1999).
4. Oda, Y., Huang, K., Cross, F. R., Cowburn, D. & Chait, B. T. Accurate quantitation of protein expression and site-specific phosphorylation. *Proc. Natl Acad. Sci. USA* **96**, 6591–6596 (1999).
5. Wu, C. C., MacCoss, M. J., Howell, K. E., Matthews, D. E. & Yates, J. R. 3rd. Metabolic labeling of mammalian organisms with stable isotopes for quantitative proteomic analysis. *Anal. Chem.* **76**, 4951–4959 (2004).
6. Washburn, M. P., Wolters, D. & Yates, J. R. 3rd. Large-scale analysis of the yeast proteome by multidimensional protein identification technology. *Nature Biotechnol.* **19**, 242–247 (2001).
7. Wu, C. C., MacCoss, M. J., Howell, K. E. & Yates, J. R. 3rd. A method for the comprehensive proteomic analysis of membrane proteins. *Nature Biotechnol.* **21**, 532–538 (2003).
8. Blonder, J. *et al.* Enrichment of integral membrane proteins for proteomic analysis using liquid chromatography–tandem mass spectrometry. *J. Proteome Res.* **1**, 351–360 (2002).
9. Brunet, S. *et al.* Organelle proteomics: looking at less to see more. *Trends Cell Biol.* **13**, 629–638 (2003).
10. Peche, H., Heslan, M., Usal, C., Amigorena, S. & Cuturi, M. C. Presentation of donor major histocompatibility complex antigens by bone marrow dendritic cell-derived exosomes modulates allograft rejection. *Transplantation* **76**, 1503–1510 (2003).
11. Raposo, G. *et al.* B lymphocytes secrete antigen-presenting vesicles. *J. Exp. Med.* **183**, 1161–1172 (1996).
12. Riteau, B. *et al.* Exosomes bearing HLA-G are released by melanoma cells. *Hum. Immunol.* **64**, 1064–1072 (2003).
13. Zitvogel, L. *et al.* Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. *Nature Med.* **4**, 594–600 (1998).
14. Thery, C., Zitvogel, L. & Amigorena, S. Exosomes: composition, biogenesis and function. *Nature Rev. Immunol.* **2**, 569–579 (2002).

15. Mears, R. *et al.* Proteomic analysis of melanoma-derived exosomes by two-dimensional polyacrylamide gel electrophoresis and mass spectrometry. *Proteomics* **4**, 4019–4031 (2004).
16. Pisitkun, T., Shen, R. F. & Knepper, M. A. Identification and proteomic profiling of exosomes in human urine. *Proc. Natl Acad. Sci. USA* **101**, 13368–13373 (2004).
17. Gagnon, E. *et al.* Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. *Cell* **110**, 119–131 (2002).
18. Desjardins, M. ER-mediated phagocytosis: a new membrane for new functions. *Nature Rev. Immunol.* **3**, 280–291 (2003).
19. Houde, M. *et al.* Phagosomes are competent organelles for antigen cross-presentation. *Nature* **425**, 402–406 (2003).
This study validated the presence of ER proteins in the phagolysosome proteome, which led to a link between ER recruitment and the phenomenon of antigen cross-presentation.
20. Ng Yan Hing, J. D., Desjardins, M. & Descoteaux, A. Proteomic analysis reveals a role for protein kinase C-α in phagosome maturation. *Biochem. Biophys. Res. Commun.* **319**, 810–816 (2004).
21. Wasiak, S. *et al.* Enthoprotin: a novel clathrin-associated protein identified through subcellular proteomics. *J. Cell Biol.* **158**, 855–862 (2002).
22. Blondeau, F. *et al.* Tandem MS analysis of brain clathrin-coated vesicles reveals their critical involvement in synaptic vesicle recycling. *Proc. Natl Acad. Sci. USA* **101**, 3833–3838 (2004).
These authors showed an important function for brain CCVs in recycling synaptic vesicles, which supports a full-fusion model for synaptic vesicle exocytosis. They also identified the novel protein enthoprotin, which has a known link to schizophrenia.
23. Ritter, B. *et al.* Identification of a family of endocytic proteins that define a new α-adaptin ear-binding motif. *EMBO Rep.* **4**, 1089–1095 (2003).
24. Ritter, B. *et al.* Two WXXF-based motifs in NECAPs define the specificity of accessory protein binding to AP-1 and AP-2. *EMBO J.* **23**, 3701–3710 (2004).
25. Pimm, J. *et al.* The epsin 4 gene on chromosome 5q, which encodes the clathrin-associated protein enthoprotin, is involved in the genetic susceptibility to schizophrenia. *Am. J. Hum. Genet.* **76**, 902–907 (2005).
26. Taylor, S. W. *et al.* Characterization of the human heart mitochondrial proteome. *Nature Biotechnol.* **21**, 281–286 (2003).
27. Gaucher, S. P. *et al.* Expanded coverage of the human heart mitochondrial proteome using multidimensional liquid chromatography coupled with tandem mass spectrometry. *J. Proteome Res.* **3**, 495–505 (2004).
28. Sickmann, A. *et al.* The proteome of *Saccharomyces cerevisiae* mitochondria. *Proc. Natl Acad. Sci. USA* **100**, 13207–13212 (2003).
29. Mootha, V. K. *et al.* Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell* **115**, 629–640 (2003).
30. Prokisch, H. *et al.* Integrative analysis of the mitochondrial proteome in yeast. *PLoS Biol.* **2**, e160 (2004).
31. Mootha, V. K. *et al.* Identification of a gene causing human cytochrome *c* oxidase deficiency by integrative genomics. *Proc. Natl Acad. Sci. USA* **100**, 605–610 (2003).
32. Marelli, M. *et al.* Quantitative mass spectrometry reveals a role for the GTPase Rho1p in actin organization on the peroxisome membrane. *J. Cell Biol.* **167**, 1099–1112 (2004).
33. Knoblach, B. *et al.* ERp19 and ERp46, new members of the thioredoxin family of endoplasmic reticulum proteins. *Mol. Cell. Proteomics* **2**, 1104–1119 (2003).
34. Breuza, L. *et al.* Proteomics of endoplasmic reticulum–Golgi intermediate compartment (ERGIC) membranes from brefeldin A-treated HepG2 cells identifies ERGIC-32, a new cycling protein that interacts with human Erv46. *J. Biol. Chem.* **279**, 47242–47253 (2004).
35. Otte, S. *et al.* Erv41p and Erv46p: new components of COPII vesicles involved in transport between the ER and Golgi complex. *J. Cell Biol.* **152**, 503–518 (2001).
36. Belden, W. J. & Barlowe, C. Role of Erv29p in collecting soluble secretory proteins into ER-derived transport vesicles. *Science* **294**, 1528–1531 (2001).
37. Taylor, R. S. *et al.* Proteomics of rat liver Golgi complex: minor proteins are identified through sequential fractionation. *Electrophoresis* **21**, 3441–3459 (2000).
38. Wu, C. C., Yates, J. R. 3rd, Neville, M. C. & Howell, K. E. Proteomic analysis of two functional states of the Golgi complex in mammary epithelial cells. *Traffic* **1**, 769–782 (2000).
39. Bell, A. W. *et al.* Proteomics characterization of abundant Golgi membrane proteins. *J. Biol. Chem.* **276**, 5152–5165 (2001).
40. Wu, C. C. *et al.* Organellar proteomics reveals Golgi arginine dimethylation. *Mol. Biol. Cell* **15**, 2907–2919 (2004).
These authors used MudPIT to study the Golgi proteome, and identified arginine dimethylation as a novel cytoplasmic post-translational modification.
41. Wu, C. C. *et al.* GMx33: a novel family of *trans*-Golgi proteins identified by proteomics. *Traffic* **1**, 963–975 (2000).
42. Novikoff, A. B., Essner, E. & Quintana, N. Golgi apparatus and lysosomes. *Fed. Proc.* **23**, 1010–1022 (1964).
43. Ladinsky, M. S., Mastronarde, D. N., McIntosh, J. R., Howell, K. E. & Staehelin, L. A. Golgi structure in three dimensions: functional insights from the normal rat kidney cell. *J. Cell Biol.* **144**, 1135–1149 (1999).
44. Smith, C. E., Hermo, L., Fazel, A., Lalli, M. F. & Bergeron, J. J. Ultrastructural distribution of NADPase within the Golgi apparatus and lysosomes of mammalian cells. *Prog. Histochem. Cytochem.* **21**, 1–120 (1990).
45. Hanada, K. *et al.* Molecular machinery for non-vesicular trafficking of ceramide. *Nature* **426**, 803–809 (2003).
46. Ferro, M. *et al.* Proteomics of the chloroplast envelope membranes from *Arabidopsis thaliana*. *Mol. Cell. Proteomics* **2**, 325–345 (2003).
47. Bagshaw, R. D., Mahuran, D. J. & Callahan, J. W. A proteomics analysis of lysosomal integral-membrane proteins reveals the diverse composition of the organelle. *Mol. Cell. Proteomics* **4**, 133–143 (2005).
48. Dermine, J. F. *et al.* Flotillin-1-enriched lipid raft domains accumulate on maturing phagosomes. *J. Biol. Chem.* **276**, 18507–18512 (2001).
49. Rout, M. P. *et al.* The yeast nuclear pore complex: composition, architecture, and transport mechanism. *J. Cell Biol.* **148**, 635–651 (2000).
One of the pioneering organelle proteomics studies. Discovered a Brownian-affinity-gating mechanism for nucleocytoplasmic transport.
50. Cronshaw, J. M., Krutchinsky, A. N., Zhang, W., Chait, B. T. & Matunis, M. J. Proteomic analysis of the mammalian nuclear pore complex. *J. Cell Biol.* **158**, 915–927 (2002).
51. Schirmer, E. C., Florens, L., Guan, T., Yates, J. R. 3rd & Gerace, L. Nuclear membrane proteins with potential disease links found by subtractive proteomics. *Science* **301**, 1380–1382 (2003).
52. Andersen, J. S. *et al.* Nucleolar proteome dynamics. *Nature* **433**, 77–83 (2005).
A quantitative temporal proteomics study, which identified proteins that are recruited to or lost from the nucleolus following transcription or proteasome inhibition.
53. Huh, W. K. *et al.* Global analysis of protein localization in budding yeast. *Nature* **425**, 686–691 (2003).
54. Andersen, J. S. *et al.* Directed proteomic analysis of the human nucleolus. *Curr. Biol.* **12**, 1–11 (2002).
55. Beausoleil, S. A. *et al.* Large-scale characterization of HeLa cell nuclear phosphoproteins. *Proc. Natl Acad. Sci. USA* **101**, 12130–12135 (2004).
56. Oh, P. *et al.* Subtractive proteomic mapping of the endothelial surface in lung and solid tumours for tissue-specific therapy. *Nature* **429**, 629–635 (2004).
A silica-coated-bead methodology was used to isolate the plasma membrane of lung endothelial cells. Two proteins were shown to be lung endothelium specific and cell-surface exposed. Annexin A1 was identified as a novel target for cancer treatment.
57. Shin, B. K. *et al.* Global profiling of the cell surface proteome of cancer cells uncovers an abundance of proteins with chaperone function. *J. Biol. Chem.* **278**, 7607–7616 (2003).
58. Tam, E. M., Morrison, C. J., Wu, Y. I., Stack, M. S. & Overall, C. M. Membrane protease proteomics: isotope-coded affinity tag MS identification of undescribed MT1-matrix metalloproteinase substrates. *Proc. Natl Acad. Sci. USA* **101**, 6917–6922 (2004).
59. Zhang, W., Zhou, G., Zhao, Y. & White, M. A. Affinity enrichment of plasma membrane for proteomics analysis. *Electrophoresis* **24**, 2855–2863 (2003).
60. Nuhse, T. S., Stensballe, A., Jensen, O. N. & Peck, S. C. Phosphoproteomics of the *Arabidopsis* plasma membrane and a new phosphorylation site database. *Plant Cell* **16**, 2394–2405 (2004).
61. Nielsen, P. A. *et al.* Proteomic mapping of brain plasma membrane proteins. *Mol. Cell. Proteomics* **4**, 402–408 (2005).
62. Amar-Costesec, A. *et al.* Analytical study of microsomes and isolated subcellular membranes from rat liver. II. Preparation and composition of the microsomal fraction. *J. Cell Biol.* **61**, 201–212 (1974).
63. Munro, S. Lipid rafts: elusive or illusive? *Cell* **115**, 377–88 (2003).
64. Foster, L. J., De Hoog, C. L. & Mann, M. Unbiased quantitative proteomics of lipid rafts reveals high specificity for signaling factors. *Proc. Natl Acad. Sci. USA* **100**, 5813–5818 (2003).
65. Li, N., Shaw, A. R., Zhang, N., Mak, A. & Li, L. Lipid raft proteomics: analysis of in-solution digest of sodium dodecyl sulfate-solubilized lipid raft proteins by liquid chromatography–matrix-assisted laser desorption/ionization tandem mass spectrometry. *Proteomics* **4**, 3156–3166 (2004).
66. Bini, L. *et al.* Extensive temporally regulated reorganization of the lipid raft proteome following T-cell antigen receptor triggering. *Biochem. J.* **369**, 301–309 (2003).
67. Chuong, S. D. *et al.* Large-scale identification of tubulin-binding proteins provides insight on subcellular trafficking, metabolic channeling, and signaling in plant cells. *Mol. Cell. Proteomics* **3**, 970–983 (2004).
68. Dougherty, M. K. & Morrison, D. K. Unlocking the code of 14-3-3. *J. Cell Sci.* **117**, 1875–1884 (2004).
69. Jin, J. *et al.* Proteomic, functional, and domain-based analysis of *in vivo* 14-3-3 binding proteins involved in cytoskeletal regulation and cellular organization. *Curr. Biol.* **14**, 1436–1450 (2004).
70. Nebl, T. *et al.* Proteomic analysis of a detergent-resistant membrane skeleton from neutrophil plasma membranes. *J. Biol. Chem.* **277**, 43399–43409 (2002).
71. Andersen, J. S. *et al.* Proteomic characterization of the human centrosome by protein correlation profiling. *Nature* **426**, 570–574 (2003).
72. Sauer, G. *et al.* Proteome analysis of the human mitotic spindle. *Mol. Cell. Proteomics* **4**, 35–43 (2005).
73. Skop, A. R., Liu, H., Yates, J., 3rd, Meyer, B. J. & Heald, R. Dissection of the mammalian midbody proteome reveals conserved cytokinesis mechanisms. *Science* **305**, 61–66 (2004).
Identified novel proteins that have a role in cytokinesis, and indicated a role for the ER chaperone GRP94 in chromosome segregation.
74. Devaney, E. & Howell, K. E. Immuno-isolation of a plasma membrane fraction from the Fao cell. *EMBO J.* **4**, 3123–3130 (1985).
75. Kamrath, F. J., Dodt, G., Debuch, H. & Uhlenbruck, G. The isolation of lysosomes from normal rat liver by affinity chromatography. *Hoppe Seyler's Z. Physiol. Chem.* **365**, 539–547 (1984).
76. Desjardins, M. & Griffiths, G. Phagocytosis: latex leads the way. *Curr. Opin. Cell Biol.* **15**, 498–503 (2003).
77. Durr, E. *et al.* Direct proteomic mapping of the lung microvascular endothelial cell surface *in vivo* and in cell culture. *Nature Biotechnol.* **22**, 985–992 (2004).
78. Gibbs, R. A. *et al.* Genome sequence of the Brown Norway rat yields insights into mammalian evolution. *Nature* **428**, 493–521 (2004).
79. Waterston, R. H. *et al.* Initial sequencing and comparative analysis of the mouse genome. *Nature* **420**, 520–562 (2002).
80. Venter, J. C. *et al.* The sequence of the human genome. *Science* **291**, 1304–1351 (2001).
81. Lander, E. S. *et al.* Initial sequencing and analysis of the human genome. *Nature* **409**, 860–921 (2001).
82. International Human Genome Sequencing Consortium. Finishing the euchromatic sequence of the human genome. *Nature* **431**, 931–945 (2004).
83. Adams, M. D. *et al.* The genome sequence of *Drosophila melanogaster*. *Science* **287**, 2185–2195 (2000).
84. Goffeau, A. *et al.* Life with 6000 genes. *Science* **274**, 563–567 (1996).
85. The *C. elegans* sequencing consortium. Genome sequence of the nematode *C. elegans*: a platform for investigating biology. *Science* **282**, 2012–2018 (1998).
86. Henzel, W. J. *et al.* Identifying proteins from two-dimensional gels by molecular mass searching of peptide fragments in protein sequence databases. *Proc. Natl Acad. Sci. USA* **90**, 5011–5015 (1993).
87. Yates, J. R. 3rd, Speicher, S., Griffin, P. R. & Hunkapiller, T. Peptide mass maps: a highly informative approach to protein identification. *Anal. Biochem.* **214**, 397–408 (1993).
88. James, P., Quadroni, M., Carafoli, E. & Gonnet, G. Protein identification by mass profile fingerprinting. *Biochem. Biophys. Res. Commun.* **195**, 58–64 (1993).

89. Pappin, D. J., Hojrup, P. & Bleasby, A. J. Rapid identification of proteins by peptide-mass fingerprinting. *Curr. Biol.* **3**, 327–332 (1993).
90. Mann, M., Hojrup, P. & Roepstorff, P. Use of mass spectrometric molecular weight information to identify proteins in sequence databases. *Biol. Mass Spectrom.* **22**, 338–345 (1993).
91. Lin, D., Tabb, D. L. & Yates, J. R. 3rd. Large-scale protein identification using mass spectrometry. *Biochim. Biophys. Acta* **1646**, 1–10 (2003).
92. Sadygov, R., Cociorva, D. & Yates, J. R. 3rd. Large-scale database searching using tandem mass spectra: looking up the answer in the back of the book. *Nature Methods* **1**, 195–202 (2004).
93. Klose, J. & Kobalz, U. Two-dimensional electrophoresis of proteins: an updated protocol and implications for a functional analysis of the genome. *Electrophoresis* **16**, 1034–1059 (1995).
94. Molloy, M. P. *et al.* Extraction of membrane proteins by differential solubilization for separation using two-dimensional gel electrophoresis. *Electrophoresis* **19**, 837–844 (1998).
95. Molloy, M. P. *et al.* Proteomic analysis of the *Escherichia coli* outer membrane. *Eur. J. Biochem.* **267**, 2871–2881 (2000).
96. Eng, J. K., McCormack, A. L. & Yates, J. R. 3rd. An approach to correlate tandem mass spectral data of peptides with amino acid sequences in a protein database. *J. Am. Soc.Mass Spectrom.* **5**, 976–989 (1994).
97. Husi, H., Ward, M. A., Choudhary, J. S., Blackstock, W. P. & Grant, S. G. Proteomic analysis of NMDA receptor–adhesion protein signaling complexes. *Nature Neurosci.* **3**, 661–669 (2000).
98. Link, A. J., Hays, L. G., Carmack, E. B. & Yates, J. R. 3rd. Identifying the major components of *Haemophilus influenzae* type-strain NCTC 8143. *Electrophoresis* **18**, 1314–1334 (1997).
99. McCormack, A. L. *et al.* Direct analysis and identification of proteins in mixtures by LC/MS/MS and database searching at the low-femtomole level. *Anal. Chem.* **69**, 767–776 (1997).
100. Liu, H., Sadygov, R. G. & Yates, J. R. 3rd. A model for random sampling and estimation of relative protein abundance in shotgun proteomics. *Anal. Chem.* **76**, 4193–4201 (2004).
101. Link, A. J. *et al.* Direct analysis of protein complexes using mass spectrometry. *Nature Biotechnol.* **17**, 676–682 (1999).
102. Wolters, D. A., Washburn, M. P. & Yates, J. R. 3rd. An automated multidimensional protein identification technology for shotgun proteomics. *Anal. Chem.* **73**, 5683–5690 (2001).
103. Yates, J. R. 3rd. Database searching using mass spectrometry data. *Electrophoresis* **19**, 893–900 (1998).
104. Yan, J. X. *et al.* Fluorescence two-dimensional difference gel electrophoresis and mass spectrometry based proteomic analysis of *Escherichia coli*. *Proteomics* **2**, 1682–1698 (2002).
105. Sam-Yellowe, T. Y. *et al.* Proteome analysis of rhoptry-enriched fractions isolated from *Plasmodium* merozoites. *J. Proteome Res.* **3**, 995–1001 (2004).
106. Florens, L. *et al.* A proteomic view of the *Plasmodium falciparum* life cycle. *Nature* **419**, 520–526 (2002).
107. Garin, J. *et al.* The phagosome proteome: insight into phagosome functions. *J. Cell Biol.* **152**, 165–180 (2001).

Acknowledgements

We would like to acknowledge support from the National Institutes of Health (J.R.Y. and K.E.H.), Genome Canada/Genome Québec (J.J.M.B.), Valorisation Recherche Québec (J.J.M.B.), and the Canada Foundation for Innovation: Cell Map Project (J.J.M.B.).

Competing interests statement

The authors declare no competing financial interests.

Online links

DATABASES

The following terms in this article are linked online to:
Entrez Gene: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
LRPPRC
Prosite: http://us.expasy.org/prosite
14-3-3 | CCV
Swiss-Prot: http://www.expasy.ch/sprot
GPP44 | Rho1

FURTHER INFORMATION

John Yates' laboratory: http://fields.scripps.edu

DATABASE SEARCHING PROGRAMS

Agilent Technologies: SpectrumMill: http://www.chem.agilent.com/scripts/pds.asp?lpage=7771
EMBL Bioanalytical Research Group: PeptideSearch: http://www.narrador.embl-heidelberg.de/GroupPages/Homepage.html
Matrix Science: Mascot: http://www.matrixscience.com
PepFrag: http://prowl.rockefeller.edu/prowl/pepfragch.html
ProbID: http://projects.systemsbiology.net/probid
ProteinProspector: MS-Tag: http://prospector.ucsf.edu/ucsfhtml4.0/mstagfd.htm
ProteinProspector: MS-Seq: http://prospector.ucsf.edu/ucsfhtml4.0/msseq.htm
SEQUEST: http://fields.scripps.edu/sequest/index.html or http://www.thermo.com/com/cda/product/detail/1,1055,22209,00.html
The GPM: X!TANDEM: http://www.thegpm.org/TANDEM/index.html
Waters: Masslynx: http://www.waters.com/watersdivision/ContentD.asp?watersit=CSAN-5K7HKQ&WT.svl=2

2DGE SOFTWARE

AMPL Software: Gel-Quant: http://www.ampl.com.au/gq_frames.htm
Dynapix Intelligence Imaging: Proteoscope: http://www.dynapix-intelligence.com/dynapix_products_proteoscope_en.html
GEHealthcare: Amersham Biosciences: DIGE: http://www.amershambiosciences.com
GeneBio: Melanie & ImageMaster 2D Platinum Software: http://www.genebio.com/products/2d_image.html
Nonlinear Dynamics: 2D gel analysis with Phoretix and Progenesis: http://www.nonlinear.com/products/2d

MASS SPECTROMETRY COMPANIES

Applied Biosystems: http://www.appliedbiosystems.com
Bruker Daltonics: http://www.bdal.de
Shimadzu: http://www.shimadzu.com
Thermo Electron Corporation: http://www.thermo.com
Waters: http://www.waters.com
Access to this interactive links box is free online.