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Review

Proteomic approaches in brain research and neuropharmacology

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Abstract

Numerous applications of genomic technologies have enabled the assembly of unprecedented inventories of genes, expressed in cells under specific physiological and pathophysiological conditions. Complementing the valuable information generated through functional genomics with the integrative knowledge of protein expression and function should enable the development of more efficient diagnostic tools and therapeutic agents. Proteomic analyses are particularly suitable to elucidate posttranslational modifications, expression levels and protein–protein interactions of thousands of proteins at a time. In this review, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) investigations of brain tissues in neurodegenerative diseases such as Alzheimer's disease, Down syndrome and schizophrenia, and the construction of 2D-PAGE proteome maps of the brain are discussed. The role of the Human Proteome Organization (HUPO) as an international coordinating organization for proteomic efforts, as well as challenges for proteomic technologies and data analysis are also addressed. It is expected that the use of proteomic strategies will have significant impact in neuropharmacology over the coming decade.

Keywords: Proteomics; Neuroscience; Neuropharmacology; Human Proteome Organization; Alzheimer's disease; Schizophrenia

Contents

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1. Introduction: genomics and post-genomics

The finalization of the genome map of man (Venter et al., 2001) and numerous other organisms (Goffeau et al., 1996; The *C. elegans* Sequencing Consortium, 1998; Waterston et al., 2002; Rat Genome Sequencing Project Consortium, 2004) as well as the rapid development of biological databases and computer algorithms (Table 1) have a tremendous impact on current research in molecular biology and pharmacology. Indeed, the availability of these powerful instruments enabled new ways of formulating and addressing biological questions. The traditional approach of studying one gene or protein at a time is now synergistically complemented by a more integrative approach, allowing the study of many genes and proteins simultaneously. Software and retrieval systems (listed in Table 1) facilitate the analysis of data from complex databases.

For large-scale screening of differential gene expression, commonly used techniques include mRNA differential display (Liang and Pardee, 1992), subtractive hybridization (Sagerstrom et al., 1997), serial analysis of gene expression (SAGE; Velculescu et al., 1995) and complementary DNA microarrays (Hu et al., 2000; Debouck and Goodfellow, 1999). In particular, microarrays have great potential as an automated and commercialized high-throughput screening method of gene expression for the investigation of disease mechanisms and for drug discovery (Marcotte et al., 2003; Mirnics and Pevsner, 2004).

In order to understand molecular processes, occurring in health and in disease states, it is necessary to unravel signal transduction pathways and intercellular and intracellular interaction networks between proteins and other molecules that influence cellular function, especially in complex systems such as the brain. It necessitates the knowledge of co- and posttranslational modifications of gene products as well as protein translocation and activity. This information is not inherently encoded in gene sequences and cannot be derived from mRNA expression, because of a lack of correlation between transcriptional profiles and actual protein levels in cells (Anderson and Seilhamer, 1997; Paulson et al., 2003). To date, more than three hundred posttranslational protein modifications have been identified (http://prowl.rockefeller.edu/aainfo/deltamassv2.html). In addition, the expression levels of genes and gene products influence each other and are altered by various epigenetic factors (Strohman, 1994).

The proteome of a cell or of an organelle provides information about the ensemble of protein isoforms expressed in that cell or organelle under specific physiological conditions and at a specific time. Investigating proteomes of healthy and diseased tissues enables the identification of molecular changes potentially underlying disease pathogenesis (Hanash, 2003; Gagnon et al., 2002). The analysis of proteomic, biochemical and physiological

Table 1
Examples of commonly used databases and retrieval systems to query those databases

Nucleic acid sequences database	GenBank	http://www.ncbi.nlm.nih.gov
Protein sequences database	PIR	http://pir.georgetown.edu/pirwww/search/textpsd.shtml
Motif databases and motif search tools	Prosite	http://kr.expasy.org/prosite
	PRINTS	http://www.bioinf.man.ac.uk/dbbrowser/PRINTS/
	Pfam	http://www.sanger.ac.uk/Software/Pfam/
	InterProScan	http://www.ebi.ac.uk/InterProScan/
Sequence databases search tools	Entrez	http://www.ncbi.nlm.nih.gov/Entrez
	SRS	http://srs.ebi.ac.uk/
	SWISS-Prot	http://www.expasy.ch/sprot/
Pairwise sequence alignment	BLAST	http://www.ncbi.nlm.nih.gov/BLAST/
	FASTA3	http://www2.ebi.ac.uk/fasta3/
	SSEARCH	http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_ssearch.html
	ALIGN	http://www.ebi.ac.uk/emboss/align/index.html
Identifying potential coding regions in genomic DNA sequences	GENSCAN	http://genes.mit.edu/GENSCAN.html
	GenWise	http://www.ebi.ac.uk/Wise2/index.html
	Procrustes	http://www-hto.usc.edu/software/procrustes/index.html
Locating promoters, sequences and splice sites	CBS Prediction server	http://www.cbs.dtu.dk/services/
Converting a DNA sequence into protein sequence or vice versa	"Protein machine" server at EBI	http://www2.ebi.ac.uk/translate/
Protein 3D structure visualization and analysis	SWISS-PDBViewer	http://us.expasy.org/spdbv/
	rasmol	http://www.openrasmol.org/
	molmol	http://www.mol.biol.ethz.ch/wuthrich/software/molmol/
	midasplus	http://www.cgl.ucsf.edu/Outreach/midasplus/
	cn3d	http://www.ncbi.nlm.nih.gov/Structure/CN3D/cn3d.shtml
	pdb	http://www.rcsb.org/pdb/
Structure classification	CATH	http://www.biochem.ucl.ac.uk/bsm/cath/
	TOPS	http://www.tops.leeds.ac.uk/
	3Dee	http://www.compbio.dundee.ac.uk/3Dee/
Peptide mass fingerprinting search tools	MOWSE	http://www.hgmp.mrc.ac.uk/Bioinformatics/Webapp/mowse
	Mascot	http://www.matrixscience.com
	MS-fit	http://prospector.ucsf.edu/ecsfhtlm3.4/msfit.htm
	Peptident	http://www.expasy.ch.ch/tools/peptident.html
	Profound	http://www.prowl.rockefeller.edu/sgi-bin/Profound

information about the healthy and diseased brain should lead to a better understanding of the interaction and function(s) of protein isoforms in brain tissues. Combining the information generated with functional genomics and proteomics will greatly advance the engineering of neuropharmacological agents with more specific molecular targets, while potentially minimizing side effects.

Since the formulation of the concept of a proteome (Wasinger et al., 1995), two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) technology (Fig. 1) is being optimized (Van den Bergh et al., 2003; Van den Bergh and Arckens, 2004), and several gel-free high-throughput screening technologies for protein analysis are being developed. These technologies include multidimensional protein identification technology (MudPIT; Washburn et al., 2001); yeast two-hybrid and reverse two-hybrid assays (Vidal and Legrain, 1999); protein microarrays (Cutler, 2003; Melton, 2004); phage-display antibody libraries (Sidhu et al., 2000) and HysTag reagent (Olsen et al., 2004). Recent advances in protein mass spectrometric methods and database searching allow accurate automated identification of proteins isolated from gel-based and other proteomic approaches (Aebersold and Mann, 2003; Pandey and Mann, 2000).

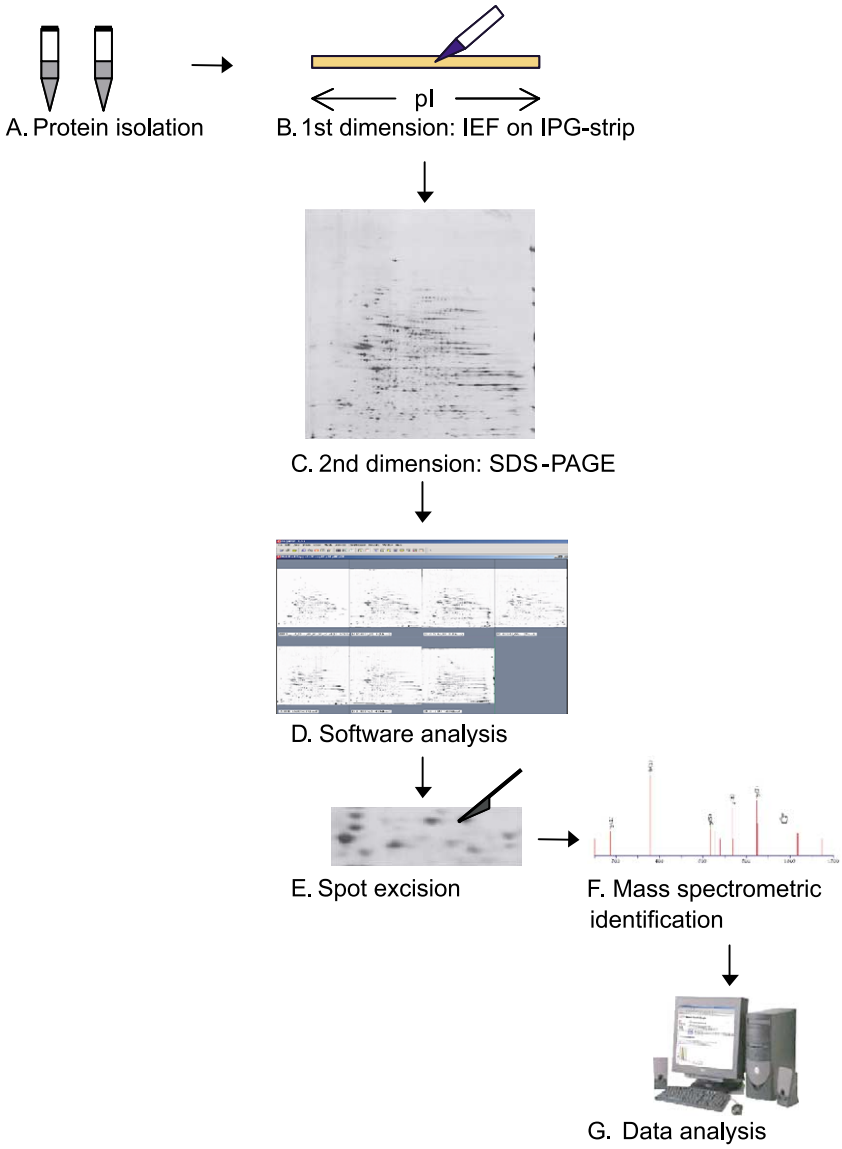

Fig. 1. Proteomic experiment based on two-dimensional electrophoresis to identify differential protein expression in different protein extracts. A typical setup of this experiment consists of seven stages. In stage A, proteins are extracted from cells or tissues, after which these proteins are separated by means of isoelectric focusing (IEF) on immobilized pH gradient (IPG) strips (B). In the second dimension of 2D-PAGE, proteins with similar isoelectric points are further separated according to their molecular weight on a sodium disulphate polyacrylamide (SDS-PAGE) gel (C), and proteomic patterns are visualized by means of, e.g. silver staining, Coomassie staining or fluorescent staining. In stage d, the proteomic patterns of the samples are compared by means of software, to identify differential spots on the gels. Differential spots are excised (E) and proteins in these spots are enzymatically degraded, usually by means of trypsin, and subsequently identified by means of mass spectrometry (F) and database searching (G).

In this review, alterations in proteomic 2D-PAGE patterns of brain tissues caused by diseases such as Alzheimer's disease, Down syndrome, schizophrenia and depression are discussed, as well as proteomic changes in brains of mouse and rat models of these diseases. The applicability of proteomic approaches for the development of molecular disease markers and the use of 2D-PAGE for the construction of brain proteome maps are illustrated. Finally, the coordinating role of the Human Proteome Organization (HUPO) in proteomic international studies and challenges encountered in current proteomic technologies and data analyses are addressed.

2. Proteomics in brain research: differential expression

2.1. Differential protein expression in Alzheimer's disease

In 2D-PAGE analyses of several regions of the Alzheimer's disease brain, alterations in the expression of a wide range of proteins have been observed, as listed in Table 2. Proteomic changes observed in these studies potentially contribute to disease mechanisms that have been associated to Alzheimer's disease, such as oxidative stress (e.g. superoxide dismutase; SOD: Schonberger et al., 2001), gliosis (e.g. glial fibrillary acidic protein; GFAP: Tsuji et al., 2001; Greber et al., 1999), energy depletion (e.g. creatine kinase BB: Schonberger et al., 2001; Aksenov et al., 2001; Castegna et al., 2002) and deranged neurotransmission (e.g. synaptosomal-associated proteins; SNAPs: Yoo et al., 2001; Greber et al., 1999). Moreover, because of the methodology used in 2D-PAGE analyses, dysregulated proteins that previously had not been linked to this disease, including histamine-releasing factor protein, DJ-1 protein, nucleoside diphosphate kinase-A, peroxiredoxins and unknown proteins, are being identified in these studies (Table 2). Furthermore, Schonberger et al. (2001) reported significant changes in the expression of proteins with a variety of functions, in brain regions that are severely affected (hippocampus, temporal and entorhinal cortices) or relatively spared (cerebellum, cingulated gyrus, and sensorimotor cortex) in Alzheimer's disease. These results underline the importance of constructing an overall picture of molecular changes occurring in all regions of the diseased brain in a given disorder.

Several animal models of Alzheimer's disease have been used to identify differentially expressed brain proteins caused by Alzheimer's disease-linked mutations (Tables 3 and 4). Interestingly, as indicated in Table 3, certain protein alterations observed in animal models are similar to changes observed in Alzheimer's disease brains. Other changes may indicate that distinct molecular mechanisms may occur in different species in response to Alzheimer's disease-linked mutant proteins and may point to the fact that Alzheimer's disease is a multifactorial disease, rendering the ensemble of biological processes in the affected tissues very complex.

One major advantage of the use of animal models for human diseases is the possibility to investigate molecular changes occurring before the onset of the disease. In one study, transgenic rats carrying Alzheimer's disease-linked mutations in amyloid precursor protein (APP) and presenilin 1 (PS1) were used to investigate hippocampal proteomic alterations in a pre-plaque stage (Vercauteren et al., unpublished results). Interestingly, long before the onset of plaque formation and/or cognitive impairments, the expression level of many hippocampal proteins that play a role in learning and memory formation was altered, eleven being particularly significant. Some proteins related to glucose metabolism and protein transport, and one unknown protein were significantly altered (Table 4). These preliminary results indicate that profound changes take place in hippocampal neurons in response to expression of these mutant proteins, preceeding plaque formation and cognitive impairments. This and similar studies may contribute to the elucidation of novel targets for disease preventing pharmacological agents.

Other proteomic studies of Alzheimer's disease brains have focused on specific posttranslational modifications of proteins, such as protein oxidation (Table 2; Butterfield, 2004). Using a novel 2D-PAGE application (Korolainen et al., 2002) or 2D Oxyblot (Aksenov et al., 2001), it was confirmed that the balance of oxidation and degradation is altered in several regions of the Alzheimer's disease brain. Castegna et al. (2002) observed that creatine kinase BB, glutamine synthase and ubiquitin carboxy-terminal hydrolase L-1 (UCH L-1) are specific targets of protein oxidation in the inferior parietal lobule in Alzheimer's disease. Loss of creatine kinase BB activity and consequent energy depletion in the Alzheimer's disease brain was shown previously (Smith et al., 1991). The increased oxidation of glutamine synthase likely contributes to decreased glutamine synthase activity in Alzheimer's disease brains. It may also be related to the fact that the glutamate transporter is oxidatively modified and dysfunctional in Alzheimer's disease. The down-regulation of UCH L-L1 in Alzheimer's disease brains observed in this and other proteomic studies (Castegna et al., 2002; Choi et al., 2004) is in accordance with defective (de)ubiquitination processes in Alzheimer's disease.

Another posttranslational modification of proteins in the Alzheimer's disease brain that is being investigated by means of 2D-PAGE is nitration. The increased nitration of proteins observed by Castegna et al. (2003) in the inferior parietal lobule of Alzheimer's disease patients (Table 2) potentially contributes to previous observations of cholinergic deficits (Auld et al., 2002), metabolic impairment as well as modifications of cytoskeletal proteins in the Alzheimer's disease brain.

Several proteins previously directly linked with amyloidosis and cell death, such as chaperones (Yoo et al., 2001a)

Table 2
Observed significant proteomic changes in the human brain in Alzheimer's disease (AD), Down syndrome (DS), Parkinson's disease (PD), schizophrenia (S), bipolar disorder (BD) and depression (depr.)

Protein	Disease	Tissue	Deregulation	Reference
Alcohol dehydrogenase	AD	CB, TC, OC	increased	Balcz et al., 2001
Alpha enolase	AD	IPL	increased nitr	Castegna et al., 2003
	AD	H, CG	increased	Schonberger et al., 2001
Alpha-crystallin B chain	AD	CG	increased	Schonberger et al., 2001
	AD	TC	increased	Yoo et al., 2001c
Alpha-internexin	AD	TC	decreased	Tsuji et al., 2002
Antioxidant protein 2	AD	TC	increased	Schonberger et al., 2001
ATP synthase beta chain, mito precursor	AD	TC	increased	Tsuji et al., 2002
Beta actin	AD	SMT	increased ox	Aksenov et al., 2001
	AD	IPL	increased nitr	Castegna et al., 2003
Beta enolase	AD	TC	decreased	Tsuji et al., 2002
Calpain 2, large subunit	AD	TC	increased	Tsuji et al., 2002
Carbonyl reductase	AD	CB, TH, CN, PC, TC	increased	Balcz et al., 2001
CNPase	AD	FC	decreased	Vlkolinsky et al., 2001
Creatine kinase beta chain	AD	SMT	increased ox	Aksenov et al., 2001
	AD	IPL	increased ox	Castegna et al., 2002
	AD	H	decreased	Schonberger et al., 2001
Diazepam binding inhibitor	AD, S	H	decreased	Edgar et al., 1999a,b
Dihydropyrimidinase related protein-2	AD	CG	decreased	Schonberger et al., 2001
	AD	TC	decreased	Tsuji et al., 2002
DJ-1	AD	H, SC	increased	Schonberger et al., 2001
Fatty acid-binding protein, heart	AD	H, CB	increased	Schonberger et al., 2001
	AD	TC	decreased	Tsuji et al., 2002
GADP dehydrogenase, liver	AD	H, SC	increased	Schonberger et al., 2001
Gamma enolase	AD	TC	increased	Schonberger et al., 2001
	AD	IPL	increased nitr	Castegna et al., 2003
GFAP	AD, DS	FC, PC, TC, OC	increased	Greber et al., 1999
	AD	TC	deregulated	Tsuji et al., 2002, 2001
Glutamine synthase	AD	IPL	increased ox	Castegna et al., 2002
GRP 75	AD	TC, PC	decreased	Yoo et al., 2001c
GRP 94	AD	TC	increased	Yoo et al., 2001c
Guanine nucleotide-binding	AD	TC	decreased	Tsuji et al., 2002
Hemoglobin beta	AD	H, EC	decreased	Schonberger et al., 2001
Hemoglobin beta, several isoforms	AD	TC, SC, CB	increased	Schonberger et al., 2001
Histamine-releasing factor protein	AD	TC	decreased	Kim et al., 2001a
HSP 60	AD	PC	decreased	Kim et al., 2001a,b,c; Yoo et al., 2001c
HSP 60, mito precursor	AD	TC	decreased	Tsuji et al., 2002
HSP 70 RY	AD	CN	increased	Yoo et al., 2001c
HSC 71	AD	TC	decreased	Yoo et al., 2001c
IEF SSP 3521	AD	CG	decreased	Schonberger et al., 2001
L-Lactate dehydrogenase	AD	IPL	increased nitr	Castegna et al., 2003
Macrophage migration inhibitory factor	AD	EC	decreased	Schonberger et al., 2001
Mito ATP synthase alpha subunit precursor	AD	TC	decreased	Tsuji et al., 2002
Neuropolypeptide h3	AD	IPL	increased nitr	Castegna et al., 2003
Nucleoside diphosphate kinase-A	AD, DS	FC, OC, PC	decreased	Kim et al., 2002
Peptidyl-prolyl *cis–trans* isomerase	AD	H	decreased	Schonberger et al., 2001
Peroxiredoxin I	AD, DS		decreased	Kim et al., 2001b
Peroxiredoxin II	AD, DS		decreased	Kim et al., 2001b
	AD, DS, FTD	FC	increased	Krapfenbauer et al., 2003a
Peroxiredoxin III	AD, DS		increased	Kim et al., 2001b
Phosphatidylethanolamine-binding protein	AD	CB	decreased	Schonberger et al., 2001
Phosphoglycerate mutase, brain	AD	CB	decreased	Schonberger et al., 2001
Profilin II	AD	CG	decreased	Schonberger et al., 2001
Protein G9I/G(S)/G(T) beta 1	AD	temporal cortex	decreased	Tsuji et al., 2002
Serum albumin precursor	AD	CB, CG	decreased	Schonberger et al., 2001
SNAP beta	AD, DS	TC	decreased	Yoo et al., 2001a
SNAP-25	AD, DS	FC, PC, TC, OC, CB	decreased	Greber et al., 1999
SOD, Cu/Zn	AD	H	increased	Schonberger et al., 2001
Stathmin	AD	FC, TC	decreased	Cheon et al., 2001
Succinyl CoA	AD	TC	increased	Schonberger et al., 2001

(continued on next page)

Table 2 (*continued*)

Protein	Disease	Tissue	Deregulation	Reference
Synaptotagmin	AD	TC	increased	Schonberger et al., 2001
SYT I (p65)	AD	CB, TC, PC	decreased	Yoo et al., 2001a
SYT I (pI 7.0)	AD	TC, PC, TH	decreased	Yoo et al., 2001a
Triosephosphate isomerase	AD	IPL	increased nitr	Castegna et al., 2003
Ubiquinone oxidoreductase, 24 kDa	AD	TC, OC	decreased	Kim et al., 2001c
Ubiquinone oxidoreductase, 75 kDa	AD	PC	decreased	Kim et al., 2001c
UCH-L1	AD	IPL	increased ox	Castegna et al., 2002
	AD, PD	FC	increased ox, carb	Choi et al., 2004
	AD	EC	decreased	Schonberger et al., 2001
VDAC1 pI 10	AD	TC, FC, OC	decreased	Yoo et al., 2001b
VDAC1 pI 7.5	AD	OC	increased	Yoo et al., 2001b
VDAC1 all isoforms	AD	FC, TH	decreased	Yoo et al., 2001b
Vesicular-fusion protein NSF	AD	CG, SC	decreased	Schonberger et al., 2001
Aconitrate hydratase, mito	DS	fetal brain	increased	Bajo et al., 2002
Antioxidant protein 2	DS	fetal brain	decreased	Gulesserian et al., 2001
APLP-1	DS	fetal brain	decreased	Engidawork et al., 2003a,b
Aspartate aminotranseferase, cytosol	DS	fetal brain	decreased	Bajo et al., 2002
Beta tubulin	DS	fetal brain	decreased	Engidawork et al., 2003a,b
Citrate synthase	DS	fetal brain	decreased	Bajo et al., 2002
CNPase	DS	FC, TC	decreased	Vlkolinsky et al., 2001
eIF3 p47 subunit 5	DS	fetal brain	increased	Engidawork et al., 2003a,b
GFAP	DS, AD	FC, PC, TC, OC	increased	Greber et al., 1999
Glutathione synthetase	DS	fetal brain	decreased	Gulesserian et al., 2001
Glutathione-*S*-transferase mu2	DS	fetal brain	decreased	Gulesserian et al., 2001
Glutathione-*S*-transferase p	DS	fetal brain	decreased	Gulesserian et al., 2001
GRP 75	DS	TC	decreased	Yoo et al., 2001d
GRP 78	DS	CB, PC	increased	Yoo et al., 2001d
Histamine-releasing factor protein	DS	TC, TH, CN	decreased	Kim et al., 2001a
HSC 71	DS	TC	decreased	Yoo et al., 2001d
HSP 70 RY	DS	TC	decreased	Yoo et al., 2001d
HSP 70.1	DS	CB	increased	Yoo et al., 2001d
HSP 75, mito precursor	DS	fetal brain	increased	Engidawork et al., 2003a,b
HSPC 140	DS	fetal brain	decreased	Engidawork et al., 2003a,b
Hypothetical protein 28.5	DS	fetal brain	increased	Engidawork et al., 2003a,b
MSF-B	DS	fetal brain	increased	Engidawork et al., 2003a,b
NADP-isocitrate dehydrogenase, mito	DS	fetal brain	increased	Bajo et al., 2002
NCK adapter protein 2	DS	fetal brain	decreased	Engidawork et al., 2003a,b
Nucleoside diphosphate kinase-A	DS, AD	FC, OC, PC	decreased	Kim et al., 2002
Periredoxin I	DS, AD		decreased	Kim et al., 2001b
Periredoxin II	DS, AD		decreased	Kim et al., 2001b
	DS, FTD, AD	FC	increased	Krapfenbauer et al., 2003a
Periredoxin III	DS, AD		increased	Kim et al., 2001b
	DS, FTD	FC	decreased	Krapfenbauer et al., 2003a
Pyruvate kinase M1 isozyme	DS	fetal brain	increased	Bajo et al., 2002
Pyruvate kinase M2 isozyme	DS	fetal brain	increased	Bajo et al., 2002
Rad 21	DS	fetal brain	increased	Engidawork et al., 2003a,b
Septin 7	DS	fetal brain	decreased	Engidawork et al., 2003a,b
SH3GLB2	DS	fetal brain	decreased	Engidawork et al., 2003a,b
SNAP beta	DS, AD	TC	decreased	Yoo et al., 2001a
SNAP-25	DS, AD	FC, PC, TC, OC, CB	decreased	Greber et al., 1999
Stathmin	DS	FC, TC	decreased	Cheon et al., 2001
SYT I (p65), SYT I (pI 7.0)	DS	TH	decreased	Yoo et al., 2001a
TCP-1 epsilon	DS	PC	decreased	Yoo et al., 2001d
Thioredoxin peroxidase-I	DS	fetal brain	decreased	Gulesserian et al., 2001
Thioredoxin peroxidase-II	DS	fetal brain	decreased	Gulesserian et al., 2001
TPM4-ALK	DS	fetal brain	decreased	Engidawork et al., 2003a,b
Ubiquinone oxidoreductase, 24 kDa	DS	OC, TC	decreased	Kim et al., 2001c
Ubiquinone oxidoreductase, 75 kDa	DS	TC, OC, CN	decreased	Kim et al., 2001c
VDAC1	DS	CB	increased	Yoo et al., 2001b
Carbonic anhydrase 1	depr.	FC	increased	Johnston-Wilson et al., 2000
GFAP, 2 isoforms	depr.	FC	decreased	Johnston-Wilson et al., 2000
Ubiquinol cyt *c* reductase complex core protein 1	depr.	FC	decreased	Johnston-Wilson et al., 2000

Table 2 (*continued*)

Protein	Disease	Tissue	Deregulation	Reference
Peiredoxin II	FTD, AD, DS	FC	increased	Krapfenbauer et al., 2003a
Peiredoxin III	FTD, DS	FC	decreased	Krapfenbauer et al., 2003a
Peiredoxin VI	FTD	FC	increased	Krapfenbauer et al., 2003a
UCH-L1	PD, AD	FC	increased	Choi et al., 2004
Diazepam binding inhibitor	S, AD	H	decreased	Edgar et al., 1999a,b
Dihydropyrimidinase related protein-2	S, BD, depr.	FC	decreased	Johnston-Wilson et al., 2000
GFAP, different isoforms	S, BD, depr.	FC	decreased	Johnston-Wilson et al., 2000
Spot containing fructose-biphosphate aldolase and aspartate aminotransferase	S, BD, depr.	FC	increased	Johnston-Wilson et al., 2000

CB: cerebellum; CG: cingulate gyrus; CN: caudate nucleus; EC: entorhinal cortex; FC: frontal cortex; H: hippocampus; IPL: inferior parietal lobule; OC: occipital cortex; PC: parietal cortex; SC: sensorimotor cortex; SMT: superior and middle temporal gyri; TH: thalamus; ox: oxidation; nitr: nitration; carb: carbonylation.

have been identified in 2D-PAGE experiments. The increased expression of small heat shock protein α crystalline B has been proposed to reflect the defensive response to prevent amyloid fibril formation in Alzheimer's disease, although it forms highly neurotoxic complexes with Aβ (Stege et al., 1999). Heat shock protein 70 (HSP70), another protein of which an increase was observed, and which is known to be increased in Alzheimer's disease, inhibits self-assembly of poly-glutamine proteins into amyloid-like fibrils (Muchowski et al., 2000). Moreover, the observed increase of glucose-regulated protein 94 (GRP94) may contribute to abnormalities of intracellular translocation of protein kinases and intracellular signal transduction in Alzheimer's disease brain. On the other hand, a significant reduction in the expression levels of heat shock cognate 71 (HSC71) and glucose-regulated protein 75 (GRP75) was observed in the temporal cortex and of heat shock protein 60 (HSP60) and glucose-regulated protein 75 (GRP75) in the parietal cortex in Alzheimer's disease (Yoo et al., 2001). In contrast to other chaperones, HSC71 is involved in proteasome function and interacts with the cytoplasmic domain of amyloid precursor protein in the presence of proteasome inhibitors (Kouchi et al., 1999). Considering the role of HSP70 in protection against stress-induced apoptosis (Mosser et al., 1997), GRP75 and HSC71 might play a role in neuronal death associated with Alzheimer's disease.

2.2. Proteomic alterations in Down syndrome

Interestingly, like in Alzheimer's disease, decreases in HSC71 and GRP75 have been observed in a proteomic analysis of the temporal cortex of patients with Down syndrome (Yoo et al., 2001b). However, in contrast to Alzheimer's disease, levels of HSP70 RY were down-regulated in Down syndrome. The group of Lubec also reported many other protein alterations in the cortex of patients and fetuses with Down syndrome, as shown in Table 2. Gulesserian et al. (2001) identified the down-regulation of several proteins, which are related to the oxidative stress response in Down syndrome brain. However, superoxide dismutase 1 (SOD1) remained unchanged. Differential protein expression has also been investigated in mitochondria of cortical tissue of SOD2 null mice, a model of endogenous oxidative stress (Hinerfeld et al., 2004). As observed in Down syndrome brains, several glutathione-*S*-transferase proteins were dysregulated in this model. Furthermore, Gulesserian et al. (2001) detected alterations in protein levels suggestive of the malfunction of the citrate cycle, amino acid synthesis and degradation, and even of the urea cycle in Down syndrome fetal cortex. Other dysregulated proteins observed in Down syndrome brains are listed in Table 2.

2.3. Differential proteome patterns in schizophrenia

A down-regulation of diazepam binding inhibitor, a neuropeptide that can regulate the action of the γ-amino butyric acid A ($GABA_A$) receptor (Bormann, 1991), has been detected in hippocampal proteome of schizophrenic brains (Edgar et al., 1999b). Further, as a model for schizophrenia, rats treated with (+)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*] cyclohepten-5,10-imine maleate (MK-801) for either 8 or 18 days have been investigated for differential gene and protein expression in the cerebral cortex (Table 4; Paulson et al., 2003). An interesting observation in this study is that different protein dysregulations were observed after varying lengths of treatment with MK-801; two proteins (HSP72, HSP70) underwent even opposite changes in their respective level after different treatment periods. This study shows that different lengths of drug treatment induce distinct proteomic changes in the brain. This observation may have important consequences for the identification of disease markers (see Section 2.4), as well as for novel drug targets.

2.4. Brain markers for neurodegenerative diseases

Because of their large-scale screening for molecular changes, proteomic approaches should prove particularly

Table 3
Significant differential protein expression in the brain of mouse models of Alzheimer's disease (AD) and oxidative stress

Disease model	Mutation	Tissue	Protein		Deregulation	Reference
AD	GSK3B tg mice	brain	aldose reductase		increased	Tilleman et al.,
			alpha enolase	(a)	increased	
			alpha internexin	(a)	decreased	
			antioxidant protein 2	(a)	increased	
			complexin		decreased	
			coronin like protein P57 fragmetn		increased	
			creatine kinase beta	(a/b)	decreased	
			D-3-phosphoglycerate dehydrogenase		increased	
			dihydropyrimidinase related protein-2	(b)	increased	
			dual specificity MAPK 1		increased	
			fascin		increased	
			glutamine synthetase	(a)	increased	
			glutathione S-transferase		increased	
			hemoglobin alpha chain		increased	
			HSP 90b		increased	
			hypoxhantine guanine-phosphoribosyl transferase		increased	
			isocitrate dehydrogenase		decreased	
			MAPK1		increased	
			mito matrix protein P1 precursor		decreased	
			mitochondrial matrix protein P1 precursor		decreased	
			NADH-ubiquitin dehydrogenase 24 kDa subunit precursor		decreased	
			NADH-ubiquitin oxidoreductase 23 kDa subunit		decreased	
			NADH-ubiquitin oxidoreductase 49 kDa subunit		decreased	
			neurofilament triplet L protein		decreased	
			nucleoside diphosphate kinase A	(b)	increased	
			peanut-like protein		increased	
			phosphatidyl inositol transferase alpha isoform		increased	
			Phosphoglucomutase		increased	
			Ser/thr protein phosphatase 1 cat gamma isoform		increased	
			Ser/thr protein phosphatase 2B cat alpha isoform		increased	
			serum albumin		increased	
			succinate dehydrogenase flavoprotein subunit precursor		increased	
			synaptosomal associated protein		decreased	
			t-complex protein 1 beta		increased	
			t-complex protein 1 e		increased	
			t-complex protein 1 theta		increased	
			Purine rich single stranded DNA binding prt. alpha		increased	
			transformation sensitive protein IEF SSP 3521		increased	
			tubulin alpha 1		decreased	
			tubulin beta 1		decreased	
			vacuolar ATP synthase beta, brain		decreased	
			vesicular fusion protein	(b)	increased	
AD	tau tg mice	brain	14-3-3 zeta protein		decreased	Tilleman et al.,
			alpha enolase	(a)	increased	2002a,b
			aspartate transaminase		decreased	
			ATP synthase alpha	(a)	increased	
			creatine kinase mito 1		increased	
			D-3-phosphoglycerate dehydrogenase		increased	
			dihydrolipoamide acetyltransferase component		increased	
			dihydrolipoamide dehydrogenase		increased	
			dynamin-1		decreased	
			gamma enolase	(b)	decreased	
			GDP dissociation inhibitor-1		decreased	
			glyceral-3-phosphate dehydrogenase		increased	
			GTP-binding alpha subunit		increased	
			guanine nucleotide binding protein beta 1 or 4		increased	
			guanine nucleotide binding protein beta 5		increased	
			hemoglobin beta chain	(a/b)	increased	
			HSP 70-2		increased	
			HSP 86-1		decreased	
			laminin receptor		decreased	

Table 3 (*continued*)

Disease model	Mutation	Tissue	Protein		Deregulation	Reference
			LIM and SH3 protein 1		increased	
			NADH-ubiquinone oxidoreductase 23 kDa subunit		increased	
			NADH-ubiquinone oxidoreductase 24 kDa subunit		increased	
			neurofilament triplet L protein		decreased	
			nucleoside diphosphate kinase		increased	
			oxidoreductase 75 kDa subunit precursor		increased	
			putative unnamed protein, glyozalase domain		increased	
			serum albumin precursor	(b)	increased	
			similarity to hemoglobin alpha family		increased	
			SIR2L2		increased	
			SNAP gamma		increased	
			triose-phosphate isomerase	(a)	increased	
			ubiquitin		increased	
			ubiquitin-conjugated enzyme E2		increased	
			unknown		increased	
ox. stress	SOD2 null mice	CT	2-oxoglutarate dehydrogenase		decreased	Hinerfeld et al., 2004
			3-mercaptopyruvate sulfurtransferase		decreased	
			GST class-mu 1		deregulated	
			peroxiredoxin 5		decreased	
			succinate dehydrogenase flavoprotein subunit precursor (2 spots)		decreased	
			triosephosphate isomerase (TPI)		increased	
			unknown		decreased	

ox.: oxidative; CT: cortex; tg: transgenic; (a): same alteration as in AD; (b): opposite alteration compared to AD.

useful to establish biomarkers of specific diseases (Table 2). Results of a proteomic study by Krapfenbauer et al. (2003a; Table 2) suggest that peroxiredoxin VI (PrxVI) may be used to discriminate frontotemporal dementia (also known as Pick's disease) from Alzheimer's disease and Down syndrome, while PrxIII may serve to discriminate Alzheimer's disease from frontotemporal dementia and Down syndrome. Peroxiredoxins are antioxidant enzymes that reduce hydrogen peroxide and alkyl hydroperoxides (Kang et al., 1998). Another study suggested the potential use of the core 1 protein of the ubiquinone cytochrome *c* reductase complex and carbonic anhydrase 1 as markers to distinguish depression from schizophrenia and bipolar disorders (Johnston-Wilson et al., 2000).

The plethora of existing pharmacological treatments, and different lengths and combinations of drug treatments are however expected to impact on the reliability of disease markers (Paulson et al., 2003). Therefore extensive studies will be required to investigate the influence of such factors on brain protein expression in order to establish reliable biomarkers of neurological disorders and mental illnesses.

3. Proteome maps of cells and organelles

Proteome maps of cells and organelles of different brain regions of various species in both health and disease are being constructed. Despite its limitation in loading capacity compared to 1D-PAGE, 2D-PAGE is often used to separate protein samples for the construction of these maps. 2D-PAGE patterns provide information about experimental molecular weight and isoelectric point of all protein isoforms identified. The reproducibility of the 2D patterns is very high: spot positions on gel patterns vary on average only few mm in *X*- and/or *Y*-axis. A shortcoming of 2D-PAGE for the construction of proteome maps is that only the most abundant proteins can be detected. It is estimated that only one in a thousand proteins is represented using 2D-PAGE, and only proteins within a certain range of isoelectric point and molecular weight are detectable on these gels. In addition, 2D-PAGE does not lend itself easily to automation. Finally, the number of proteins identified depends strongly on protein separation techniques, the amount of protein loaded and the sensitivity of the mass spectrometric technique utilized (Rabilloud, 2002).

2D-PAGE proteome maps of several human brain regions are being established, including the parietal cortex (Langen et al., 1999), the adult (Edgar et al., 1999a) and embryonic (Oguri et al., 2002) hippocampus, the fetal brain (Fountoulakis et al., 2002) and the Alzheimer's disease brain (Tsuji et al., 2002). Whole cell 2D-PAGE maps of the rat brain (Fountoulakis et al., 1999; Shin et al., 2004; Klein et al., 2003; Svensson et al., 2003; Taoka et al., 2000) and the mouse brain (Tsugita et al., 2000; Svensson et al., 2003; Gauss et al., 1999; Soreghan et al., 2003) are being constructed.

The combination of organelle isolation and multidimensional protein separation methods before analyzing these complex protein mixtures by means of mass spectrometry substantially increases the number of proteins that can be identified (Brunet et al., 2003). For example, by using subcellular fractionation and ion-exchange chromatogra-

Table 4
Proteomic alterations in the brain of rat models of Alzheimer's disease and schizophrenia

Disease model	Mutation/treatment	Tissue	Protein		Deregulation	Reference
AD	APP/PS1 tg rat	HC	alpha 1 enolase	(a)	increased	Vercauteren et al., unpublished results
			copine 1, isoform 2		increased	
			glycogen phosphorylase, muscle		decreased	
			unknown		increased	
Schizophrenia	MK-801-treated rats (8 days)	CT	beta-actin		decreased	Paulson et al., 2003
			gamma enolase		decreased	
			H+-transprotein ATP synthasebeta-subunit		decreased	
			HSP72		increased	
			mitochondrial HSP 70		decreased	
			pyruvate dehydrogenase lipoamide		decreased	
			soluble superoxide dismutase		decreased	
			stathmin (2 isoforms)		increased	
Schizophrenia	MK-801-treated rats (18 days)	CT	alpha enolase		decreased	Paulson et al., 2003
			gamma enolase		decreased	
			HSP 60		increased	
			HSP 72		decreased	
			mitochondrial HSP 70		increased	
			stathmin		decreased	

CT: cortex; H: hippocampus; 18 days: animals were treated for 18 days; (a): same alteration as in AD.

phy, Krapfenbauer et al. (2003b) identified in the cytosol, mitochondria and microsomes of the rat forebrain hundreds of proteins that were not identified when the 100,000×*g* supernatant of a total forebrain homogenate was run (Karlsson et al., 1999). It is thus expected that such an approach will be favored in future studies. Organelle proteome maps are also being established of the synaptic (Stevens et al., 2003) and the postsynaptic plasma membrane fraction (Li et al., 2004), myelin (Taylor et al., 2004) and the mitochondria (Mootha et al., 2003).

4. The human proteome organization

The HUPO (http://www.HUPO.org) is a global organization with the ultimate mission of identifying all protein isoforms in cells of organisms such as human and mice, both in health and in disease. HUPO has launched international initiatives to elucidate protein expression profiles in the liver (Human Liver Proteomics Project), plasma (Human Plasma Proteomics Project) and brain (Human Brain Proteomics Project; HBPP). In a first phase of the HBPP, researchers from both academia and industry investigate brain proteomes of mice at different ages and of mouse models of human diseases. These studies will complete mouse databases and prove the applicability of different proteomic approaches for the investigation of brain proteomes. Subsequently, human brain tissues will be studied, focusing on areas most relevant to Alzheimer's disease, Parkinson's disease, Down syndrome and ageing. Current studies involve whole cell proteomics of the brain and cerebrospinal fluid, as well as organelle proteomics. The goal of the fourth major HUPO initiative is the construction of a human antibody library, which will greatly facilitate large-scale screening of protein expression by means of protein arrays.

Moreover, HUPO has a major role in setting standards for obtaining, handling and processing samples, as well as for the representation of proteomic analyses, and for statistical data analysis and interpretation. By formulating standard requirements for data processing and analysis, the HUPO Proteomics Standards Initiative (http://psidev.sourcefourge.net; Orchard et al., 2003) is of fundamental importance for the construction of reliable public proteome databases, in which data verification, comparison and exchange are encouraged and even straightforward. Investigating protein degradation rates in postmortem human brain tissue by comparing biopsy and autopsy tissues is an essential part of the HBPP. Moreover, proteomic studies underline the necessity of setting standards to match tissues for postmortem delay times and handling (Franzen et al., 2003). The Fountoulakis et al. (2001) reported important changes in protein expression when rat brain samples were kept at 23 °C for 0, 6, 12, 24, 48 or 72 h before protein extraction and 2D-PAGE analysis. Twenty-nine proteins with altered expression level were identified, including structural proteins and enzymes, such as SNAP25, dihydropyrimidinase-related protein-2, GFAP and heat shock proteins. Because of the difference in the volume of mouse, rat and human brains, effects on protein expression caused by difference in cooling time of these tissues therefore need to be taken into account when comparing proteomic changes in these species.

The use of animal models for the investigation of human disease obviously has many advantages and disadvantages,

which will not be addressed in this review. Noteworthy, however, is that certain proteomic changes observed in animal models for Alzheimer's disease have also been observed in proteomic studies of Alzheimer's disease brains (Tables 3 and 4), while other proteomic alterations are different or even opposite, as discussed in Section 2.1. Furthermore, one particular proteomic study cautions for false positive results of proteomic analyses of transgenic mice, due to the genetic modifiation itself (Skynner et al., 2002). Moreover, by comparing the 2D-PAGE patterns from *mus musculus* with those of *mus spretus*, more than 1000 genetically variant spots were detected (Klose et al., 2002). Therefore, certain proteomic changes inherent to animal strains should be given special attention before extrapolation to human tissues.

5. Present and future challenges

Despite significant advances in technological development, difficulties are still being encountered in currently available proteomic approaches for brain studies. Firstly, certain characteristics of brain peptides and proteins require special adaptation of proteomic techniques. Many proteins of primary interest in the neuronal network of the central nervous system (CNS) are transmembrane and membrane-associated proteins, including metabotropic and tonotropic receptors, ion channels and G-proteins. Unfortunately, most of these proteins are rather insoluble, are expressed in small quantities and tend to precipitate when they reach their isoelectric point during isoelectric focusing. Despite the availability of solubilizing agents (Taylor and Pfeiffer, 2003) and the addition of salt and urea washes in 2D-PAGE experiments, these factors considerably reduce the applicability of current 2D-PAGE technologies to separate membrane-associated proteins. Non-gel-based techniques such as isotope-coded affinity tagging (ICAT; Gygi et al., 1999) and liquid chromatography-mass spectrometry (Gygi and Aebershold, 2000) may be more suitable for the analysis of these protein populations. Further, the minimum size of proteins analyzable by means of 2D-PAGE is generally 6 kDa, excluding most neuropeptides, which are of great interest in brain function and neuropharmacology.

As stated above, the copy number of many neuropeptides and CNS proteins is very low, while the amount of available tissue is usually limited. As a consequence, the presence of very abundant proteins, such as structural proteins and metabolic enzymes, hampers mass spectrometric analysis of many neuropeptides. This problem might be overcome by utilizing subcellular fractionation (Section 3), narrow pH gradients for isoelectric focusing, as well as peptidomic technologies such as nanoscale capillary liquid chromatography systems (Baggerman et al., 2004). In addition, in contrast to normal tryptic peptides, which lead to y-type ions in mass spectrometric analysis, neuropeptides generate a mixture of ion types, rendering the mass spectrometric identification of these peptides more complex. Finally, labile posttranslational modifications, such as sulfatation and glycosylation, often occur on amino acids of neuropeptides rendering software identification and the localization of posttranslational modifications more difficult (Baggerman et al., 2004).

The second aspect of proteomic analyses in which major challenges are encountered, relates to data storage and processing. Protein expression in the CNS is an extremely dynamic and complex phenomenon. Therefore, the plethora of "omics" techniques, together with other research areas, is generating terabytes of information and the amount of biological data being generated is growing exponentially. Hence, the development of data mining technologies and softwares for proteomic data analyses requires the joint efforts of academic and clinical researchers with specialists in bioinformatics, mathematics and statistics (Buckingham, 2003; Orchard et al., 2003; Quakenbush, 2004).

In conclusion, the results of proteomic investigations described above illustrate the suitability and advantages of proteomic approaches to investigate brain diseases. Often, results from these studies confirm observations made previously utilizing more traditional approaches. On the other hand, the large-scale screening capacity of 2D-PAGE has allowed the observation of differential expression of many proteins that previously had not been linked to a given disease. These data may lead to new molecular mechanisms and treatment approaches of these disorders. Further development and optimization of high-throughput experimental and computational approaches, complemented with traditional experiments to investigate the biological meaning of proteomic data should allow for a better understanding of the etiology of brain disorders. This integrative knowledge will ultimately enable the development of more specific diagnostic markers and neuropharmacological agents to treat these diseases.

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