Review

The use of proteomics in meat science

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Abstract

Characterising the function of genes is a major challenge in the post-genomic era. Post-genomic tools and technologies have dramatically changed the experimental approaches by which complex biological systems can be characterised.

Proteomics is an important cornerstone in functional genome characterisation, and like all other functional genomics tools, including transcriptomics and metabolomics, the aim of proteome studies is to translate genome information into useful biological insight, that will allow scientists to build and test better hypotheses, with the ultimate goal to find better solutions to challenges in food production, medicine and environmental management.

In agricultural sciences as well as in all other life sciences, the implementation of proteomics and the other post-genomic tools is an important step towards achieving better product quality and a more sustainable animal production.

The aim of this review is to introduce the developing field of proteomics, and to discuss the use of proteomics in meat science projects. The most frequently used technologies for characterising cellular protein expression patterns will be introduced, and some early examples of applying proteomics to meat quality research will be discussed.
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Contents

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1. Introduction

All biological traits and mechanisms are controlled by and associated with complex regulation of genes and proteins. Meat quality traits are closely related to the biological traits of the live animal, hence biological sciences, including genetics, physiology, cell biology and biochemistry has been widely employed for decades to characterise the biological mechanisms behind major variability of meat quality traits, like tenderness, and water holding capacity of meat, but also the physiology of meat animals, with special attention on muscle growth, development, and carcass composition has been well characterised. It has become increasingly clear that meat quality traits are complex, and multigenic in nature, hence detailed characterisation would benefit from experimental approaches and technologies aimed at parallel analyses of numerous genes and proteins simultaneously. These tools and approaches have only recently become available, and are frequently referred to as post-genomic and functional genomic tools. Post-genomic tools and technologies have dramatically changed the experimental approaches and above all the speed by which we currently characterise molecular mechanisms. The post-genome era brings hope that we can move beyond the more reductionistic approaches of classic biochemistry and genetics. In agricultural sciences as well as in all other life sciences, the implementation of post-genomic tools is an important step towards improving product quality and developing more sustainable animal production methods.

Proteomics is an important cornerstone in post-genome sciences, and the aim of this review is to introduce the developing field of proteomics, and to discuss its use in meat science projects. The most frequently used technologies for characterising cellular protein expression patterns will be introduced, and examples of proteome research related to animal production and meat quality will be discussed.

1.1. Defining proteomics

According to the classic definition, a proteome is the protein complement of a genome (Wilkins et al., 1996), hence proteomics refers to the scientific area of characterising the entire complements of proteins that are expressed in a cell or tissue type. The aim of proteomics is to obtain information about cellular protein expression, and thereby also reveal the function of genes, and ulti-

mately to explain how heredity and environment interact to control cellular functions, and form the physiological traits of living organisms. Mapping proteomics and global proteomics are terms frequently used to reflect the systematic efforts aimed at cataloguing entire protein complements of cells, tissues and organisms. The first reports of complete proteome maps, as recently reported for yeast (Ghaemmaghami et al., 2003) illustrates well the complexity of this task, and serves as a forecast to the complexity of mammalian tissues. The terms comparative proteomics and expression proteomics refer to comparative studies that are based at parallel quantitative protein analyses of expression patterns, with the aim that relative quantitation of protein species in complex tissue samples will provide comparative "snapshots" of given cellular protein states. The aim of expression proteomics is often to find molecular markers, commonly termed biomarkers, that for instance in human medicine would allow a more accurate diagnosis of a disease state. Searching for biomarkers has currently gained much attention in all biological sciences, as biomarkers can be used to improve a wide range of applications, including the methods which are used in production and processing of meat (Pan et al., 2005).

As technologies and applications of proteomics has become more widely used, the term itself is currently expanding from its classic status as "biochemistry at an unprecedented high throughput scale" to a much broader definition of "panoramic protein characterisation", which includes global characterisation of functionality, modification states and isoform patterns of proteins. For a general review see Tyers and Mann (2003).

2. Proteome technologies – a state of the art

Over 30 years ago, the early 2DE (two-dimensional electrophoresis) technologies (O'Farrell, 1975) were aimed at characterising cellular protein expression patterns. However, the development of currently used proteome technologies was greatly facilitated by the information that came from the human genome sequencing project in the late 1990s. Since then, the genomes of other species followed quickly, and presently the genomes of more than 250 organisms have been completed, and will also soon include the genomes of cattle, pig and chicken (Kyrpides, 1999).

Not only did the genome sequencing era make clear that DNA sequence information is an essential step to-

wards understanding the function of genes, but the availability of genome information also allowed interpretation of complex mass spectra of proteins and peptides, and set the stage for developing mass spectrometry (MS) based methods for characterising proteins and peptides at unprecedented high speed and low cost. Further advances of MS-based methods currently dominate the technological frontiers of proteomics, and will be discussed in more detail later in this chapter.

Mammalian tissue samples typically contain between 10,000 and 30,000 different protein species, hence a wide range of technologies must be interfaced in order to prepare, separate and quantify the relative expression levels of thousands of proteins in parallel. All these technologies have their individual advantages and limitations, and none of them are equally well suited for the analysis of all protein species present in a complex tissue sample, hence a major challenge of any proteome project is to apply and combine the optimally suited techniques and instrumentations in order to answer the questions of interest. The rest of this chapter is aimed at introducing some of the most frequently used technologies in current proteomics, and the interplay of proteome technologies has been summarised in Fig. 1.

2.1. 2 DE-based comparative proteomics

2DE (two-dimensional electrophoresis) is an electrophoretic method that allows the parallel separation and analysis of 500–2000 individual protein species extracted from complex samples such as biopsies, tissues and cell cultures. In a 2DE analysis, spot patterns are formed, where every single spot represents an individual protein species that migrates to its specific coordinates, due to its specific molecular weight and charge. The intensity of an individual spot indicates how much the cell has produced of that actual protein, Fig. 2 shows a typical 2DE pattern of drip loss from porcine muscle. 2DE maps of many species and tissues have been published within the past 2 decades (SIB, 2005), including maps of porcine (Lametsch, Roepstorff, & Bendixen, 2002; Morzel et al., 2004), cattle (Bouley, Chambon, & Picard, 2004), and chicken (Doherty et al., 2004) muscles. Some of these maps have been used to find molecular markers for meat quality traits.

From the earliest 2DE analyses (O'Farrell, 1975), this technology has been developed into reproducible and stable IPG-based systems with excellent resolving power (Gorg & Weiss, 1999; Gorg et al., 2000). Methods for visualisation of the protein patterns include using coomassie (Rabilloud, 2000), silver (Rabilloud, 1999), and fluorescence methods (Rabilloud, Strub, Luche, Van Dorsselaer, & Lunardi, 2001). In particular the DIGE system, based on differential fluorescence-tagging of comparable samples, prior to their multiplexed separation and analysis, has improved the quantitative properties of 2DE based comparative proteomics (Unlu, Morgan, & Minden, 1997). 2DE based proteomics is a multi-step procedure unfit for automation, but in spite of this, 2DE technology has remained useful in compar-
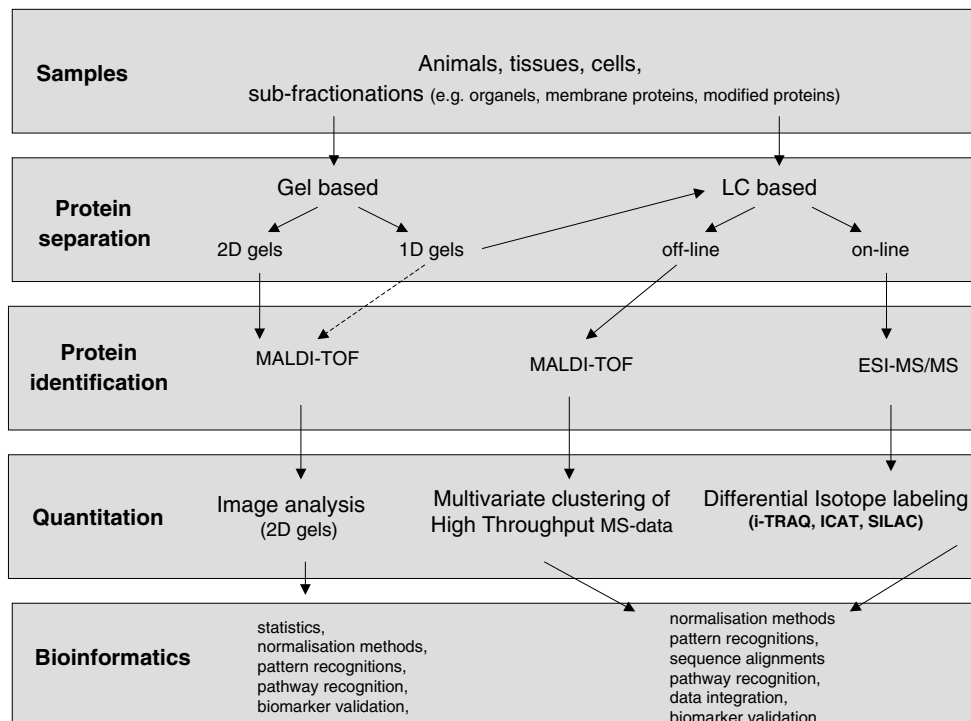

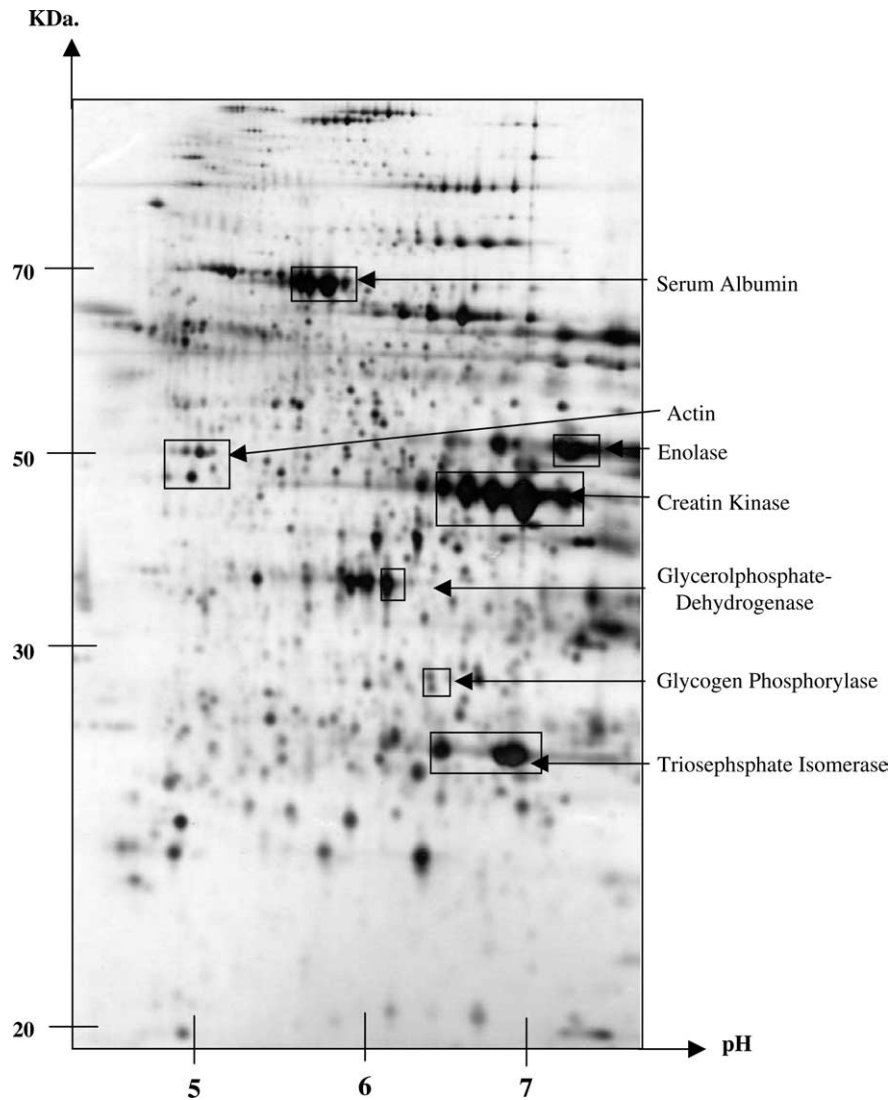Fig. 1. Diagram of methods and workXows in comparative proteomics.

Fig. 2. Two dimensional electrophoresis (2DE) of drip loss from porcine *Longissimus dorsi* muscle. 2DE analysis was performed using the IPG gradient of 3-9, and SDS–PAGE of 11%T.

ative proteomics (Rabilloud, 2002). This is partly due to the fact that 2DE is an excellent preparative medium for further protein characterisation by MS-based methods (Scheler et al., 1998), but also because better alternatives for visual protein-display methods have not yet been presented.

A serious shortcoming of 2DE is that analysis is constrained to only a limited subset of the cellular protein complement (Pedersen et al., 2003). This is partly due to chemical properties of IPG-based 2DE systems that discriminate mainly against basic (Gorg, 1999), and hydrophobic proteins (Rabilloud, 1998; Santoni, Molloy, & Rabilloud, 2000) and therefore excludes analysis of most receptors and transmembrane proteins (Tyers & Mann, 2003). Characterisation of the Yeast proteome indicates that 50% of the proteins have a pI above 8, while 30% of the predicted ORFs encode a transmembrane protein (Pedersen et al., 2003). Current advances

have markedly improved the 2DE based analysis of basic proteins, and recent reports of analyses over the entire range of pH 7–11 have been reported (Corton et al., 2004).

However, the most severe limitation of 2DE based analyses is due to the limited dynamic range, which only spans 2–3 orders of magnitude (pico–nanomolar range) while the ranges of cellular protein expression in most tissues spans more than 8 order of magnitude (micro–femtomolar range). Hence 2DE analysis of unfractionated tissue samples is constrained to the analysis of the most abundant proteins (Pedersen et al., 2003). A wide range of methods for prefractionation of complex samples has been developed to bypass this problem (Gorg et al., 2002; Spandidos & Rabbitts, 2002) but the currently rapid development of MS-based methods for comparative proteomics will to some extent replace some of the classic 2DE based applications.

2.2. The use of mass spectrometry in proteomics

Within the past 20 years, MS has been developed from methods that allow analyses of small volatile molecules, to a wide range of applications, which includes characterisation of proteins and peptides. For a recent review see Yates (2004).

In proteomics, two main applications of MS have emerged. Firstly MS-based methods are used to identify proteins and peptides from both 2DE analyses, and from more crude tissue extracts, but more recently MS-based methods have been developed that allow comparative protein profile analyses of complex tissue proteomes, and similar to the 2DE based proteomics aims at characterising the relative expression patterns of thousands of molecular markers within comparable tissue samples. The fundamentals of these applications will be discussed in a little more detail in the following.

The discoveries of soft ionisation techniques, most importantly electrospray ionisation (ESI) (Fenn, Mann, Meng, Wong, & Whitehouse, 1989; Whitehouse, Dreyer, Yamashita, & Fenn, 1985) and matrix-assisted laser desorption ionisation (MALDI; Karas, 1996) were major steps towards applying MS for protein identification. These ionisation methods are the core technologies of MALDI-TOF and ESI-MS/MS, based instruments, and their combined use has become a classic approach in proteomics (Bodnar, Blackburn, Krise, & Moseley, 2003; Yates, 2004). In MALDI technology, laser energy is used to convert matrix-embedded peptides into gas-phase ions (Bahr, Stahl-Zeng, Gleitsmann, & Karas, 1997; Karas, 1996). When MALDI is combined with a time-of-flight (TOF)-based mass analyser, MALDI-TOF instruments become robust, low in cost, have low-sample consumption and high-speed, and are ideally suited for protein identification using peptide mass fingerprinting (PMF), which is currently the most widely used method for analysis of 2DE separated proteins and peptides. Briefly, PMF-based identification is an analysis of proteolytically digested proteins. Trypsin is frequently used to digest separated proteins or crude protein mixtures, as tryptic peptides have optimal size and charge for MALDI-based analysis. The experimental masses obtained by MALDI-TOF analyses can be compared with theoretical peptide masses of in silico-digested protein sequence databases (Magnin, Masselot, Menzel, & Colinge, 2004). The quality of PMF data depend mainly on the accuracy of the MS data, and of the availability of comprehensive sequence databases, hence PMF-based analysis is greatly facilitated in organisms for which the genomes has been fully sequenced. However, due to extensive sequence homology between species, PMF analyses have also been successfully applied to identify proteins from organisms with less well characterised genomes, which at the moment include all domestic animals. Proteins from pig (Morzel et al.,

2004), cattle (Bouley et al., 2004), and chicken (Doherty et al., 2004; Stagsted, Bendixen, & Andersen, 2004) have been successfully characterised in spite of lacking fully sequenced genomes. For porcine proteins, typically 90% of 2DE-separated proteins could be identified with typical sequence coverage of 20% (Lametsch et al., 2002). For cattle, 70% of the 2DE proteins could be identified by PMF analysis, with typical sequence coverage around 30%. Forty percent of the identified proteins were aligned to bovine sequences. Not yielding true sequence information has been a major disadvantage of MALDI-TOF analyses, but this can now be overcome by addition of tandem TOF/TOF units separated by a collision cell, so true sequence data from MALDI-instruments can routinely be achieved through fragmentation, and repeated TOF analysis of the fragment ions (Suckau et al., 2003).

Unlike MALDI-based ionisation, the complimentary ionisation technique of ESI is easily interfaced with a wide range of single or tandem mass analysers. ESI-MS/MS analysis typically yields sequence information of small 3–10 amino acid peptides from each protein, and these sequence tags allow protein identification through PFF (peptide fragment fingerprint) based matching of experimental data and database information. ESI-MS/MS based instruments are also easily interfaced with a wide range of LC (liquid chromatography) methods, and are therefore a key technology in MS-based comparative proteomics, as will be discussed in the following.

2.3. MS-based comparative proteomics

Considering the shortcomings of 2DE technology, there is a need to develop alternatives to 2DE-based comparative methods, and in particular there is a great interest in extracting quantitative proteome information from MS data. However, MS of proteins and peptides is a qualitative rather than quantitative method, mainly due to unpredictable ionisation capabilities of individual peptides (Lim et al., 2003). Hence, a wide range of approaches for bypassing the non-quantitative nature of MS analyses exists. Some of the most promising within this rapidly expanding area will be discussed in the following section. For more detailed reviews, see Aebersold and Mann (2003) and Ong et al. (2003a).

One of the first methods that allowed relative quantification of mass spectra were based on Isotope Coded Affinity Tag labelling (ICAT), that allows differential chemical tagging of proteins from different samples. By using heavy versus light isotope coded tags in two comparable samples, the relative abundances of individual proteins can be analysed as peak-doublets in the same MS spectra, hence analytic noise can be reduced, and increasing the quantitative nature of compared MS data (Gygi, Rist, Griffin, Eng, & Aebersold, 2002).

Similarly, stable isotope labeling by amino acids in cell culture (SILAC) based methods allow differential metabolic labelling followed by MS analysis of comparable cell culture samples (Ong, Kratchmarova, & Mann, 2003b). The most recent advance in differential mass tagging is the iTRAQ based chemistry that allows a 4-fold multiplex peptide tagging, where samples can be combined after tagging, and the relative quantitative protein expression profiles of 4 comparable proteomes can be processed in parallel (Ross et al., 2004). This approach also allows absolute quantitation of proteins and peptides, through the use of internal peptide standards.

Comparing proteome patterns by MS analyses requires that complexity of proteomes must be reduced prior to MS analysis. A well-established method is the use of multidimensional LC fractionation steps, frequently referred to as multi dimensional protein identification technique (MudPIT) (Link, 2002). A classic approach is a serial combination of micro- and nanocapillary columns containing strong cation exchange (SCX) as well as reversed phase resins, but a wide range of separation methods can be used to reduce complexity, by selective enrichment of specific subsets of peptides, including cysteine containing peptides (Olsen et al., 2004) and phosphopeptides (Peters, Brock, & Ficarro, 2004), while serial lectin chromatography allows selective enrichment of glycosylated peptides (Graumann et al., 2004).

The isotope based MS quantitation has mainly been developed for the use of MS/MS (tandem MS) instruments, like quadrupole-TOF instruments, which allow high resolution (2–5 ppm), but rather low throughput proteome analyses. Alternatively, high throughput proteomics approaches for biomarker searching are based on the use of MALDI-TOF analysis followed by multivariate analysis and clustering of thousands of spectral datasets. This approach yields less accurate mass analyses (10–50 ppm), but a much higher throughput, hence a large number of samples can be compared to allow a statistics-based semiquantitative MS analysis, and much broader biomarker searches can be attempted by a high throughput approach than the use of MS/MS analyses normally allow. Surface enhanced laser desorption ionisation (SELDI) technology (Tang, Tornatore, & Weinberger, 2004) is one examples of a high throughput based analytic approach, aimed to reduce the complexity of proteomes by interfacing specially coated chromatography chips that allow selective enrichment and subsequent MALDI-TOF analysis of subproteomes to be performed. A wide range of similar approaches, including LC-MALDI-based methods (Bodnar et al., 2003) has been published. For a more thorough introduction to high throughput based biomarker analyses, see the reference by (Pan et al., 2005). New hybrid instruments and MS-based platforms rapidly appear. Specially, hybrid linear ion trap systems (Le Blanc

et al., 2003), and Q-FTCR (Belov et al., 2004) instruments are emerging technologies that may soon find widespread use due to increased speed and sensitivity, and will allow more sophisticated methods for MS-based quantitative proteomics to evolve.

2.4. The use of bioinformatics in comparative proteomics

Storing and making sense of enormous amounts of data is a major challenge in all post-genomic sciences. As the throughput and capacity of analytical technologies rapidly increase, data overload is easily created. Computational biology, commonly termed bioinformatics, has become increasingly important, and high speed computers, computational techniques and increasingly sophisticated algorithms (for analysing, storing, sorting, searching and integrating data) is currently being developed with amazing speed. For a recent review see (Blueggel, Chamrad, & Meyer, 2004).

Moreover, the complexity of statistical analysis of comparative proteome data also requires innovation of statistical tools.

Bioinformatics and statistical analyses of expression data are scientific areas in their own rights, and covering the recent development of post-genomic data handling is beyond the scope of this review. However, a few issues of highest importance for comparative proteomics will be briefly introduced in order to highlight the necessity and complexity of this field.

Digital image analysis provides increasingly sophisticated software for analysing 2DE images has greatly improved the quantification of comparative 2DE analysis. A wide range of software is commercially available and information from 2DE databases is accessible at public Web sites.

Thousands of MS spectra are generated during a comparative proteome study, and extracting information from MS data includes multiple analytical steps, like noise extraction, mass calibration, and deconvolution of complex peak patterns, for which improved algorithms and software is continuously being created. For a recent review see Chamrad et al. (2003). In addition, both PMF and PFF-based protein identification require alignment of the experimental MS data to in silico-digested sequence databases, for which a wide range of publicly available tools exist. Although probability based scoring methods are continuously improved in order to facilitate automatic data interpretation, the very time consuming step of manually reviewing scoring data is still a critical step in avoiding false identifications.

Statistical analyses of comparative proteome data involve a large number of variables (typically the expression level of hundreds or thousands of proteins), which greatly outnumbers the repeated observations (typically 10–20 animal samples). This implies that data normalisation and statistical approaches must be

improved for accurate detection of differential protein expression. In addition, automatic processing and software development is needed for handling large data sets. In this respect, statistical analyses of proteome data have very much in common with data analysis of microarray based mRNA expression. In order to extract biological knowledge from microarray data, a wide variety of pattern recognition methods has been developed (Valafar, 2002), of which hierarchical clustering (Eisen, Spellman, Brown, & Botstein, 1998), self organising maps (Tamayo et al., 1999), and K-means clustering (Tavazoie, Hughes, Campbell, Cho, & Church, 1999), are the most widely used. Some of these statistical tools are also available through commercially available 2DE-softwares, but their use is not yet widely applied in proteomics.

2.5. Integrating omics-data

Understanding the vast amounts of complex data that emerge from comparative proteomics often requires integration of many additional sources of data, including phenotypic descriptions, as well as structural and functional genome data. Systems biology is a term that is frequently used to describe the science of integrating data from biological sciences at many different levels, and across a wide range of disciplines, in order to understand the interrelations and pathways that create biological systems and organisms. Data integration and data mining is a major challenge in all post-genomic sciences, and the development of increasingly sophisticated algorithms and computational methods that aid the discovery process is rapidly developing (Hood, 2003).

Improved database technologies are a major bioinformatic resource, and a number of open source as well as commercially available database platforms for storing and analysing proteome data exist (Taylor et al., 2003a; Taylor et al., 2003b). Data mining, and integration of different data types, like linking proteins and genes into assemblies and interlinked pathways, is greatly facilitated by open-source websites. For a review see Blueggel et al. (2004).

Currently, these databases cover studies of human as well as classic model organisms like mouse, rat, and yeast, and although not much information is to be found about cattle and pig proteomics, these repositories offer knowledge that can be applied to proteomics in animal production sciences as well.

3. Applying proteomics in meat quality research

Biomarkers for muscle growth and meat quality traits will allow scientists to build and test better hypotheses, and meat producing industry and consumers will benefit from better indicators for meat quality. Presently, the application of proteomics to meat sciences is in an early stage, and only a limited amount of studies have been published so far. However, proteome studies of metabolism, growth and development of muscle tissues from man, and of classic model organisms like mouse, may provide valuable information when implementing proteome technologies to muscle- and meat studies of livestock species.

A major challenge for improving meat quality is to understand the variability of tenderness. Although many of the biochemical factors are well known (Koohmaraie, 1996; Maltin, Balcerzak, Tilley, & Delday, 2003) and a number of QTLs for tenderness have been determined (Burrow, Moore, Johnston, Barendse, & Bindon, 2001; Rothschild, 2004) the complex mechanisms of postmortem processes, including proteolysis and interactions of soluble muscle proteins, intracellular pH and ion transport during the very early postmortem phase, and their effect on meat texture remains a major challenge. Also, understanding the molecular mechanisms that influence water-holding capacity of pork and the interrelationships between muscle growth, development, and meat quality traits, would greatly benefit production methods and processing technologies. Early examples of how comparative proteomics have been applied to approach some of these problems will be discussed in the following.

3.1. Growth and development

Skeletal muscle tissues are heterogeneous populations of muscle fibers, generally classified according to their metabolic traits as well as their contractile properties, and in all species, muscle fiber properties are modified during post-natal life, relative to age, sex, and breed as reviewed by (Picard, Lefaucheur, Berri, & Duclos, 2002).

The relationships of fiber types and meat quality traits like juiciness, flavor and tenderness has been much disputed, and especially the influence of fiber types on meat tenderness remains unclear. For a review, see Maltin et al. (2003). Animal models with well characterised growth phenotypes and unique tenderness traits, including double muscling in cattle (Fiems, Hoof, Uytterhaegen, Boucque, & Demeyer, 1995) and the callipyge trait in sheep (Taylor & Koohmaraie, 1998) have already contributed greatly to our understanding of how muscle growth and meat quality traits are interrelated, and are obvious choices for models to study by proteome approaches, in order to learn more about the molecular mechanisms that relate growth- and meat quality traits.

Recently, the first proteomic study of a bovine hypertrophy was reported (Bouley et al., 2005). This study describes molecular markers associated with an 11-base-pair deletion in the myostatin gene. This mutation

results in expression of normal levels of inactive myostatin protein. Proteome patterns from semitendinosus muscles from young Belgian Blue bulls were compared, and the study revealed thirteen differentially expressed proteins, including contractile- and metabolic proteins. Most interestingly, the isoform patterns of troponin-T seem to be affected by the myostatin mutation. Based on their observations, the authors suggested that myostatin mainly controls proliferation of fast-twitch glycolytic muscle fibers, supporting previous studies of bovine muscle hypertrophy, which associated muscle hypertrophy with increased proliferation of secondary myoblasts in the fetuses, and relatively increased ratios of glycolytic muscle fibers in the adult animals (Deveaux, Picard, Bouley, & Cassar-Malek, 2003).

As muscle hypertrophy also seems to influence perimysium organisation (Boccard, 1981), calpain/calpastatin activities (Uytterhaegen, Claeys, & Demeyer, 1994), and fat metabolism (McPherron & Lee, 2002), further proteome studies specially aimed at enlightening these mechanisms may be informative about how muscle growth and meat quality traits are interrelated.

The Callipyge mutation in sheep causes muscle hypertrophy due to a mutation in chromosome 18 (Freking et al., 2002) that results in a two to threefold increase in calpastatin (Koohmaraie, Shackelford, Wheeler, Lonergan, & Doumit, 1995). The postmortem structural changes in muscle from callipyge sheep seem to be similar to that of wild type animals, but occur at a slower rate, emphasizing the important role of the calpain/calpastatin system in tenderisation of meat. Proteome studies of callipyge sheep have not been reported so far, but this will certainly yield new information about some of the mechanisms that link muscle growth, postmortem metabolism and tenderness in meat.

Proteome studies of muscle growth in chicken have also been reported (Doherty et al., 2004). Due to intensive breeding and differential selection for meat production (broilers) or egg production (layers), chicken are potentially interesting model organisms for analyzing molecular mechanisms behind muscle growth. Although the presented work do not primarily relate their studies of muscle growth to meat quality traits, the proteome observations may become useful in understanding regulation of skeletal muscle growth, and could in future studies be involved in relevant meat quality models.

3.2. Postmortem metabolism

Understanding the relations of postmortem metabolism and meat quality, is of major interest in meat sciences, as it is well established that the biochemical events that take place during the early postmortem period greatly affect meat quality. Changes related to physio-chemical factors (Goll, Henderson, & Kline, 1964), histo-chemical properties (Brocks, Hulsegge, & Merkus, 1998), temperature (Monin, Lambooy, & Klont, 1995), genotypes, and many other factors, influence postmortem metabolism, but the relationship to meat quality has remained poorly understood. In general, factors which alters the onset of rigor may have a significant effect on tenderness, including electrical stimulation (Hildrum, Solvang, Nilsen, Frøystein, & Berg, 1999), stress (Scanga, Belk, Tatum, Grandin, & Smith, 1998), chilling rate, and nutritional status of the living animal, hence establishing animal models for characterising proteome changes during postmortem storage is a challenging task. A series of papers (Lametsch & Bendixen, 2001; Lametsch et al., 2003; Lametsch et al., 2002; Morzel et al., 2004) have reported proteome changes of postmortem processes in pork. Postmortem markers detected during the first 48 h of post-slaughter storage, included structural proteins (actin, myosin and troponin T) as well as metabolic enzymes, like myokinase, pyruvate kinase and glycogen phosphorylase (Lametsch et al., 2002), and accumulation of specific metabolic enzymes, as well as specific actin and myosin fragments were observed to correlate to meat tenderness (Lametsch et al., 2003). Although postmortem tenderness development is classically thought of as a process mainly related to degradation of myofibrillar components, the roles of metabolic enzymes as tenderness markers has not gained much attention so far, but the existing proteome data indicate that metabolic enzymes may be excellent biomarkers for meat tenderness. However, these studies must be regarded as a starting point, and further studies are needed to understand some of the complex mechanisms that rule postmortem metabolism of muscles and meat.

3.3. Calpains role in tenderness

It is well established that calpains play a key role in tenderisation of meat (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995), and that the rate-limiting factor is actually calpastatin-mediated inhibition of postmortem calpain activity (Koohmaraie et al., 1995). Callipyge sheep have very tough meat and also a low postmortem calpain activity but are normal in other postmortem changes. A very important recent finding is that transgenic mice that over-express calpastatin have very limited postmortem calpain activity (Kent, Spencer, & Koohmaraie, 2004). A recent proteome study has reported the specific calpain-mediated degradation patterns of myofibril proteins (Lametsch, Roepstorff, Møller, & Bendixen, 2004). This study was carried out in vitro, by co-incubating myofibril subfractions with calpain. Specific degradation patterns of actin, desmin, troponin, and several tropomyosin isoforms could be detected. Correlation of these peptide patterns to meat tenderness remains to be further analysed.

3.4. Water holding capacity

Water holding capacity is an important meat quality trait, closely related to variations in the postmortem metabolism, but little progress has been made within the past decades. Several single-gene models of porcine drip loss phenotypes exist, including variant of the ryanodine receptor (Ervasti, Strand, Hanson, Mickelson, & Louis, 1991), and variants of the PRKAG3 gene, commonly termed the RN gene (Le Roy et al., 2000). Recently, a comparative proteome study of the RN-gene effect has shown that the expression profiles of several enzymes of the glycogen storage pathways are differentially regulated in a pattern, and the integrated data from this proteome study indicates that regulation of glucose transport is severely affected in mutant animals (Hedegaard et al., 2004). Further studies of these mutations are of great interest in order to explain molecular mechanisms that influence drip loss in porcine meat. A microarray-based analysis of the same animal material is in progress.

4. Future applications of proteomics in meat quality

4.1. Markers for technological processing

Understanding the protein changes that are induced during technological processing, and why the outcome of processing is so variable, will greatly aid the improvement of processing technologies. Numerous studies have shown that variation in postmortem glycolytic rates in muscles from different carcasses yields aged meat of varied tenderness (O'Halloran, Troy, & Buckley, 1997). As slowly glycolysing muscles yield tough meat, electrical stimulation (ES) is widely used to accelerate postmortem glycolysis; however, the mechanisms that are unleashed by ES stimulation are not well understood. As a consequence, this process is rather erratic and deleterious to fast glycolysing muscles (Hwang, Devine, & Hopkins, 2003), due to lack of knowledge on how to optimize the technologies. Moreover, a wide range of meat processing techniques like fermentation, marination, drying, heating, freezing and high-pressure treatment influence the quality of processed meat by modifying the proteins. As with electrical stimulation, the principles and mechanisms behind these processes are often insufficiently understood, hence proteome studies will be of great value in optimisation meat processing technologies.

4.2. Proteomics in food safety and authenticity

As proteome technologies are optimised to characterise complex protein mixtures, these technologies will certainly be valuable also in characterising the protein components of complex foods, which traditionally has been difficult to control. For instance proteome studies of ground beef could tell which tissues and species have been mixed in a product, and at which ratios. This may be useful in controlling niche products, e.g. related to religious regulations. As an example, for kosher-food this would imply not mixing milk and meat, and not mixing pork into food products.

Proteomics will also be useful for developing analytic assays for authenticity of food products, like the verification of an animals origin, or related to whether regulations and food policies has been kept, e.g., related to regulations on animal transport. Pineiro, Barros-Velazquez, Vazquez, Figueras, and Gallardo (2003) and Martinez and Jakobsen (2004) give examples of how proteomics can be used in food science.

4.3. Some consideration on applying proteome based approaches to meat sciences

Meat quality is determined by complex interactions of biological and environmental factors, but is often classified according to the appearance of the raw material, like texture, colour and taste. This is useful for production and consumers, but may be insufficiently precise for classifying comparative proteome groups, and may result in too complex variations within a compared dataset. One way to reduce this problem is to use experimental designs where genetically well defined groups of animals are analysed, and preferentially, the influence of single genes could be characterised in detail. So far, only a limited list of single gene effects on meat quality has yet been described, but these animal models are valuable for finding new molecular markers for meat quality traits through proteome and expression analyses, as exemplified by the proteome study of the RN genotype (Hedegaard et al., 2004). Currently, a wealth of genome information, including Atlas related to meat quality traits is being established (Rothschild, 2004). This information will be valuable for hunting biomarkers that can link phenotypes to genotype, and help to spot the biological mechanisms and pathways that are important for production and quality traits of domestic animals.

When established, biomarkers for growth-, development-, and meat quality traits may be used in selective breeding, while other markers may be further developed into technological markers that can facilitate more precise labelling and sorting of meat and for optimising production methods and meat processing technologies.

5. Conclusions

Post-genome technologies will allow us to work beyond the reductionistic models of classic biology. Genes and proteins do not function independently, but partic-

ipate in complex networks that give rise to cellular functions, tissues, organs and organisms. The challenge of defining biological systems is far beyond the capabilities of any single functional genomic tool like proteomics; hence data from many levels of biology and many technologies, including those of post-genomic tools must be integrated, in order to understand complex biological systems, and their influence on animal production and product quality. This truly requires interdisciplinary collaborations between a broad range of sciences, including those of physiology, genetics, cell biology, computer sciences, as well as from the animal production and from food industry.

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