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Understanding meat quality through the application of genomic and proteomic approaches

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Abstract

During the past few decades, advances in molecular genetics have led to the identification of multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including single genes of large effect and QTL (genomic regions that affect quantitative traits). Transcriptomics enables analysis of the complete set of RNA transcripts produced by the genome at a given time and provides a dynamic link between the genome, the proteome and the cellular phenotype. Through a functional genomics approach to understanding the molecular basis of meat quality, we can gain further insight into the complex interplay of gene expression events involved in the development of meat quality. Proteomics permits visualisation of the protein content of the cell under varying conditions, combining powerful separation techniques with highly sensitive analytical mass spectrometry. To date, both the human and bovine genome projects have advanced our understanding of gene expression and helped elucidate the function of large portions of the genome. Advantages from this research have permeated through to a broader spectrum of research including that of meat science. Meat quality is manifested through a complexity of events in the muscle and their interactions with many environmental stimuli in both the live animal and during the post-mortem period. A lot of progress has been made in our understanding of the biological processes that contribute to the delivery of consistent quality meat. Through the application of tools of genomics and proteomics we are gaining a deeper insight into these processes and their interaction with environmental factors. Knowledge gained from these approaches can be beneficial in defining and optimising management systems for quality, providing assurance of meat quality and in tailoring quality to suit market needs.

Keywords: Transcriptome; Meat management system; 2-D Electrophoresis; QTL; Marker assisted selection; Microarray

1. Introduction

According to McIlveen and Buchanan (2001) flavour, tenderness and juiciness appear to be the three most important determinants of sensory enjoyment for the UK consumer. Of the three attributes mentioned Ouali (1990), Warkup, Marie, and Harrington (1995), Szczesniak (1998), Koohmaraie (1998) among others all concluded that beef tenderness was the primary determinant of satisfaction among beef consumers. In Norway, a recent study found that beef consumers were willing to pay 50% more for very tender beef and 25% more for tender beef compared with less tender beef (Alfnes, Rickerten, & Ueland, 2005). Therefore, providing consistently tender beef should be key priority for the beef industry. While there have been many successful efforts at improving the tenderness of beef research has shown that an unacceptable level of variability still remains in beef tenderness (Maher, Mullen, Moloney, Buckley, & Kerry, 2004).

Many factors affect the quality of meat, including the way animals are fed, managed, slaughtered and both carcass handling and processing post-slaughter. While there is often emphasis on the management systems that can be implemented to meet market specifications there has, until

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recent years, been little emphasis on factoring in the molecular or biological components of meat quality. We are now in an exciting period where many new opportunities are presented to researchers through the application of genomics, proteomics and other 'omic' approaches. Extracting useful information from the large amounts of data stemming from this research is a major challenge being addressed by the field of bioinformatics. Here we review these approaches and provide a synopsis of the current status of genomic and proteomic meat science research.

2. DNA polymorphism analysis and meat quality

In a competitive commercial environment, the benefits to be gained from selection for slight improvements in production and/or a more consistent quality within the population are significant enough to have resulted in an expansion of research in this area. If desirable quantitative trait loci (QTL) alleles for traits of economic importance can be identified which have significant physiological associations with meat quality, these may be combined with estimated breeding values (EBV's) and incorporated into best linear unbiased prediction (BLUP) models in a process known as marker-assisted selection (MAS) (Kuhn et al., 2005). MAS has particular advantages for traits that challenge traditional selection, including difficult to measure traits, those that are measured for only one sex and traits that can only be assessed after the end of an individual's reproductive period, such as lifetime fecundity or those that must be measured post-mortem, such as many meat quality traits (Dekkers, 2004). The additional genetic gains to breeding programmes from MAS are greatest for these traits (Dekkers, 2004).

2.1. How are markers for meat quality identified?

There are several approaches to identifying markers for MAS. The candidate gene approach begins with an examination of the physiological pathways underlying the trait. Sequencing phenotypically divergent individuals at candidate loci may lead to the identification of single nucleotide polymorphisms (SNP's) or insertions/deletions (indels) that can be investigated for associations with traits of interest. SNP's in coding regions can cause a change in an amino acid sequence, some of which will be causative to phenotypic variants (Williams, 2005) and indels can result in the appearance of e.g. premature stop codons as in the musculature hypertrophy locus in cattle (Switonski, 2002). Mutations in introns can affect the phenotype as well, if located in regulatory regions, as can those outside genes, like insulin-like growth factor 2 (IGF-2) in pigs (Williams, 2005). The limitation of this approach is the requirement for prior knowledge on the physiology of the trait, which is not always available. Also, other genes that are not part of the known physiological pathways may contribute to the trait under investigation.

In the second approach, a mapping population of pedigrees (usually an inter-breed cross) is selected in which the phenotype of interest is segregating and the genes (QTL's) for that phenotype are mapped to large chromosomal regions using e.g. restriction fragment length polymorphism (RFLP) markers, microsatellites, SNP's and/or expressed sequence tags (EST's) (Eggen & Hocquette, 2003), followed by fine mapping to narrow the region of interest (Vignal, Milan, San Cristobal, & Eggen, 2002). To date, a disadvantage of this approach is that it has been very slow to produce markers, taking many years in some cases. An expected impact of the sequencing of the bovine genome is a reduction in time and expense required to get from gene discovery in cattle to specific nucleotide variation (Womack, 2006). In practice, the candidate gene approach is often combined with the mapping technique (Williams, 2005), e.g. the localisation of a QTL for subcutaneous fat in a region of chromosome 19, known to contain a candidate gene, the growth hormone locus (Taylor et al., 1998). Novel markers for functional polymorphisms can also be generated based on comparative genomics and expression analysis, e.g. the mapping of the muscular hypertrophy locus (*mh*) to QTL-containing, syntenic regions of cattle based on the location of *mh* on the human map (Switonski, 2002). Identifying conserved sequence among EST's in the genomes of related species may signal functional significance. Where these EST's are found to be differentially expressed in individuals (or breeds) divergent for phenotypes for the trait of interest, subsequent sequencing may yield polymorphisms associated with this divergence (Womack, 2006).

2.2. Integration of marker information in breeding programmes

How a marker is applied in breeding programmes depends on the information provided on the functional mutation underlying variation in the target trait and is reviewed by Dekkers (2004). Dekkers has classified markers for MAS into: (a) direct markers that are causative for the trait of interest, (b) linked markers, which are in linkage disequilibrium (LD) across the population with the causative mutation, and (c) unlinked markers which are in linkage equilibrium with the mutation, when screened in the population as a whole, but quite close to the mutation. Gene assisted selection with direct markers (GAS) is currently the most practical and commercially viable system, because GAS results in certain inheritance of the desired trait and so can be used for selection across the population. However, these are also the hardest markers to identify. In contrast, linkage equilibrium (LE) markers are readily identifiable, but difficult to commercialise. Within-family studies must be done to determine the phase of alleles at marker locus and functional mutation in each generation and different relationships occur between marker and trait locus in different families. LE studies are currently most useful in the initial stages of marker identification, i.e. finding QTL's that segregate between breeds (Kuhn et al., 2005). Because the LD marker is a sta-

tistical rather than direct association, the utility of LD-MAS depends on the extent of linkage in the genome and the population history (Dekkers, 2004). Fortunately, because linkage disequilibrium extends quite far in cattle breeds due to historical inbreeding (Farnir et al., 2000) it becomes possible to detect QTL in crosses between breeds, using markers that are in linkage equilibrium with the QTL in the general population (Dekkers, 2004). However, these markers can be almost as difficult to identify as direct markers and only few have been detected in livestock populations to date (Freking et al., 2002).

2.3. Progress in marker identification for meat quality traits

Exploiting MAS for the delivery of consistent meat quality challenges traditional selection and is currently benefiting from GAS and LD-MAS. Important traits for meat quality that may benefit from MAS include selection on meat pH, marbling and tenderness (Kuhn et al., 2005) and many SNPs have been described and some patented (Hocquette, 2005). The importance of the calpain family of proteases in post-mortem tenderness has been well documented. The calpain system is proving fruitful in association studies for meat tenderness (Casas et al., 2006; Page et al., 2002; Smith, Casas, Rexroad Iii, Kappes, & Keele, 2000; Zhang, DeNise, & Ax, 1996). Several markers for tenderness have been developed at the gene for the inhibitor of calpain, calpastatin (Barendse, 2002) and the calpain I gene (Casas et al., 2006; Page et al., 2002; White et al., 2005). Candidate genes for marbling are the leptin gene (Buchanan et al., 2003), the thyroglobulin gene, the DGAT gene, which is also involved in the regulation of milk fat level (Grisart et al., 2001; Thaller et al., 2003) and the growth hormone gene (Di Stasio, Brugiapaglia, Destefanis, Albera, & Sartore, 2003). Another group of important candidate genes for muscle growth are the myogenic regulatory factors (MRFs) gene family. The Myo-D and myf-5 genes regulate proliferation of myoblasts and satellite cells (te Pas, 2003) and are associated with growth traits in pigs (te Pas et al., 1999). Markers for lamb quality are fewer to date. The callipyge phenotype is related to a SNP on ovine chromosome 18 with a complex mode of inheritance (Freking et al., 2002), and causes hypertrophy in sheep buttocks, which are, however, less tender and palatable as a consequence. A marker for the Texel breed-related hypertrophy has also been described (Marcq et al., 2002). Many of the markers developed in cattle may also be useful in sheep due to the high similarity of their genomes (Womack, 2006). The porcine genome is more divergent from the ungulate genomes, therefore it will require further testing to establish whether markers for tenderness and intramuscular fat content developed for the bovine genome can be adapted to porcine breeding programmes.

With pork, MAS has been most successful in the elimination of undesirable traits, ensuring more consistent meat quality from the population (van der Steen, Prall, & Plastow, 2005). The occurrence of a recessive mutation at the ryanodine receptor (HAL gene), that governs Ca^{2+} transport across muscle cell membranes, results in susceptibility to stress induced death in pigs or porcine stress syndrome (Fujii et al., 1991). A dominant mutation in the rendement napole (RN) gene results in poor meat quality that has a low pH, is associated with poor appearance and taste and high drip loss. Markers for the mutation are being used to select for more consistent quality meat (Milan et al., 2000; van der Steen et al., 2005). Resource populations of inter-breed crosses with divergent traits have been used to identify candidate genes for intramuscular fat in pigs (Williams, 2005). Traditionally in Europe, there has been strong selection for lean growth, whereas in China, pork meat with high fat content has been selected for. Mapping inter-breed crosses of Chinese and European pigs resulted in the identification of IGF-2 as a likely candidate present in the QTL region (van Laere et al., 2003). Although there were 258 polymorphisms in the gene, a single SNP was identified (G to A transition in intron 3 of the gene) that appears to be either a causative mutation or a quantitative trait nucleotide (QTN) (van Laere et al., 2003). Genes in the leptin pathway are proving profitable in association studies with growth and backfat, e.g. the MC4R gene (Kim, Kim, Dekkers, & Rothschild, 2004). A particular haplotype in the calpastatin (CAST) gene in pork is associated with quantitative variation in eating quality (Ciobanu et al., 2004).

2.4. Commercial exploitation of molecular markers in selection programmes

Most commercial applications to date exploit direct or LD markers (GAS or LD-MAS), but cost-benefit analysis of MAS for production traits in pig-breeding programmes indicates that, given assumed costs of implementation, even LE-MAS is economically feasible due to increased profit at the production level from the extra genetic gain (Hayes & Goddard, 2003). However, at present, the increased profit due to the incorporation of molecular markers in selection programmes is derived mainly from bulls with favourable allelic combinations achieving increased market share of breeding stock (Dekkers, 2004). At present commercial markers for meat quality are mostly direct markers such as GeneStar® Marbling and GeneStar® Tenderness of Genetic Solutions, Australia Inc. (http://www.geneticsolutions.com.au), both of which test for favourable SNP's at major genes that have been shown to be involved in meat marbling and tenderness. Other companies have commercialised tests based on single or multiple markers, e.g. GeneSTAR Quality & Tenderness markers (http://www.bovigensolutions.com), Igenity TenderGene and Igenity-L (http://www.igenity.com), Geneseek (http://www2.geneseek.com). Implementation of MAS on a commercial basis requires careful consideration of issues ranging from sample collection and storage, genotyping and data analysis (Dekkers, 2004).

2.5. Future prospects for marker assisted selection

While much research on haplotype diversity in the bovine genome is currently under license (De Nise, 2004), the advances in sequencing of the bovine genome and the proposed bovine haplotype mapping project may increase research into candidate gene polymorphisms in the public domain (Eggen & Hocquette, 2003). In October 2004, the first draft of the bovine genome sequence was deposited into free public databases for use by biomedical and agricultural researchers around the globe. In June 2005, the Bovine Genome Sequencing Project released the second version of the bovine genome, Btau_2.0, which is a 6.2× whole genome shotgun (WGS) assembly. The sequence is available in GenBank, EMBL, and DDBJ, and by BLAST. With minor exceptions, the cattle genome sequence will serve as a platform for the genome of goats, sheep, buffalo, and other artiodactyls whose chromosomes are remarkably similar (Gallagher, Derr, & Womack, 1994). Plans for a porcine genome project are underway and this will permit cross-species comparison of the effects of candidate gene allelic polymorphisms on meat quality.

It is likely that progress in the identification of differentially expressed genes and expressed sequence tags with RT-PCR and microarray analysis will further stimulate the search for markers. Routine genotyping of these SNP's by widely used methods, i.e. SSCP and PCR-RFLP is labour-intensive and screening effort is directly proportional to the (rapidly growing) number of candidate loci, therefore this may represent a limiting factor in the advancement of marker-assisted selection. More recently, novel, rapid and high-throughput automated assays have been developed, including mass-spectroscopy and array based allele-specific hybridisation and mini-sequencing (Syvaenen, 2005; Twyman & Primrose, 2003). The European MolTools project (http://www.moltools.org) aims to greatly improve the speed and accuracy of genotyping by examining limiting technical factors (Hocquette, 2005). If such methods can be adapted to meat quality analysis, association mapping with meat quality traits will be greatly expedited.

While the importance of genetic markers in selection is likely to grow in the coming years, a balanced approach must be taken to ensure they enhance but not supplant traditional selection. MAS may divert resources from selection on polygenes and traits without known associated QTL, yet which influence overall genetic merit. It is even possible that overall genetic merit in the population may decline with strong unbalanced selection (Williams, 2005). Additionally, marker and gene assisted selection must be applied with care to ensure improvements in selected economically important traits do not conflict with natural selection for reproductive success and disease resistance (Williams, 2005). While it is difficult to quantify the success of MAS programmes on enhancing overall genetic merit, suggestions of how this might be achieved have been presented (Dekkers, 2004).

3. Functional genomics: transcriptomics

In the last 20 years techniques for evaluating gene expression have progressed from methods developed for the analysis of single specific genes (northern, slot and dot blotting, semi-quantitative polymerase chain reaction, PCR) to those focused on identifying a range of genes that differ in expression between experimental samples. Broad-spectrum approaches to identify differences in gene expression include suppressive subtractive hybridisation (SSH) (Mohan, Hurst, & Malayer, 2004; Wan, Wright, Cai, Flament, & Lindpaintner, 2002), differential display (Davis, De Sousa, & Schultz, 1996; Liang & Pardee, 1992), sequence analysis gene expression (SAGE; Graff, Behnke, Radke, White, & Jutila, 2006) and microarray hybridisation (Moody, 2001; Rinaudo & Schultz, 2004; Schena, Shalon, Davis, & Brown, 1995).

The evaluation of gene expression using microarray technology was originally described by Schena et al. (1995). At that time the technology was largely inaccessible to animal scientists due to numerous reasons, the most important of which, was that little was known about the genome of domestic animals. Also the specialised equipment for producing and reading arrays was largely unattainable from an economic perspective. Over the past decade we have witnessed major developments in array technologies. Microarrays are now an invaluable exploratory tool to provide information on differentially expressed genes and enhance our understanding of the biological pathways that underlie the delivery of consistent quality meat (Fig. 1).

3.1. DNA microarray technology

Two types of microarrays are commonly used for expression profiling. These arrays differ primarily in the length of the probes that make up the array elements. For microarrays that are printed on membranes or glass, the array elements are fragments of genes approximately 400–800 base pairs (bp) long, typically produced through PCR amplification. Two probes labelled with different fluorophores are placed on a single slide simultaneously and competitively hybridise to the genes/EST's spotted onto the array. In contrast, oligonucleotide-based microarrays (also termed DNA chips) have array elements comprising of short (~25 bp), synthetic DNA molecules and instead of hybridising two samples on one array, a single sample is hybridised on the array and comparisons are then made between two or more chips.

There are broadly two approaches that can be taken when carrying out microarray experiments; examine a vast number of genes, i.e. genome wide (using Affymetrix arrays; 23,000 bovine genes) or focus on tissue specific transcripts that are identified through other discovery means in targeted arrays. To date, published studies in livestock microarray experiments have used the second more tissue-specific approach (Bai et al., 2003; Byrne et al., 2005;

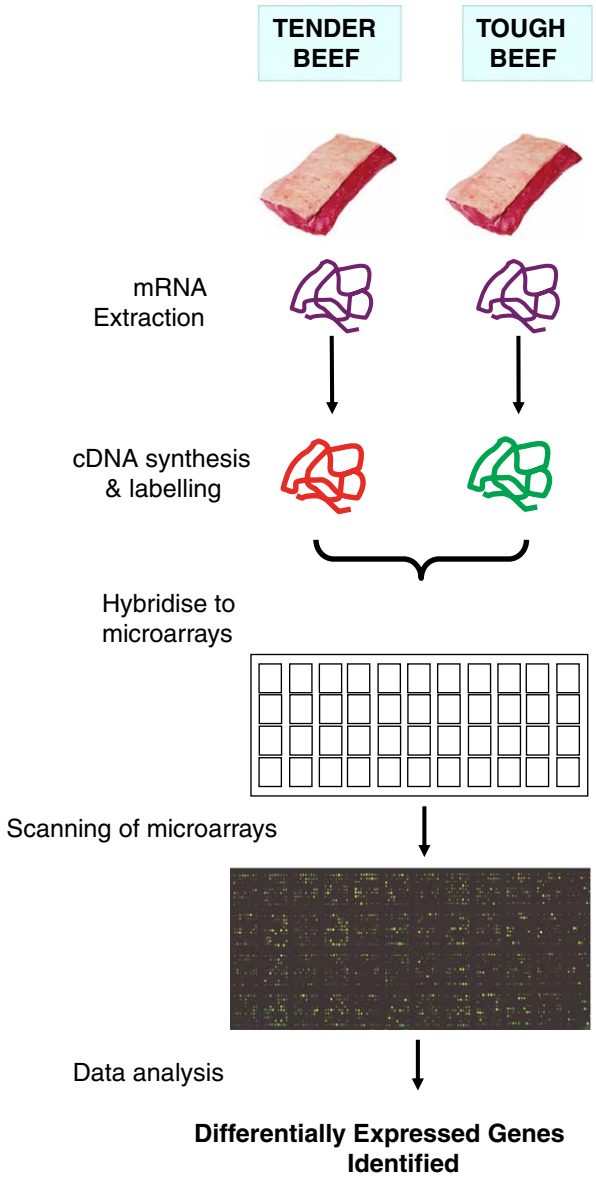

Fig. 1. Outline of microarray experiment comparing gene expression profiles in beef divergent for quality.

Cagnazzo et al., 2006; Yao, Coussens, Saama, Suchyta, & Ernst, 2002). For example The Centre for Animal Functional Genomics, Michigan State University, USA has constructed cDNA microarrays from a normalised porcine muscle library (Yao et al., 2002) and another porcine cDNA microarray comprising 5500 clones has been used to analyse differential transcript expression in phenotypically distinct muscle (Bai et al., 2003).

3.2. Limitations of microarray experiments

Microarray studies are not without their limitations or difficulties (Hocquette, 2005). Before commencing any experiment for example, the amount of different sequences or genes represented on the array immediately determines or limits the number of genes that will be assessed within the samples being analysed. A major issue is in understanding the sources of variation and accounting for this in the analysis of the data. Variability can arise within each of the steps taken to produce and hybridise an array. For example, low specificity of printed probes affects hybridisation, there can also be inconsistent fidelity across arrays due to differing printing pins, and low quality RNA reduces reverse transcription efficiency and subsequent dye labelling efficiency.

A huge component of microarray experiments is the image analysis procedure and subsequent data analysis including normalisation and statistical assessment of differential expression. Normalisation is the attempt to remove non-biological influences on biological data and allows comparison from one array to another. Normalisation is applied to fluorescent values recorded for each spot on the array after scanning. Differences in dye labelling efficiencies is one of the most common sources of bias; this can be seen in an experiment where two identical mRNA samples are labelled with different dyes and subsequently hybridised to the same slide (Yang & Speed, 2002). In this instance it is rare to have the dye intensities equal across all spots between the two samples. Dye biases can stem from a variety of factors, including physical properties of the dyes (heat and light sensitivity, relative half-life), efficiency of dye incorporation, experimental variability in hybridisation and processing procedures, or scanner settings at the data collection step (Yang & Speed, 2002). Even though such systematic biases may be comparatively small, they may be confounding when searching for subtle biological differences. Many inconsistencies within microarray experiments can be overcome through good laboratory practice and assessment of the quality of the samples at critical points throughout the process, e.g. RNA quantity and quality measurements following extraction, assessment of both labelling and hybridisation efficiency.

3.3. Data analysis

Because of the large volume and intrinsic variation of the data obtained in each microarray experiment, statistical methods are used as a way to systematically extract biological information and to assess the associated uncertainty. A common task in analysing microarray data is to determine which genes are differentially expressed across two different tissues or at two different time points or conditions. Initially the simple method of fold changes was used whereby the log ratio of fluorescent values between two conditions was evaluated and all genes that differed by more than an arbitrary cut-off value were deemed to be differentially expressed (DeRisi, Iyer, & Brown, 1997; Schena et al., 1996). It is now known to be unreliable (Chen, Dougherty, & Bittner, 1997) because statistical variability was not taken into account. Since then, many more sophisticated statistical methods have been proposed (Efron & Tibshirani, 2002; Ideker, Thorsson, Siegel, & Hood, 2000; Newton, Kendziorski, Richmonds, Blattner, & Tsui, 2001; Pan,

2002; Pan, Lin, & Le, 2003; Tusher, Tibshirani, & Chu, 2001). To aid researchers extract accurate results from the data generated during microarray experiments, software programs have been created and many of which are listed at the following URL: http://www.endosociety.org/external/Bioinformatic/analysis_microarraybiosites.cfm.

3.4. Transcriptomics and meat research

The significance of array technology lies in the potential to tie specific changes in gene expression to a phenotype of interest. In recent years the focus of much research has turned to the functional aspects of genes and how expression controls protein production and ultimately the phenotypic characteristics of a trait. The target of this research is RNA, the key molecule which gives life to cells. Functional genomic studies allow the detection of genes that are actively transcribing at any given time, depending on environmental factors (Hocquette, 2005).

In 2002, the only commercially available microarray for livestock species was a small one for cattle (Band, Olmstead, Everts, Liu, & Lewin, 2002; http://www.pyxisgenomics.com/) and the only microarrays for pigs and chickens were those within individual laboratories. Several research efforts are underway to develop livestock cDNA and EST library resources needed to identify and obtain clones for preparing DNA to spot onto cDNA microarrays (Abdrakhmanov et al., 2000; Bai et al., 2003; Band et al., 2002; Bernard et al., 2005; Davoli, Zambonelli, Bigi, Fontanesi, & Russo, 1999; Fahrenkrug et al., 2002; Nobis et al., 2003; Sudre, Leroux, Cassar-Malek, Hocquette, & Martin, 2005; Tirunagaru, Sofer, Cui, & Burnside, 2000; Tuggle et al., 2003). An on-going collaborative project between the Ashtown Food Research Centre, University College Dublin and the National Diagnostics Centre has applied SSH to beef samples showing extremes of quality to create a muscle specific cDNA library.

An EST is a small sequence from an expressed gene. They are typically identified by purifying mRNAs, converting to cDNAs, and then sequencing a portion of the cDNAs. Currently there is a lack of genome-wide farm animal cDNA arrays, as construction requires access to cDNA resources from many tissues and developmental stages in order to obtain adequate gene representation. However, an extensive commercially available bovine array has become available (GeneChip® Bovine Genome Array, Affymetrix). This array is based on the content from Bovine Unigene Build 57 and GenBank mRNAs and contains 24,027 probe sets designed to monitor expression of approximately 23,000 bovine transcripts (http://www.affymetrix.com/products/arrays/specific/bovine.affx).

The extensive research being carried out by many different groups in an effort to expand the knowledge base regarding gene expression in livestock is reflected in the fact that the number of EST's in public databases doubled on average from December 2001 to August 2004 (Hocquette, Cassas-Malek, Listrat, & Picard, 2005). Examples of gene expression discovery studies in meat research include the construction of a porcine biceps femoris muscle cDNA library which identified 72 unique clones (Davoli et al., 1999). Two porcine cDNA libraries (MARC 1PIG and MARC 2PIG), derived from embryonic and reproductive tissues, respectively, were also constructed, sequenced and analysed (Fahrenkrug et al., 2002). A *Sus scrofa* gene index (SsGI) including all sequences in public repositories was developed to facilitate further characterisation of porcine genes (Fahrenkrug et al., 2002). In the most recent SsGI release (Release 11.0) there were 104,327 unique porcine sequences documented on the database: (http://www.tigr.org/tigr-scripts/tgi/T_index.cgi?species=pig).

Five thousand five hundred clones from the *longissimus dorsi* of a 50-day porcine foetus and the gastrocnemius of a three-day old pig were created to form the basis of a skeletal muscle cDNA microarray (Bai et al., 2003). Also, a porcine brain cDNA library was generated and a cDNA microarray produced using 877 unique porcine brain EST amplicons spotted in triplicate on glass slides (Nobis et al., 2003). Prior to this, only two publicly available porcine cDNA microarrays existed, both constructed from skeletal muscle cDNA libraries (Bai et al., 2003; Yao et al., 2002). Since this, however, there has been a large increase in the volume of studies examining a multitude of animal functions across a number of species using the microarray technique. Pomp, Caetano, Bertani, Gladney, and Johnson (2001) have used cDNA derived from ovary and follicular RNA from animals from either an index line selected for higher litter size or a control line and co-hybridised them with 4600 follicle-derived probes to study gene expression patterns related to reproductive efficiency (Caetano, Johnson, Ford, & Pomp, 2004; Pomp et al., 2001). In cattle studies, array work has already helped to identify differential gene expression between resistant or susceptible animals following infection by pathological organisms (review by Moody, Rosa, & Reecy, 2003), in placenta and uterine tissue between non-pregnant and pregnant cows (Ishiwata et al., 2003), in the mammary gland between non-lactating and lactating cows (Suchyta et al., 2004), in oocytes during maturation (Dalbies-Tran & Mermillod, 2003), between embryos produced in vitro or in vivo (Corcoran et al., 2006), between different tissues and organs (Cho, Han, Kang, Lee, & Choi, 2002), during muscle ontogenesis (Sudre et al., 2003) and between different feeding regimes (Reverter et al., 2003; reviewed by Hocquette et al., 2005).

Campbell et al. (2001) demonstrated that the use of array technology can provide insights into muscle biology. They compared white and mixed red fibre types and developed a global gene expression profile, allowing a better understanding of the gene regulation that underlies the differences between muscle fibre types. Forty-nine genes were identified to be differentially expressed between the muscle types. Carson, Nettleton, and Reecy (2002) compared rat muscles submitted to a work overload and a negative con-

trol to investigate the global changes in gene expression induced by work overload.

Reverter et al. (2003) developed a bovine cDNA microarray with 19,200 spots for the profiling of bovine muscle and fat tissue. A total of 9600 elements were printed in duplicate onto glass slides. There were 9222 cattle probes, comprised of 7291 anonymous cDNAs from bovine skeletal muscle and fat cDNA libraries and 1915 bovine EST's selected from various library sources. These slides have been used in three experiments so far. Firstly, the gene expression profiles of muscle in steers fed varying quality diets were compared. In another experiment the expression profile between two breeds of cattle were compared at three time points in development 11, 15 and 20 months of age. Thirdly, mechanisms underlying in vitro adipogenesis were studied in fibroblast cell cultures.

Wang et al. (2005) used a bovine fat/muscle cDNA microarray to investigate differential gene expression in the LD muscle of Japenese Black (JB) and Holstein (HOL) cattle at 11.5 months of age. A porcine skeletal cDNA created by Bai et al. (2003) was used to detect gene expression differences between red and white muscle types.

Another option when investigating mRNA expression using microarray technology is to use commercially available human or mouse cDNA microarrays for cross species hybridisation with livestock cDNA (Gladney, Bertani, Johnson, & Pomp, 2004; Moody, Zou, & McIntyre, 2002). One of the most recent publications in the area of animal functional genomics compared prenatal muscle tissue expression profiles of two pig breeds (Duroc and Pietrain) differing in muscle characteristics (Cagnazzo et al., 2006). Samples from each breed were hybridised onto arrays containing more than 500 genes affecting myogenesis, energy metabolism, muscle structural genes and other genes from a porcine muscle cDNA library (Davoli et al., 1999, 2002).

3.5. Outlook for the future

Global gene expression profiling at the mRNA or protein level will provide a better understanding of gene regulation that underlies certain biological functions for example myogenesis and its control by nutrition but also quality issues such as what genes are involved in determining the eating quality of meat. The full value and applications of the species genome projects will be realized only when the actual genes and gene products (proteins) that coordinate and regulate important animal traits are known and understood.

To date there have been few studies directed at uncovering the genes regulating the key attributes of beef eating quality: toughness, flavour and juiciness. With the expanding knowledge of transcripts expressed in bovine/porcine skeletal muscle it should be possible to identify those genes differentially regulated as a consequence of the nutritional status, breed, age, pre- and post-mortem handling of an animal, which may in turn affect the overall eating quality of the meat. While DNA-based techniques are suitable for aiding genetic selection for livestock quality improvement across generations, functional genomics will have major applications with respect to characterisation of muscle and meat (Hocquette et al., 2005).

4. Proteomics

Proteomics is the study of the whole protein cell content or proteome. Since proteins are frequently the functional molecules, they are most likely to reflect differences in gene expression. Proteomics can be defined as the systematic determination of protein sequence, quantity, modification state, interaction partners, activity, subcellular localisation, and structure in a given cell type at a particular time (Campbell, 2003). Proteome analysis is a direct measurement of proteins in terms of their presence and relative abundance (Wilkins et al., 1996). Neither genomic DNA code nor the amount of mRNA that is expressed for each protein yields an accurate picture of the state of a cell. This is because genes may be present but not transcribed and the number of mRNA copies does not always reflect the number of functional proteins present (Celis et al., 2000). The aim of proteomics is to obtain information about cellular protein expression and hence to reveal the function of genes, with the ultimate goal of explaining how heredity and environment interact to control cellular functions (Bendixen, 2005). However, global proteome analysis is a difficult task, as described by Ghaemmaghami et al. (2003), who successfully reported a complete protein census for yeast. Equally, this approach is beneficial when focusing on a selected set of proteins. For example, with regard to proteins relevant to meat quality, working with myofibrillar, exudate or sarcoplasmic extracts may be more manageable than attempting to examine the whole protein complement and can facilitate detection of some of the lower abundance proteins (Sierra, O'Reilly, White, Mullen, & Troy, 2005). Proteomics can address problems that cannot be approached using DNA analysis. As well as functional aspects, these problems include estimation of the relative abundance of the protein product, its post-translational modification, subcellular localisation, turnover and interaction with other proteins (Celis et al., 2000; Stagsted, Bendixen, & Andersen, 2004).

There are two approaches to proteome characterisation, namely comparative proteomics and mapping proteomics. Mapping proteomics is similar to genome sequencing projects and aims to characterise and make comprehensive databases of "cellular proteomes" (Bendixen et al., 2005). However, this is a huge task, partly due to the complex variety of modification forms most proteins possess (Mann & Jensen, 2003), and also because the proteome constantly changes with time and physiological state. In every sense, every single cell and organism has an infinite number of proteomes. Comparative proteomics aims to characterise the biological mechanisms that form the link between observable phenotypes and genotypes, thereby making moment-by-moment snapshots of cellular responses at the protein level (Hunter, Andon, Koller, Yates, & Haynes, 2002).

4.1. Protein technologies

Due to the abundance and diversity of proteins and the vast amount of data that can be generated, the production, processing and interpretation of proteomic data is complex. Mammalian tissue samples typically contain between 10,000 and 30,000 different protein species, hence a wide range of technologies must be used to prepare, separate and quantify the relative expression levels of thousands of proteins in parallel (Bendixen, 2005).

4.1.1. Two-dimensional electrophoresis (2DE)

Two-dimensional electrophoresis (2DE) involves two separation parameters, isoelectric point and molecular weight, which can improve resolution in the fractionation of complex mixtures of proteins, allowing multiple proteins to be separated for parallel analysis (Fig. 2). In a 2DE analysis, spot patterns are formed, with each spot theoretically representing an individual protein and the intensity of a particular spot indicating how much of that protein is present. However, due to the complexity of 2DE electrophoresis patterns, a single spot may actually be composed of more than one protein. Care should be taken because MS identification is more reliable and sensitive where few or a single protein co-electrophorese in a single spot (Pietrogrande, Marchetti, Dondi, & Righetti, 2006). Another shortcoming of the technique is that according to Pedersen et al. (2003), 2DE analysis is confined to a limited subset of the total protein cell content. This is because IPG-based 2DE systems discriminate against basic (Gorg, 1999) and hydrophobic proteins (Rabilloud, 1998; Santoni, Molloy, & Rabilloud, 2002), thus excluding the analysis of many receptors and transmembrane proteins (Tyers & Mann, 2003). However, this may also be viewed as an advantage for early stage biomarker discovery as it limits the amount of data to be processed at the initial stages while still producing a range of protein spots to be analysed. Recently, successful analysis over the entire range of pH 7–11 has been reported (Corton et al., 2004). Another shortcoming of 2DE analysis is that when it is applied to complex unfractionated samples, the limited dynamic range of 2DE only allows for the most abundant proteins to be analysed (Pedersen et al., 2003). However, pre-fractionation of complex samples may be used to by-pass this problem (Gorg et al., 2002; Spandidos & Rabbitts, 2002).

4.1.2. Mass spectrometry (MS)

In proteomics two main applications of MS have emerged. The first is the identification of protein spots from 2DE analysis or cruder extracts, the second is comparative proteomics. Soft ionisation techniques such as electrospray ionisation (ESI) (Fenn, Mann, Meng, Wong, & Whitehouse, 1989) and matrix-assisted laser desorption ionisation (MALDI) (Karas, 1996) are used for protein identification. These methods involve laser energy which is used to convert peptides into gas phase ions (Bahr, Stahl-Zeng, Gleitsmann, & Karas, 1997). MALDI can then be combined with a time-of-flight (ToF) mass analyser. This technology is ideally suited to protein mass fingerprinting (PMF) which analyses proteolytically digested proteins, i.e. trypsin digest. For cattle, 70% of 2DE proteins could be identified by PMF analysis, with sequence coverage of around 30% (Bendixen,

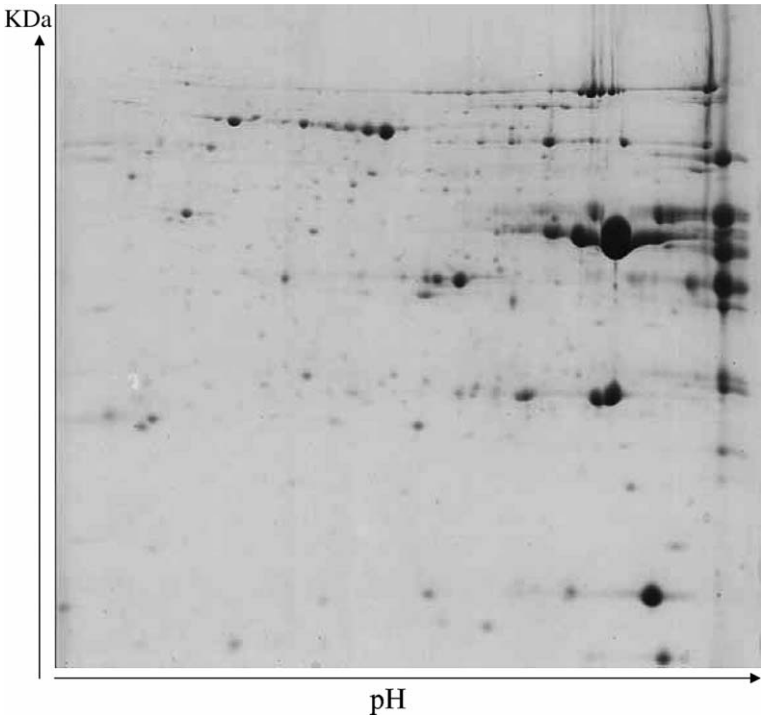

Fig. 2. 2-D electrophoretic separation of porcine sarcoplasmic proteins from M. *semimembranosus* (AFRC/INRA).

2005). A drawback of using MALDI-ToF technology is not receiving true sequence information. However, using tandem ToF/ToF units separated by a collision cell allows this problem to be overcome (Suckau et al., 2003). ESI together with tandem mass analysers yields information on short peptides, which allow for protein identification through peptide fragment fingerprints (PFF), which entails matching experimental data and database information. Surface enhanced laser desorption ionisation (SELDI) technology involves the interfacing of specially coated chromatography protein chips. These chips allow selective enrichment so that MALDI-ToF analysis can be performed on sub-proteomes rather than proteomes (Tang, Tornatore, & Weinberger, 2004; Zhu & Snyder, 2003).

MS of peptides is not quantitative, but qualitative, as the ionisation capabilities of peptides are unpredictable (Lim et al., 2003). Quantitative MS-based comparative proteomics methods, for example isotope coded affinity tag (ICAT) labelling, can overcome the shortcomings of 2DE analysis. ICAT labelling allows for chemical tagging of proteins from different samples.

New technologies with increased speed and sensitivity allow MS-based proteomics to become a more powerful tool. Examples of these include hybrid linear ion trap and fourier transform ion resonance cyclotron (FTICR) (Belov et al., 2004; Le Blanc et al., 2003).

4.1.3. Microarray technology

2DE and MS, while highly effective, have limitations, including the fact that proteins expressed at low abundance may be missed. It is possible that many of the changes affecting protein levels may involve low abundance peptides (Celis et al., 2000). Therefore, highly sensitive methods of proteome analysis are called for, such as protein microarrays, which are miniaturised solid phase ligand binding assay systems using immobilised proteins. The technique involves using a probe that is specific for a particular analyte, which is placed at a defined position on a surface. The basic principals have been discussed by Elkins, Chu, and Biggart (1989). However, the application of protein microarrays to proteomics is not very advanced when compared to that of DNA. DNA is a fairly uniform molecule which binds to complementary targets according to the base pairing principle, therefore it is easy to predict highly selective and specific DNA capture sequences from the primary sequence of the target DNA. In contrast the diversity of both the proteins themselves and their interactions renders it impossible to predict high affinity capture molecules. Potential capture molecules must be screened for specificity to individual target proteins. The development of a PCR equivalent for proteins is necessary for the low cost and high throughput production of capture proteins and protein targets (Stoll, Bachmann, Templin, & Joos, 2004). Burbulis, Yamaguchi, Gorden, Carlson, and Brent (2005) describe a new method for detecting and quantifying proteins that is almost as sensitive as PCR for the quantification of nucleic acids.

4.2. Data analysis

Developments in the area of proteomics have increased the amount of data produced for analysis. An efficient use of the large amount of data generated is vital to achieve the most from proteomic research. Patterson (2003) stated that our ability to generate data now outstrips our ability to analyse it. For this reason image and data analysis is of major importance to proteome research. The task of image analysis by comparing the relative volumes of individual spots on different gels in order to identify differentially expressed proteins is time consuming; therefore methods for image and data analysis have been suggested. Meunier et al. (2005) reported that statistical methods used for microarray analysis which identify a small number of differentially expressed genes is a useful method for quantitative determination of differentially expressed proteins. Recent developments in mathematical approaches to data analysis are helping to decode complex 2D-PAGE maps (Jessen, Lametsch, Bendixen, Kjaersgard, & Jorgensen, 2002). Examples include the statistical model of peak overlapping (SMO), which is used for the statistical quantification of the degree of spot overlapping present in a map and the 2-D autocovariance function (2D-ACVF) method. 2D-ACVF enables simple display of a comprehensive description of the whole map and offers simplified qualitative and quantitative information on the composition of the complex mixture (Pietrogrande et al., 2006). Computational analysis for proteomics has also been developed, e.g. automated trend analysis (Malone, McMarry, & Bowerman, 2006).

Data handling from non-gel based systems must also be considered. Difficulties in data handling include the fact that many MS/MS spectra are not of peptides, but of instrument noise or minor contaminants and their analysis consumes considerable computing time (Patterson, 2003). Thousands of MS spectra are generated during a comparative proteome study and extracting information from the data involves a series of analytical steps. However, improved algorithms and software are continuously been created (Bendixen, 2005; Chamrad et al., 2002). For ease of visual inspection of large data sets and immediate identification of relevant differences, it may be a useful technique to represent MS-based data in a similar way to a 2DE gel. For these reasons 2D-MS mapping may be used which is the visualisation of MS data in a pseudo-two-dimensional map (Roesli, Elia, & Neri, 2006).

4.3. Proteomics and meat research

There are many factors which contribute to meat quality such as growth and development of muscle tissue. Bouley et al. (2005) reported the first proteomic study of bovine hypertrophy, which focused on the comparison of proteome patterns from muscles with a mutation resulting in the expression of normal levels of inactive myostatin protein. Proteome studies of muscle growth in chicken have also been reported by Doherty et al. (2004).

As mentioned earlier there is a lot of variability present in the eating quality of meat much of which is attributed to variation within the muscle during the early post-mortem period. As the muscle cell receives a large insult after exsanguination it is likely that there are many other cellular pathways and processes altered as a result. The interaction of pH, temperature and time post-mortem affect the biochemical dynamics of early post-mortem meat and hence the rate of proteolysis (White, O'Sullivan, O'Neill, & Troy, 2006). Calpains, whose activity is dependant on the biochemical state of the muscle post-mortem, play a key role in tenderisation of meat (Taylor, Geesink, Thompson, Koohmaraie, & Goll, 1995). The specific calpain mediated degradation patterns of myofibril proteins has been reported (Lametsch, Roepstorff, Moller, & Bendixen, 2004). At present the application of proteomics to meat science is at an early stage, with some initial studies published in this area (Lametsch, Roepstorff, & Bendixen, 2002; Morzel et al., 2004). Interesting results have been obtained from one-dimensional electrophoresis (1DE) analysis of muscle proteins in the search for markers of tenderness (Kolczak, Pospiech, Palka, & Lacki, 2003; Sawdy, Wick, Kaiser, & St-Pierre, 2004; Sierra et al., 2005). Expanding this research to incorporate methods described above holds great potential for further elucidation of cellular processes which influence meat quality traits (Sayd et al., 2006). Understanding how these processes interact with environmental factors such as post-mortem processing (for example, Lametsch & Bendixen, 2001; Lametsch et al., 2003; Lametsch et al., 2002; Morzel et al., 2004) in the delivery of meat quality, can contribute to optimisation of whole chain management systems for consistent quality meat.

Proteome studies of muscle tissue from man and model organisms, like mouse, may provide valuable information when implementing proteome technologies to muscle and meat studies of live stock species (Bendixen, 2005). As technologies improve in sensitivity and reproducibility, and data sets become more complete, proteomics should also facilitate the application of systems approaches for modelling complex phenomena, and reveal protein biomarkers that can be used in diagnostic and predictive screens for meat quality.

5. Outlook for the future

Global gene expression profiling at the mRNA or protein level will provide a better understanding of gene regulation that underlies certain biological functions such as those pertinent in the delivery of consistent quality meat. However, just as our increased understanding is uncovering the complexity of the genome and providing information regarding the extensive interactions to produce gene products, i.e. the proteome, there is the growing realization that the interrelationships within the proteome are even more complex. Primary and secondary metabolites represent the end products of genetic expression and the comprehensive analysis of large numbers of metabolites has been termed metabolomics (see Blackstock & Weir, 1999; Glassbrook, Beecher, & Ryals, 2000; Trethewy, 2001). Employing both proteomics and metabolomics approaches can help address the issue of appreciating all of the activities taking place in the muscle cell that are relevant to the delivery of consistent quality. An emerging academic field is that of systems biology which seeks to integrate different levels of information from a variety of disciplines (see Hood, 2003; Hocquette, 2005; and Bendixen et al., 2005, for review). Another discipline currently developing rapidly is that of bioinformatics. In order to extract meaning from the large amounts of data that stems from these high-throughput techniques, sophisticated mathematical algorithms and computational methods are emerging. Bioinformatics has advanced greatly to keep pace with the 'omics' fields of study and is enabling detection of associations between differential gene expression and a phenotype (Fadiel, Anidi, & Eichenbaum, 2005). Although linking this information mechanistically to the biology driving the phenotype is still a challenge (Quackenbush, 2005), this holds much promise for the future.

Many exciting discoveries have been made through our investigation of the genome and proteome in relation to meat quality which are of relevance to the meat industry. Potential applications of this research encompass improvements to traditional breeding programmes, diagnostic tests for quality and management systems for quality. While there is often emphasis on the management systems that can be implemented to meet market specifications, there has, until recent years, been little emphasis on factoring in the molecular or biological components of meat quality. Through our ongoing appreciation of muscle molecular signatures and how they interact with environmental stimuli, management systems can be optimised on the basis of genotype to deliver consistent quality meat.

6. Conclusion

The expanding development and rapid advances in molecular and quantitative genetics, reproduction technologies, animal nutrition and muscle science carry with them a huge potential. Genes and proteins do not function independently; they participate in complex networks that ultimately give rise to cellular functions, tissues, organs and organisms. We have gained great insights through investigating single proteins or single pathways within the muscle cell. However, we now need to adopt a more holistic approach to understand how cellular processes interact within an organism, in response to environmental factors and in the delivery of consistent quality meat. Knowledge gained will benefit scientists and industry alike. Incorporation of this data into a beef management system such as MSA (Meat Standards Australia) will assist in defining management systems, which are designed for genotype.

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