

## PRODUCT QUALITY, HEALTH and WELL-BEING

# Recent advances in cattle functional genomics and their application to beef quality

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*The advent of high-throughput DNA sequencing techniques, array technology and protein analysis has increased the efficiency of research in bovine muscle physiology, with the ultimate objective of improving beef quality either by breeding or rearing factors. For genetic purposes, polymorphisms in some key genes have been reported for their association with beef quality traits. The sequencing of the bovine genome has dramatically increased the number of available gene polymorphisms. The association of these new polymorphisms with the variability in beef quality (e.g. tenderness, marbling) for different breeds in different rearing systems will be a very important issue. For rearing purposes, global gene expression profiling at the mRNA or protein level has already shown that previously unsuspected genes may be associated either with muscle development or growth, and may lead to the development of new molecular indicators of tenderness or marbling. Some of these genes are specifically regulated by genetic and nutritional factors or differ between different beef cuts. In recognition of the potential economic benefits of genomics, public institutions in association with the beef industry are developing livestock genomics projects around the world. From the scientific, technical and economical points of view, genomics is thus reshaping research on beef quality.*

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### Introduction

Beef consumers increasingly seek meat of high and consistent quality. As a consequence, the beef industry is investing in research aimed at the identification of indicators of quality and an increased understanding of muscle biology to control quality traits.

Beef quality includes sensory quality traits (tenderness, flavour, juiciness, colour, etc), nutritional value, healthiness and technological quality (Geay *et al.*, 2001) and also other issues such as animal welfare, environmental concerns, traceability, etc (Hocquette and Gigli, 2005). Whereas the latter are more complex or subjective issues, the former are directly associated with muscle biology traits of live animals and during post-mortem processing treatments.

Biotechnology is widely regarded as one of the most promising life science frontiers for the next decade. Together with informatics, biotechnology is expected to lead to revolutionary changes in our society and economy. This scientific revolution, sometimes referred to as the genomic revolution, is global and is creating new opportunities in all biological

sciences including medicine, human health and nutrition, agronomy and, the subject of this paper, animal science.

This paper aims to illustrate how animal scientists can take advantage of the recent developments in functional genomics to get a better understanding of the factors which determine gene expression and hence muscle physiological traits controlling beef eating quality.

### 'Genomics': definition, history, potential benefits and limitations

#### *Definitions and history of genomics*

In the past, molecular biologists studied one gene at a time in isolation from the larger context of other genes. This was particularly true in physiology where only a few genes characterising a specific metabolic pathway were studied together. In more recent times, high-throughput analyses of gene expression have allowed a global view of gene expression thanks to the development of techniques such as DNA arrays and proteomic approaches (Hocquette, 2005).

The first high-throughput molecular biology methods to be developed were sequencing methods. Therefore,

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sequencing of genomes from many species has been achieved or is in progress. This has facilitated a better understanding of the genome structure and the identification of polymorphisms (mainly single nucleotide polymorphisms or SNP), which allow more precise gene mapping. In beef science, this will contribute to the more rapid identification of genetic markers which will be useful for animal breeding, with the objective to improve beef quality traits as previously reviewed (Kühn *et al.*, 2005).

Now, the application of genomics to biology is gradually evolving from the discovery of genetic determinants (structural genomics) to 'biological signatures' (functional genomics). The latter is a typical pattern of gene expression, or even a typical profile of proteins or metabolites characterising a specific physiological or pathological state. The methods to determine these biological signatures are currently reshaping biology and are referred to as transcriptomics, proteomics and metabolomics/metabonomics (Hocquette, 2005). Functional genomics therefore describes a new scientific field midway between genetics and physiology. The current challenge for biological research is to integrate structural and functional genomics (Tuggle *et al.*, 2006) and also to associate data from the different '-omic' sciences with phenotypic data (Hocquette, 2005). This process is facilitated by rapid developments in computational biology or bioinformatics, which has followed the rapid expansion in genomic research. Several analytical approaches, bioinformatic methods and tools have indeed been suggested to integrate data, for instance expression data at mRNA and protein levels (Cox *et al.*, 2005) in association with metabolite profiling (Weckwerth *et al.*, 2004) to identify real biochemical networks. More generally, bioinformatics is becoming crucial for the analysis of expression data with the ultimate objective to extract biologically meaningful information from the lists of differentially expressed genes. A variety of bioinformatic tools are available for data-mining depending on the question being asked (Hanai *et al.*, 2006). Many experts indicated that bioinformatics and computational biology approaches will be major areas of emphasis in the next few years in both research and practical usage even for livestock (Henderson *et al.*, 2005).

#### *Potential benefits of genomics*

The development of the new genomic high-throughput technologies is reshaping biology due to four new paradigms in science. The first one of these paradigms, can be described as a shift away from characterising a small number of key molecules (such as high differentially expressed genes or proteins), but on molecular signatures (cluster of genes, of proteins, of metabolites, etc), which are characteristic of a biological process or of a specific phenotype. This shift has been made possible with the advent of high-throughput methods of data acquisition and of bioinformatics methods to handle large amounts of data.

Another new paradigm is the change in research conception. Up to now, hypothesis-driven research has been conducted with the aim of testing new biological hypotheses which have emerged from previous studies or

theoretical considerations. High-throughput genomic techniques are capable of uncovering associations between previously unknown molecules (DNA, RNA, proteins, metabolites) or previously uncharacterised DNA/protein sequences and physiological traits of interest. Genomics is thus capable of generating new biological hypotheses which can then be further studied by more focused approaches. This will impact most markedly on the characterisation of complex traits, which are governed by interactions between many genes with small effects. An example of such a complex trait is beef tenderness, which depends on connective tissue characteristics, lipid content, fibre composition and meat ageing, all complex biological phenomena in themselves.

The third new paradigm is the goal of capturing the structure-function relationships of any genome as a whole. Gene and protein studies, as well as metabolomics, will provide the link between the genome and the biological processes which give rise to the phenotype. The most important characteristics of biological organisms are their ability to change in response to changing environments. Genomics (and especially functional genomics) will thus help to understand physiology, in addition to promoting progress in genetics (Andersson and Georges, 2004).

The last important new paradigm associated with genomics is that high-throughput gene interrogation systems will help to integrate knowledge about genes associated with different physiological functions. Knowing what genes are activated following stress by the environment, infections by pathogens and increasing production levels of milk or meat will enable researchers to assess interactions between all these biological mechanisms. This will help to develop novel management and breeding strategies that simultaneously promote animal health, well-being, food quality and safety.

In medicine, genomics will be used for effective prediction of a patient's disease risk and drug reactions, and the interaction between both. Preventive medicine and medical therapy will be personalised. The development of genomic applications for personalised medicine will require associations of gene marker profiles and gene expression patterns with clinical data (Hocquette, 2005).

In agriculture and animal science, the outcomes of genomics will include improvements in food safety, in crop yield, in traceability and in quality of animal products (dairy products and meat) through increased efficiency in breeding and better knowledge of animal physiology. DNA-based techniques have been developed for the detection of bacterial contamination (beef safety), for species and animal identification (breed and individual traceability), and for genetic selection (genetic improvement of beef quality). On the other hand, RNA- and protein-based techniques may one day be used to characterise the health, physiological and nutritional status of the animals according to their breed, their genetic type, the rearing factors or the place where they are bred. These techniques may also be adapted to characterise each muscle type. For all these

reasons, genomic techniques may contribute to quality control of beef. Protein-based techniques may be employed to monitor protein degradation during post-mortem ageing of beef, a process which controls tenderness. Therefore, genomics is likely to play a major role in sustainable agriculture due to the wide range of its potential applications (Harlizius *et al.*, 2004). The current knowledge indicates that the major genes causing variability for similar traits in different species or breeds are rarely the same. Therefore, the study of the species of interest is needed even if research in farm animals strongly relies on the genomic knowledge in other species such as humans and model organisms (Harlizius *et al.*, 2004).

#### *Limitations of genomics*

Many of the limitations to genomics research are of a technical nature. The technical limitations of expression array technology are still under active discussion (Shields, 2006; Sievertzon *et al.*, 2006). Array technology is based on the principle that probes (cDNA from expressed sequence tag (EST) libraries or oligonucleotides) can be arrayed on membranes or glass slides for hybridisation with labelled targets, which originate from the biological samples of interest. The flatness of the glass support makes it possible to miniaturise the procedure. The resulting images of hybridisation are captured and analysed using software that quantifies the signal of each spot. The intensity of each spot is proportional to the amount of mRNA of the corresponding gene expressed in the studied sample.

The major technical problems that have affected expression array technology are associated with the spotted cDNA arrays which were the first forms of DNA expression arrays available and which are now almost superseded by spotted oligo arrays or manufactured oligo arrays. Problems with cDNA arrays apply generally to all species and range from the availability and inconsistent quality of molecular probes to be printed on the arrays and difficulties in array preparation (inconsistent fidelity across arrays, low specificity or low concentration of printed probes) and utilisation (Murphy, 2002; Drobyshev *et al.*, 2003). Commercial microarray platforms, particularly those that employ *in situ* synthesis of oligomer probes to construct arrays are now available for the bovine and are reducing these sources of variation to very low levels. Consistent protocols for array experimentation (RNA extraction from samples, labelling, and hybridisation) and data acquisition (image capture and normalisation procedures) are emerging and microarrays are considered a reliable method for quantitating gene expression data (Canales *et al.*, 2006).

The statistical analysis of genomic data is a challenge shared by all genomics researchers. The challenge begins with the design of large animal experiments that nevertheless contain sufficient biological replication to allow the discovery of gene expression associations with a high level of confidence. The animal genomics community has contributed to the development of microarray analysis methods by adapting the use of mixed model analysis to

microarray experimentation (Reverter *et al.*, 2003; Pfister-Genskow *et al.*, 2005). In proteomics, research by INRA scientists has also allowed improvement in statistical analyses by adapting the SAM (significance analysis of microarray) method (Meunier *et al.*, 2005) and clustering methodologies (Meunier *et al.*, 2007) to two-dimensional gel electrophoresis experiments.

A last challenge is to share, within the bovine community, the genomic data related to beef quality (from genetic information, transcriptomics and proteomics to phenotypes). Consequently, it is now accepted that the comparison of results from different laboratories requires standardisation procedures which have been proposed for both transcriptomic (Brazma *et al.*, 2001) and proteomic (Taylor *et al.*, 2003) analyses. Methods to jointly analyse microarray experimentation have been developed for a large set of bovine muscle microarray results (Reverter *et al.*, 2005). In addition, methods have been developed for computing networks of correlated gene expression from bovine muscle gene expression data (Reverter *et al.*, 2006).

Powerful data management tools and computational techniques are also required to store, analyse, and compare the increasing amount of genomic information in cattle. The number of expressed sequenced tags in NCBI's GenBank has now surpassed 1 million for cattle. Other specific bioinformatic databases have been developed.

Some of the existing databases and associated bioinformatic tools are devoted specifically to applications within livestock species, especially cattle (Table 1). For instance, most of the single-locus traits in livestock are listed in the OMIA (Online Mendelian Inheritance in Animals) database. Other databases have been constructed in UK (Hu *et al.*, 2001), Australia (Hawken *et al.*, 2004) and France (Table 1).

### **Bovine genome sequencing and applications for breeding**

#### *Bovine genome sequence*

Sequencing of the bovine genome started in December 2003. It is under way at the Baylor College of Medicine Sequencing Centre in Houston (Texas, USA).

As set out below, the availability of the bovine genome sequence will be useful for both agricultural purposes and human health. Accordingly, the contributors to the \$53 million international effort were: the National Human Genome Research Institute, USDA (NRI and ARS), State of Texas, Genome Canada, Kleberg Foundation, Commonwealth Scientific and Industrial Research Organisation (CSIRO) Australia, New Zealand, United States Beef Council, Texas Beef Council and South Dakota Beef Council.

The animal whose DNA was sequenced is a Hereford cow, named L1 Dominette 0144. The second draft of the bovine genome sequence has been deposited into free public databases. The current assembly (Btau 3.0, August 2006) is based on a 7.1-fold coverage of the bovine

**Table 1** Some specific bioinformatics tools and browsers for bovine genomics (extracted, adapted and updated from Fadiel *et al.* (2005))

| Name   | Features   | Developer  | Web address   |
|--|--|--|---|
| OMIA (Online Mendelian Inheritance in Animals)<br>CSIRO Livestock Genomics | Database for animal genetics (single trait loci)<br>Ruminant-Human Genome Browser, Bovine genome browser, EST-based SNP database (IBISS)                                   | ANGIS, Australia<br>CSIRO, Australia   | omia.angis.org.au/<br>www.livestockgenomics.csiro.au                                  |
| The Bovine Genome Database<br>Information System of AGENAE programme       | QTL viewer, bovine genome browser, EST database<br>Farm Animals EST Contigs Access through Ensembl and BioMart   | Texas A&M University, USA<br>INRA, France  | www.bovinegenome.org/<br>www.sigenae.org/   |
| Bovine genome project<br>NCBI Cow Genome Resources<br>UCSC Genome Browser  | Current genome assembly and BLAST search facility<br>Comprehensive links to bovine genome resources<br>Bovine genome browser, particularly useful for comparative genomics | Baylor College of Medicine, USA<br>NCBI, USA<br>University of California at Santa Cruz | www.hgsc.bcm.tmc.edu/projects/bovine/<br>www.ncbi.nlm.nih.gov<br>www.genome.gucsc.edu |
| Ensembl Genome Browser   | Bovine genome browser  | Wellcome Trust Sanger Institute in<br>Cambridge, England                               | www.ensembl.org   |
| AgBase: a functional genomics resource<br>for agriculture                  | Structural and functional annotation of agricultural<br>annotation to facilitate systems biology   | Mississippi State University   | www.agbase.msstate.edu<br>McCarthy <i>et al.</i> (2006).                              |

genome. The sequence data can be accessed via GenBank at NIH's National Centre for Biotechnology Information (NCBI), or via EMBL Bank at the European Molecular Biology Laboratory's Nucleotide Sequence Database and via the DNA Data Bank of Japan. A number of browsers have been developed for the bovine genome: NCBI's Map Viewer, UCSC Genome Browser at the University of California at Santa Cruz, the Ensembl Genome Browser at the Wellcome Trust Sanger Institute in Cambridge, England and the Ruminant-Human Genome Browser at CSIRO Australia (Table 1).

Sequencing at lighter coverage, to derive additional SNP, is being carried out in other breeds, such as the Holstein, Angus, Jersey, Limousin, Norwegian Red and Brahman and there are plans to also sequence the bovine Y chromosome.

In a white paper (Gibbs *et al.*, 2004), the rationale and objectives of the bovine genomic sequencing initiative were set out. Eight different biological objectives were considered. The first one is to improve human health. Understanding genetic interactions with environmental factors will indeed be a major aim of medical research in the next decades. The bovine is a valuable model to achieve this goal because a vast amount of research has been and will be conducted to study the genetic  $\times$  environment interaction in this species in the case of multigenic physiological traits. The second objective is informing mammalian biology. The bovine is indeed a good model for understanding endocrinology, developing reproductive techniques and studying reproductive or metabolic diseases. For example, different cattle breeds have different capacity to deposit fat. Detailed knowledge of the molecular regulation of this trait is important for both the production of marbled beef and the understanding of obesity and diabetes in human beings. The third and fourth objectives of the bovine genome sequencing are to contribute to the annotation of the human genome sequence and to provide additional links between the sequence of humans and other species by comparative genomics. This was recently done by a high-resolution whole-genome cattle-human comparative map: 3 204 ordered markers in the bovine genome over a total of 3 484 are anchored in the human genome. The comparative coverage relative to the human genome is about 91%. This will facilitate identification of candidate genes for economically important traits (Everts-van der Wind *et al.*, 2005). The fifth objective is to expand our knowledge of the biological mechanisms related to health. It is suggested that alternative transcripts and post-translational modifications must play a major role in phenotypes. The phenotypic diversity of many cattle breeds throughout the world is a tremendous resource to study the molecular mechanisms which control tolerance to pathogens, altitude or heat tolerance, susceptibility to any type of illness, etc. The sixth and seventh objectives are to provide surrogate systems for human experimentations and to facilitate the ability to do experiments, especially in genetics. The last objective is to contribute to the understanding of evolutionary processes.

To summarise, it is expected that the bovine genome sequencing, and more generally, any genomic work in cattle will have an impact not only on the livestock industries, but also on all other mammalian research, especially research into human genetics and health.

#### *Current knowledge of genetic markers in bovine*

The practical genetic issues of genomics have been described elsewhere (Renand *et al.*, 2003; Smith *et al.*, 2003; Kühn *et al.*, 2005). In a few words, the objective of breeders is to exploit the variability among animals by selecting those with a superior genetic potential for the trait of interest. Many traits suitable for improvement using quantitative genetic methods are easy or inexpensive to measure on a great number of animals, but many of the important traits are not. Several of the meat quality traits, for example, are difficult to improve, such as meat tenderness and marbling, so researchers are currently looking for genes that influence genetic potential for these traits to select animals. This is a promising approach because genotyping is now increasingly easy and inexpensive to perform. One important benefit of high-throughput techniques of genomics will indeed be the identification and the practical use of polymorphisms within DNA sequences of those genes (Garnier *et al.*, 2003; Barendse, 2005). Single nucleotide polymorphisms (SNP) are the most frequent form of DNA variation in mammals. They are abundant, normally biallelic and easy to detect by automatic techniques.

So far, polymorphisms in some genes have been reported for their association with tenderness or beef marbling (i.e. the amount of visual intramuscular fat) (Barendse, 1997; 2001 and 2003 and Barendse *et al.*, 2004). They include the genes encoding  $\mu$ -calpain (Page *et al.*, 2002; White *et al.*, 2005) and lysyl oxidase and calpastatin (Barendse, 2001; Drinkwater *et al.*, 2006) for tenderness. They also include the genes encoding leptin (Buchanan *et al.*, 2002), DGAT1 (Thaller *et al.*, 2003), TG (Barendse *et al.*, 2004), RORC (Barendse, 2003), GH1 (Schlee *et al.*, 1994), SCD, mitochondria (Mannen *et al.*, 1998; 2003), mitochondrial transcription factor A (Jiang *et al.*, 2005) and FABP4 (Michal *et al.*, 2006) for marbling. Summaries of the tests have been published (e.g. Dekkers, 2004; Jeon *et al.*, 2006) although no listing of markers is comprehensive. Other efforts are devoted to the identification of SNP in a large set of candidate genes which are associated with meat quality traits measured in 15 different European breeds. This is the EU-funded project (GeMQual, <http://www.gemqual.org/>). So far, a total of about 710 SNP have been identified in 209 genes and these SNP are being genotyped in 450 bulls from the 15 breeds. The expected results are SNP that may have an effect on the carcass, muscle or meat quality traits (Hocquette *et al.*, 2006). Table 2 contains a list of all the commercially available DNA tests for cattle, which can be obtained from web searches, although there are more tests in the literature than there are tests being offered to farmers.

Many of these associations have been confirmed in independent studies, although some have failed to be confirmed. Confirmations for the calpain and calpastatin genes have been reported by several authors (Page *et al.*, 2004; Casas *et al.*, 2006; Morris *et al.*, 2006; Schenkel *et al.*, 2006). For marbling several of the genes have been confirmed in some studies while in others some tests have failed to be confirmed (Thaller *et al.*, 2003; Nkrumah *et al.*, 2004; Barendse *et al.*, 2005; Kononoff *et al.*, 2005; Nkrumah *et al.*, 2005; Pollak, 2005; Schenkel *et al.*, 2005; Barendse *et al.*, 2006; Rincker *et al.*, 2006). Marbling is a more difficult trait to work with than tenderness, since it is a visual inspection of the carcass and hence is a measurement with a greater error, which means that it will require larger samples than normal to be detected. The failure to confirm an association usually occurs in samples where there are fewer than 1000 animals in the study. At present there are no guidelines on the composition of confirmatory studies, and different approaches are being taken in different countries. Most of the confirmations are *ad hoc*, however, using whatever samples are to hand. In some confirmations, the commercialiser is asked what samples they want used to evaluate the gene. Such approaches are unsatisfactory since they can lead to the type II error of accepting a null hypothesis as true when it is false, due to inadequacies in the experimental design, primarily due to sample sizes being too small. Clearly, sample sizes need to be large enough to ensure that this sort of error is minimised (Barendse, 2005).

A number of high throughput SNP genotyping platforms have recently become commercially available for the bovine. The MegAllele™ Genotyping Bovine 10K SNP Panel assays approximately 10 000 SNP discovered by the Bovine Genome Sequencing Project and the CSIRO. A 32 000 SNP update of this panel is about to be commercially released. Similarly, the bovine BeadChip™ genotyping system allows around 10 000 bovine SNP to be assayed in parallel. A new version of the bovine iSelect BeadChip™ is under development in collaboration with the USDA-ARS, the University of Missouri-Columbia and the University of Alberta. This genotyping platform will allow the analysis of 12 samples in parallel on a single microarray with over 48 000 SNP per sample. Research groups all over the world are currently applying these genotyping tools to populations of cattle extensively phenotyped for beef and dairy production and health traits. In the future, more than 1 million SNP within the *Bos taurus* breeds and well over 2 million with the Brahman animal included are predicted by comparing the Hereford genome assembly with traces from other breeds (Womack, 2006).

Rapid progress in discovering patterns of genetic variation that can predict animal performance can be expected from these international efforts. The increased knowledge of functional candidate genes, which will be discovered from functional genomics approaches (array data, proteomic studies) is expected to further contribute to the progress of this field.

**Table 2** Commercialised DNA tests for cattle QTL

| Gene <sup>†</sup> | Trait               | Year <sup>‡</sup> | Discoverer                     | Commercialiser <sup>§</sup> |
|-------------------|---------------------|-------------------|--------------------------------|-----------------------------|
| TG                | Marbling            | 2000              | CSIRO/MLA                      | Genetic Solutions P/L       |
| CAST              | Meat tenderness     | 2002              | CSIRO/MLA/Beef CRC             | Genetic Solutions P/L       |
| CAPN1             | Meat tenderness     | 2003              | USDA/AgResearch NZ             | Open                        |
| DGAT1             | Milk fat yield      | 2003              | Univ. of Liege/Tech Uni Muench | Merial                      |
| GH1               | Marbling            | 2003              | NIAS, Japan                    | Prescribe Genomics CO       |
| LEP               | Marbling/fat traits | 2003              | Univ. of Saskatchewan          | Merial                      |
| Multiple tests    | Tenderness          | 2003/4            |                                | Genetic Solutions P/L       |
| GHR               | Milk yield          | 2004              | Univ. of Liege                 | Merial                      |
| SCD               | Fatty acid ratios   | 2004              | Kobe University                | Prescribe Genomics CO       |
| Multiple tests    | Marbling            | 2004              |                                | Genetic Solutions P/L       |
| Multiple tests    | Multimark dairy     | 2004/5            |                                | Merial                      |
| CAPN3             | Meat tenderness     | 2006              | CSIRO/MLA/Beef CRC             | Genetic Solutions P/L       |
| Multiple tests    | Feed efficiency     | 2006              | CSIRO/MLA/Beef CRC             | Genetic Solutions P/L       |

<sup>†</sup> Multiple tests are those based on several genes, although some tests are not based on sequences directly associated with genes, and for some tests the identity of the sequence has not been revealed.

<sup>‡</sup> Year is year commercialised.

<sup>§</sup> First commercialiser, some of the tests, such as those based on CAPN1, CAST and LEP are now offered by several laboratories.

There are other discoveries of DNA markers but these are not yet available as commercialised DNA tests.

Tests for Casein and Lactoglobulin, originally milk protein variants, now DNA tests, not included in this Table, these were originally described and worked on in studies from the 1960s to mid 1984, see for example Genmark AG.

Table does not include (1) colour mutations, (2) disease mutations, (3) mutations for discrete traits such as poll/horn.

Henderson *et al.* (2005) predict that there are three challenges currently facing the beef industry with the use of genetic markers in genetic selection: (i) the adequate collection of DNA marker information by breed associations, (ii) quality control mechanisms for use and interpretation of DNA marker information, and (iii) increased expertise to use genetic markers discovered by the scientific communities. Furthermore, the improvement of the efficiency of marker-assisted selection does require the characterisation of regulatory sequences of gene expression, the development of methods to rapidly screen genomes of multiple individuals, a better understanding of gene functions and interactions, and more emphasis on complex traits with low heritabilities.

### Available molecular tools for transcriptomic studies

#### *Tissue-specific cDNA arrays for beef quality research*

Before the development of bovine cDNA microarrays, some groups used available human microarrays in cross-species hybridisation studies. For instance, RNA from cattle skeletal muscle (Sudre *et al.*, 2003 and 2005a) and oocyte (Dalbies-Tran and Mermillod, 2003) have been hybridised on human cDNA microarrays. However, because cross-species hybridisation may generate spurious results, researchers have paid careful attention to data analysis. Therefore, interesting results were obtained despite loss of information associated with the technical constraints.

Many different research teams throughout the world have published the construction of bovine cDNA libraries from various tissues including liver and intestine (Dorroch *et al.*, 2001; Herath *et al.*, 2004), embryos (Potts *et al.*, 2003; Renard *et al.*, 2004b), endometrium (Renard *et al.*, 2004a), uterus and ovaries (Anderson *et al.*, 2004) and

several pooled tissues (Smith *et al.*, 2001). Several different bovine cDNA arrays were generated, specifically covering the transcriptome of, for example immune cells and tissues (Donaldson *et al.*, 2005; Jensen *et al.*, 2006) and other tissues of interest (Moody *et al.*, 2003). None of these molecular tools is specifically tailored for functional genomics studies of beef quality. Therefore, arrays have been constructed from cDNA libraries derived from bovine muscles or muscle and adipose tissue (Cho *et al.*, 2002; Lehnert *et al.*, 2004). The number of array elements printed on these tissue-specific arrays was generally lower than that derived from multiple tissues (Suchyta *et al.*, 2003).

Researchers from the CRC for Cattle and Beef Quality in Australia constructed a bovine microarray of 9600 elements comprising ca. 2000 EST and ca. 7300 anonymous cDNA clones from muscle and fat-derived cDNA libraries (Lehnert *et al.*, 2004). The microarray also includes a collection of 'candidate genes' from the adipogenesis and protein turn-over literature. The main classes of genes represented on the microarray are coding for: contractile proteins (e.g. myosins, actins, etc.), extracellular matrix genes (e.g. collagens), mitochondrial enzymes (e.g. ATPases, cytochrome-c oxidase, etc.) and adipogenesis-related molecules or binding proteins (e.g. SCD, FABP4). These gene families are associated with the main cellular components of muscle tissue: the muscle fibres themselves, connective tissue and intramuscular fat islands. The bovine fat/muscle microarray has been used in a number of experiments to track gene expression changes in muscle tissue during nutritional treatments (Byrne *et al.*, 2005; Lehnert *et al.*, 2006) and in breed comparisons (Wang *et al.*, 2005a and b). The array has clear limitations for the discovery of regulatory mechanisms in muscle tissue development, as it was based on adult tissues only and therefore has very

poor representation of probes for transcription factor genes and other regulatory factors.

One muscle specific library was prepared in France from selected various muscle types (oxidative or glycolytic) sampled from different bovine animals of various age (foetal and post-natal) and various breeds. Among the 1440 cDNAs characterised so far, 1019 from several gene families could be exploited: mitochondrial genes (133), genes encoding ribosomal proteins (100), contractile proteins, metabolic enzymes, etc. Finally, 353 cDNAs from this library and 75 cDNAs individually prepared by RT-PCR were considered as a first bovine muscle cDNA repertoire (Sudre *et al.*, 2005b).

#### Pooled-tissue cDNA arrays

Tissue specific microarrays, particularly when they are based on adult tissues, have very clear limitations with respect to discovery of regulatory interactions, as they do not capture the whole transcriptome of the organism. cDNA microarrays with genome-wide representation have therefore been a goal of the scientific community since many EST collections became available and could be pooled (Table 3).

A bovine cDNA array (CattleArray 7600 from Pyxis Genomics) became available commercially in 2003. Since this array has been made with cDNAs derived from spleen and placenta, it may not be suitable to explore muscle biology and hence beef quality. A number of high-density cDNA arrays constructed for assessing genome-wide gene expression changes in cattle now include genes from muscle-specific libraries.

The NBFGC in the USA has selected 18263 genes from the pooled tissues MARC 1–4 libraries for printing on a high-density microarray (Suchyta *et al.*, 2003). Scientists at the Iowa University have followed the same strategy. They selected a set of 10608 EST from MARC 1–4 libraries which include all the known genes and unknown, putative or hypothetical genes expressed in muscle, liver or adipose tissue. The selected genes include 4484 known genes, 1170 hypothetical proteins, 497 unknown and 4364 not assigned genes. From a database of human genes expressed in muscle, liver and adipose tissue (<http://telethon.bio.unipd.it/GETProfiles/Index.html>), it was calculated that the collection contains 66.37%, 71.02% and 67.34% of the human genes expressed in these three tissues respectively. This new library was spotted with a set of negative and positive controls. This new molecular array has been called the *BoviAnalyser*. It is advertised as suitable to analyse cell proliferation and differentiation in response to, for instance, disease resistance or the ability of the animals to produce marbled beef.

Scientists at the Roslin Institute's ARK-Genomics Centre in the UK have constructed high-density microarrays based mainly on its collection of immune-specific EST (McGuire and Glass, 2005) and AgResearch has been using a 10 204 bovine cDNA microarray with broad genome coverage in bovine and ovine transcriptome studies (Diez-Tascón *et al.*, 2005).

**Table 3** Some bovine gene expression arrays suitable for beef quality studies (extracted, adapted and updated from Moody *et al.* (2003)). The reader should note that cDNA arrays are becoming superseded by oligo arrays and whole genome arrays

| Array   | Type   | Developer                         | Details  | Reference or site  |
|---|--|-----------------------------------|--|--|
| MEM array   | cDNA   | France                            | 1896 selected cDNAs from muscle, mammary gland and embryo                            | Bernard <i>et al.</i> (2005).  |
| NBFGC Microarray  | cDNA   | Michigan State University         | 18 000 transcripts, multiple tissue libraries (MARC 1–4)                             | Suchyta <i>et al.</i> (2003)   |
| BoviAnalyser  | cDNA   | Iowa State University             | 10 608 cDNAs from MARC libraries,  | <a href="http://www.ans.iastate.edu/report/air/2004pdf/AS1877.pdf">www.ans.iastate.edu/report/air/2004pdf/AS1877.pdf</a> |
| Beef CRC muscle and fat array                             | cDNA   | CSIRO and Beef CRC, Australia     | 9000 probes from skeletal muscle and adipose tissue cDNA libraries                   | Lehnert <i>et al.</i> (2004)   |
| <i>Bos taurus</i> (bovine) OpArray                        | Long oligo array                             | Operon Biotechnologies            | 8329 longer probes plus additional controls representing 8329 bovine EST transcripts | <a href="http://www.operon.com">www.operon.com</a>   |
| GeneChip®   | <i>In situ</i> synthesised short oligo array | Affymetrix                        | Ca. 23 000 genes, probes (March 2004 GenBank content)                                | <a href="http://www.affymetrix.com">www.affymetrix.com</a>   |
| Bovine whole genome long oligonucleotide expression array | Long oligo set                               | Bovine Microarray Consortium, USA | unique genes, based on 2005 EST databases  | Elsik <i>et al.</i> (2006)   |
| <i>Bos taurus</i> eArray                                  | Long oligo array (60 mer)                    | Agilent                           | 21 475 unique probes, representing ca. 19 500 distinct bovine genes                  | <a href="http://earray.chem.agilent.com/earray">earray.chem.agilent.com/earray</a>                                       |

French scientists have also prepared cDNA libraries from bovine mammary gland (Le Provost *et al.*, 1996) and embryos (Renard *et al.*, 2004b). Their strategy is now to combine high-quality cDNA clones from their different libraries (namely from muscle, mammary gland and embryo) in the same molecular tool for gene expression profiling in bovine tissues involved in reproduction and production traits. This was reported as successful (Bernard *et al.*, 2005). In addition, a selection of 13 168 clones from the MARC 1–4 libraries (75%) and from INRA clones (25%) has been made to prepare an array suitable for many applications in cattle (<http://sigenae.jouy.inra.fr/>).

While cDNA libraries and cDNA arrays have allowed the bovine research communities to make rapid progress in transcriptomics, this technology platform is currently becoming superseded by oligo arrays and whole genome arrays.

#### *Oligo arrays*

Microarrays of synthetic oligonucleotide probes are becoming increasingly popular for transcriptomic studies, including for bovine (Table 3). The power of this approach lies largely in the opportunity to tap into the bovine genome and EST sequencing effort without the need to own libraries that contain probes of interest. Oligo arrays avoid some of the technical pitfalls of cDNA arrays, namely their lack of reliability and spot variability. The reliance on microbiological libraries of clones and their inherent contamination and handling risks has caused problems with reliability for many practitioners. The majority of the variability of microarray spots on cDNA arrays is due to the non-uniform performance of PCR reactions in probe generation. Oligo arrays are therefore of much better quality than cDNA arrays, even in heterologous systems. For instance, muscle-specific oligo arrays initially prepared for studies in mice and humans were shown to be well adapted to muscle gene expression studies in cattle (Bernard *et al.*, 2006b). Many bovine-specific oligo arrays have also been developed for transcriptomic studies in cattle (Table 3).

Operon technologies, Inc. market a bovine oligo probe set for microarray printing. It is derived from TIGR assemblies of bovine EST available in 2004 and contains 70mer probes (about 8 300 bovine genes with a known sequence with a hit to a known human, mouse, or cattle transcript). A bovine long oligo array project has been developed among a consortium of researchers at Texas A&M University, University of Missouri, Iowa State University, and the University of Minnesota. GenBank sequences are being analysed to remove duplicated sequences, vector sequences and other artefacts of the cloning process. This array was made available to the international community in 2006 (Elsik *et al.*, 2006). Another oligo array project is currently developed between the University of Illinois, the University of Connecticut and INRA in France.

Affymetrix markets a high-density microarray, the GeneChip<sup>®</sup> Bovine Genome Array. It is representative of the publicly known, high-quality bovine sequences that were

available in March 2004. It contains 24 027 probes. It is advertised to be suitable for studies related to disease resistance, meat and dairy production as well as stress tolerance.

#### **Applications of functional genomics**

Numerous results from transcriptomic studies have been obtained so far in bovine health, normal physiology or pathology. Gene expression profiling has been used to document changes in gene expression for example following infection by pathological organisms (Meade *et al.*, 2006), during the metabolic changes imposed by lactation in dairy cows (Loor *et al.*, 2005), in cloned bovine embryos (Smith *et al.*, 2005; Somers *et al.*, 2006) and in various other models (reviewed by Henderson *et al.*, 2005 and by Hocquette *et al.*, 2005).

It has been forecast that the identification of differentially expressed genes may be of high importance for muscle growth and development (Reecy *et al.*, 2006) and also for both genetic and physiological studies related to beef quality (Eggen and Hocquette, 2004). Despite this enthusiasm, some pitfalls need to be avoided. There are several reasons why the differentially expressed genes may not necessarily be the causal genes: (i) the lack of information collected on low-expressed genes (such as transcription factors and cellular regulators) is a limitation of the technology, (ii) muscle tissue is a complex mixture of cell types (myofibres, connective tissue fibroblasts, endothelial cells and adipocytes) and array studies can be simply affected by proportional changes in all these cell populations. Efforts to characterise the histological phenotype of the tissue alongside the gene expression phenotype will go some way towards addressing these issues. Notwithstanding these limitations, gene and protein expression profiling approaches have been directed at muscle of cattle (Table 4) and other livestock species, such as the pig (Lin and Hsu, 2005; Plastow *et al.*, 2005; Cagnazzo *et al.*, 2006). Some recent insights into bovine muscle biology that have been obtained by cattle muscle profiling are described, as follows.

#### *Comparison of gene expression between muscle types*

The first aim when looking at gene expression in various muscle types is to get a better understanding of muscle characteristics, which determine meat quality traits across muscles since the major factor of variability of tenderness is the muscle type rather than cattle breed or animal type (heifer, young bulls or cull cows) (Dransfield *et al.*, 2003). Furthermore, some beef quality indicators, such as shear force, which is an indication of tenderness, are poorly correlated between different muscle types (Shackelford *et al.*, 1995). Therefore, understanding differences between muscle types is of major importance in meat science.

Differences between bovine muscle types were studied by using human macroarrays (Sudre *et al.*, 2003 and 2005a). As these arrays were initially prepared for other



**Table 4** Some examples of current initiatives in bovine genomics research applied to beef quality (extracted, adapted and updated from Henderson *et al.* (2005) for US projects)

|   | Location  | Objectives  |
|---|---|---|
| NC-1010   | USA   | (i) Location, structure, function and expression of genes affecting health, reproduction, production and product quality in cattle, (ii) interpret and apply genomics and proteomics information by statistical/bioinformatics methods and utilising molecular tools in cattle, and (iii) educational materials about genomics research to consumers and stakeholders |
| NRSP-8  | USA   | (i) Genetic and physical maps of ruminants for cross-species comparisons and sequence annotations, (ii) genomic, transcriptomic, proteomic and metabonomic approaches toward better understanding of biological mechanisms underlying economically important traits, and (iii) bioinformatics tools to extract, analyse, store and disseminate information            |
| ARK-Genomics Centre for Comparative & Functional Genomics in Farm Animals | UK <a href="http://www.ark-genomics.org/">http://www.ark-genomics.org/</a>            | (i) Identification of traits controlling genes of agricultural and biomedical importance in farm animals, (ii) integration of genomics and physiological approaches to gene discovery, (iii) development of a functional genomics toolkit, resources and facilities for research in farm animals  |
| FUGATO (Funktionelle GenomAnalyse im Tierischen Organismus)               | Germany <a href="http://www.fugato-forschung.de/">http://www.fugato-forschung.de/</a> | (i) Host-pathogen interactions, (ii) biology and biotechnology of reproduction and (iii) product quality (the QualIPID project aims at the functional analysis of genes involved in lipid metabolism)   |
| AGENAE (Analyse du GENome des Animaux d'Eleavage)                         | France <a href="http://www.inra.fr/agenae/">http://www.inra.fr/agenae/</a>            | Analyse of genes determining physiological functions of four species (bovine, pig, poultry and trout). Specific projects concern bovine muscle characteristics and beef quality: MUGENE (integrated biology of beef quality including genetic markers, transcriptomics and proteomics) and QUALVIGENE (genetic markers of beef quality)                               |
| Beef CRC (Cooperative Research Centre for Beef Quality)                   | Australia <a href="http://beefcrc.org.au">http://beefcrc.org.au</a>                   | (1) High quality beef for global consumers, (2) feed efficiency, maternal productivity and responsible resource use, (3) adaptation and cattle welfare, (4) female reproductive performance   |

research objectives in humans, few differences were detected between muscles, however some genes of interest for muscle biology were revealed. Some of them were linked to the contractile (nebulin, actinin-associated LIM protein) or metabolic (ICDH  $\beta$ -subunit precursor) properties of muscles. Two interesting differentially expressed genes were LEU5 and Trip 15. LEU5 is a tumour suppressor gene associated with B-cell chronic lymphocytic leukemia. Trip 15 is a thyroid receptor interacting protein. They are probably involved in the regulation of muscle development. The differences between muscle types appeared to depend on the stage of development and the genetic types of animals (Sudre *et al.*, 2003 and 2005a).

Similar studies should also be performed to detect the specificity of intramuscular adipose tissue (which is important for beef flavour) in comparison to other fat tissues of the carcass undesirable for beef producers.

#### *Gene expression according to the production system*

The influence of two production systems (pasture *v.* maize-silage indoors) on muscle gene expression was studied in France. Transcriptomic analyses using a multi-tissue bovine

cDNA repertoire were performed to compare gene expression profiling in *rectus abdominis* and *semitendinosus* muscles between both production groups. Variance analysis showed that the muscle type has an important effect on the expression level of genes. The effect of the production system was less marked. A list of the 30 most variable genes was established, of which 15 muscle genes were considered. Amongst them, the Selenoprotein W, which was found to be under-expressed on pasture, could be considered as an indicator of pasture-based system (Cassar-Malek *et al.*, 2005a). However, whether variation of Selenoprotein W gene expression is linked to the feeding regime (grass *v.* maize-silage), or to the selenium status of the diet is still unclear.

#### *Gene expression throughout development*

Before the development of bovine cDNA arrays, human muscle-specific arrays were used to monitor gene expression throughout development (Sudre *et al.*, 2003). This allowed the identification of up to 110 genes differentially expressed from 110 days post-conception to 15 months of age after birth. Among these genes, 33% have

unknown functions so far. Most of the differentially expressed genes are either up-regulated or down-regulated at 260 days of foetal development. This confirms the importance of the last three months of gestation in bovine muscle differentiation (Picard *et al.*, 2002). Indeed, the number of muscle fibres is roughly fixed at two thirds of foetal development and muscle fibres differentiate before birth making cattle a relatively mature species at birth compared to other mammals. Not only did the microarray experiment confirm this general view, but it also helped in the discovery of putative interesting genes regulated throughout development and involved in cell homeostasis (e.g. Sialyltransferase 8) or cell regulation (e.g. activin A, the thyroid receptor interacting protein 15) and in metabolic (e.g. oxoglutarate dehydrogenase) or contractile (e.g. nebulin) muscle properties (Sudre *et al.*, 2003). Further studies are needed to explore the biological functions of the novel identified genes.

#### *Muscle gene expression profile associated with marbling*

Some sectors of the beef industry are looking for gene markers that would identify animals that have a high propensity to accumulate intramuscular fat in order to produce tasty and tender meat. In addition to research on gene expression in perirenal adipose tissue before and after fattening (Oishi *et al.*, 2000), research by Childs *et al.* (2002) aimed to identify genes associated with intramuscular fat development. Differential-display polymerase chain reaction allowed the identification of a known gene (NAT1, a translational suppressor) by comparing muscle RNA from different finishing periods on high grain feeding. NAT1 was not previously suspected to play a role in fat accumulation. Putative functional genes were found to be differentially expressed (e.g. ATP citrate lyase) or, surprisingly, not differentially expressed (e.g. PPAR $\gamma$ ) between extreme animals.

Australian and Japanese scientists undertook a microarray-based comparison of the *longissimus* muscle (LM) from Japanese Black (JB) and Holstein (HOL) cattle over an extended intensive feeding period to identify genes that may be involved in determining the unique ability of JB cattle to deposit intramuscular (IM) fat with lower melting temperature (Wang *et al.*, 2005a). Three consecutive biopsies from LM tissue were taken and RNA isolated from three JB (Tajima strain) and three Holstein (HOL) animals at age 11 to 20 months. The gene expression changes in these samples were analysed using a bovine fat/muscle cDNA microarray (Lehnert *et al.*, 2004). A mixed-ANOVA model was fitted to the intensity signals. Three hundred and thirty-five (4.8%) array elements were identified as differentially expressed genes in this breed  $\times$  time comparison study. Genes preferentially expressed in JB are associated with monounsaturated fatty acid (MUFA) synthesis, fat deposition, adipogenesis development and muscle regulation, while examples of genes preferentially expressed in HOL come from functional classes involved in connective tissue and skeletal muscle development. The gene expression differences detected between the LM of

the two breeds give important clues to the molecular basis for the unique features of the JB breed, such as the onset and rate of adipose tissue development, metabolic differences, and signalling pathways involved in converting carbohydrate to lipid during lipogenesis. These findings will impact on industry management strategies designed to manipulate intramuscular adipose development at different development stages to gain maximum return for beef products.

#### *Gene and protein expression profile according to muscle growth potential*

Muscle hypertrophy is of particular interest in beef meat production as it has a strong economic importance. High muscle development is associated with specific muscle characteristics in favour of meat tenderness but not of its flavour. To address the consequences of selection for increased growth rate and reduced fat content of the carcass, transcriptomic analyses of two muscles (*rectus abdominis* oxidative and *semitendinosus* glycolytic) from young bulls divergently selected on muscle growth potential were performed. The selection process in favour of muscle growth decreased oxidative muscle activity especially in *rectus abdominis*, which is the most oxidative muscle of both. Some genes were differentially expressed in *rectus abdominis* and/or *semitendinosus* between the two groups of bulls with high or low muscle growth potential. Many of them are involved in muscle structure (e.g. sarcosin, titin) or in cellular regulation (e.g. thyroid hormone receptor interacting protein 10, LEU5, an heat shock protein) and were more expressed in muscles from low muscle growth potential bulls compared to high muscle growth potential bulls (Sudre *et al.*, 2005a).

Similarly, studies were conducted in double-muscling (DM) animals which are known to be characterised by a higher muscle mass and a lower fat content in the carcass and in the muscle tissue due to mutation in myostatin (a negative regulator of skeletal muscle growth which belongs to the transforming growth factor (TGF)- $\beta$  superfamily). One challenge is nowadays to elucidate the specific mechanisms, through which myostatin signals to inhibit the growth of skeletal muscle. About 20 genes were reported to be differentially expressed between DM and normal-muscling bovine embryos at 31 to 33 days of gestation. These differentially expressed genes fall into general classes including transcription factors ( $n = 5$ ), genes involved in protein synthesis and degradation ( $n = 8$ ), cell proliferation ( $n = 3$ ), or altered metabolism ( $n = 4$ ). Three of these genes were physically mapped to bovine chromosome 5 very close to a QTL for Warner Bratzler shear force (WBS) at day 14 *post mortem* (an indicator of the final toughness of beef). This may be a first step to explain the link between changes in meat tenderness associated with muscle hypertrophy (Potts *et al.*, 2003).

A recent study was conducted in order to compare the expression profile of muscle genes in the *semitendinosus* of double-muscling and non-double-muscling (NDM)

260-day-old fetuses using muscle-dedicated oligochips. The differential expression of several gene categories was found. Genes involved in slow contractile properties (e.g. TNNC1, TPM3, MYH7), extracellular matrix (e.g. collagen I and III, etc.) and ribosomal proteins (e.g. RPL3, RPL23, RPS24, RPS20) were found to be under-expressed in the DM fetuses. On the other hand, genes belonging to the regulation of cell cycle (e.g. p21cip1, E2F1, CTBP1), DNA metabolism and the regulation of transcription (e.g. HMGB1, mcm6, HDAC4, MEF2A, MyoD), and the protease (e.g. furin, TIMP4) were found to be over-expressed in the DM fetuses compared to normal ones. Interestingly, the expression of three differentially expressed genes (Foxc2, SIX3 and ZFX1B) was also found in DM cows, suggesting the putative involvement of these genes in the maintenance of muscle hypertrophy (Cassar-Malek *et al.*, 2005b). Further work is needed to understand their physiological implication in the development and modulation of muscle mass.

Similar transcriptomic studies were conducted in myostatin-knockout mice compared with wild-type mice using the Affymetrix GeneChip system. It was shown that myostatin may act upstream of Wnt pathway components, Wnt signalling being known to play a role in embryonic myogenesis. Several Wnt, including Wnt4, are capable of inducing some myogenic regulatory genes. Wnt4 is also capable of stimulating satellite cell proliferation. Based on gene expression results, a model was proposed in which Wnt4 is inhibited in the presence of myostatin, and activated in the absence of myostatin leading to stimulation of satellite cell proliferation. These results offer new insight into genes which may interact with myostatin to regulate skeletal muscle growth (Steelman *et al.*, 2006).

To further understand how muscle hypertrophy is controlled, a proteomic analysis was developed to identify some markers of muscle mass. Two models of cattle with different origins of muscle hypertrophy: monogenic (DM Belgian Blue bulls) or polygenic (divergent lineages of Charolais bulls: with high (H) or low (L) muscle growth rate) were studied. Differential proteomic analysis of *semitendinosus* muscle was performed using two-dimensional gel electrophoresis (4 to 7 pH gradient in the first dimension and 11% SDS-PAGE in the second) followed by mass spectrometric analysis of interesting spots (MALDI-TOF) as described in Bouley *et al.* (2004a). Proteomic analysis revealed 13 proteins corresponding to 28 protein spots significantly altered between DM and NDM muscles (Bouley *et al.*, 2005). Among the proteins differentially expressed between the two genotypes, eight were related to contractile apparatus including myosin-binding protein H, several myosin light chains and troponin T (TnT) including slow TnT and fast TnT. Two proteins were involved in metabolic pathways, including phosphoglucosmutase and the heart fatty acid-binding protein. Finally, three proteins were significantly altered including sarcosin, sarcoplasmic reticulum 53 kDa glycoprotein and p20. According to these data it seems that contractile properties are more modified than metabolic ones in DM muscles. Most of the modifications

of protein expression are in favour of fast glycolytic properties of DM muscle. This is coherent with the data of the literature showing an orientation of muscle type towards the fast glycolytic type in case of muscle hypertrophy (reviewed by Hocquette *et al.* (1998)). A similar analysis was conducted on *semitendinosus* muscle from H and L young bulls. Interestingly, it revealed that the proteins differentially expressed in DM muscle were also differentially expressed in H muscle (Picard *et al.*, 2005). Among these proteins, eleven spots of fast TnT (fTnT) were analysed more precisely. The expression levels of five of them were significantly increased in DM and H muscles. The levels of expression of the other six were unchanged or decreased in DM and H muscles. These fTnT isoforms differ in terms of the presence of the alternative splicing region corresponding to mutually exclusive exons 16 and 17 (Bouley *et al.*, 2005, Muroya *et al.*, 2003). All these data demonstrated that the TnT fast isoforms over-expressed in the muscle of high muscle growth potential (DM and H) contained the exon 16 and the others contain the exon 17. This could constitute a good protein marker of muscle hypertrophy. This higher expression of fTnT with exon 16 in muscles of high growth potential showing an over expression of fast contractile proteins is coherent with the data from the literature: indeed, Bucher *et al.* (1989) showed a correlation between the expression of fTnT exon 16 and fast contractile status of muscle. Also, Jin *et al.* (1998) found a higher proportion of fTnT cDNA encoding the mutually exclusive fTnT exon 16 in a fast glycolytic muscle (Pectoralis).

#### *Gene and protein expression associated with tenderness*

Transcriptomic and proteomic analysis of bovine muscle was also carried out in order to identify some markers of meat tenderness. For transcriptomic studies, samples of *longissimus* muscles from 14, 15- and 19-month-old Charolais bulls were analysed using oligo chips. Gene expression profiles were compared between high and low meat quality scores of tenderness, flavour and juiciness. Several genes were differentially expressed. Fourteen of them were highly correlated with flavour and juiciness, and one of them had a strong negative correlation with tenderness (Bernard *et al.*, 2006b). This gene called DNAJA1 was patented because it is responsible for up to 63% of the variability in meat tenderness (Bernard *et al.*, 2006a) although these results have to be validated using a greater number of samples. DNAJA1 encodes a protein which is a member of the large heat shock 40kDa protein family. This protein is a co-chaperone of Hsc70 and could to play a role in protein import into mitochondria. Its involvement in beef tenderness remains unknown.

Similarly, samples of the *semitendinosus* muscle previously sorted according to different eating quality classes on the basis of the results of sensory analysis (Dransfield *et al.*, 2003) were analysed by proteomic approaches. These muscles had been taken from 15-month-old bulls of three French breeds: Charolaise, Limousine and Salers. For

the Charolaise and Limousine breeds, five protein spots were significantly altered between two classes of tenderness (Bouley *et al.*, 2004b). Myosin binding protein H, Acyl-coA-binding protein, parvalbumin and a non identified protein were over-expressed in muscles of the high tenderness class. Myosin regulatory light chain 2 was under-expressed in the high tenderness class. However, these changes were not observed for the hardy Salers breed in which other proteins were altered. Because these preliminary results show that the same indicators were found in both beef breeds, this indicates that in hardy breeds such as Salers the determinism of tenderness might be different. Further analyses are in progress to complete these results on a more consequent number of samples.

## Conclusion

Genomic research in cattle has to be considered in its different aspects. From the technical point of view, cattle genomics cannot be considered in isolation but will be heavily influenced by the general progress in mammalian genomics. From the scientific point of view, we must recognise that some interesting results have been obtained so far with regard to linking genomics results with aspects of beef quality. These two aspects will be dramatically reshaped with the availability of the next draft of the bovine genome sequence. The last point of view, which is of tremendous importance for economic considerations, is the prospect of practical applications of genome-based technologies to the beef industry.

From the technical point of view, genomic research of cattle is somewhat lagging behind that of model organisms despite a great deal of technical research which was recently done in cattle. Many bovine cDNA libraries have been constructed, and various bovine arrays were prepared either from cDNA libraries or from oligonucleotide probe sets designed from publicly available bovine sequences. Basic improvements in genomics using model organisms are likely to produce new tools and knowledge useful for genomics in cattle. On the other hand, the involvement of statisticians experienced in genetic statistics in cattle genomic research teams has led to some innovative developments in basic statistic methods of general interest to the microarray community. The bovine genome sequencing is forecast to be very useful for mammalian biology and health by comparing the bovine genome sequence to that of other species.

From the scientific point of view, this review has reported differentially expressed genes which were detected during bovine muscle development, between different breeds or different genotypes, between different nutritional levels or between different types of diet. A few markers of muscle hypertrophy or of beef tenderness were also identified at the mRNA or protein levels. The challenge is now to check the relevance of those key genes or proteins with the aim to use them in the near future to improve breeding or rearing strategies. The muscle tissue is

however a complex tissue with different cell types (multi-nucleated fibres, adipocytes, etc.) in various proportions between muscles or even within the same muscle. Another challenge is to investigate which cell population is responsible for any change in gene expression.

From the economic point of view, the importance of genomics in modern agricultural practice is likely to increase. The education of producers is therefore a key issue in the adaptation of genomic research results to agricultural practice. The application of genomics and proteomics research has the potential to generate tools for beef producers that will help them to improve beef quality or to produce differentiated products. Over the longer term, these tools have the potential to change production methods in cattle breeding, husbandry and nutrition. In order to ensure efficient adoption of the outcomes of genomic research, ethical, legal, environmental, consumer and any societal concerns with the technology have to be addressed. A first success in that direction is that private partners representative of the beef industry are working together with public institutions to allow the development of the genomic research.

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