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New Indicators of Beef Sensory Quality Revealed by Expression of Specific Genes

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To identify new molecular markers of beef sensory quality, the transcriptomes of Longissimus thoracis muscle from 25 Charolais bull calves were analyzed using microarrays and compared between high and low meat quality groups; 215 genes were differentially expressed according to tenderness, juiciness, and/or flavor. Among these, 23 were up-regulated in the tenderest, juiciest, and tastiest meats, and 18 were highly correlated with both flavor and juiciness (e.g., *PRKAG1*), explaining up to 60% of their variability. Nine were down-regulated in the same meats, but only *DNAJA1* [the results relating to *DNAJA1* and its relationship with tenderness have been patented (Genomic marker for meat tenderness; Patent EP06300943.5, September 12, 2006)], which encodes a heat shock protein, showed a strong negative correlation with tenderness that alone explained 63% of its variability. This protein, known for its anti-apoptotic role, could be involved in meat aging. Thus, *DNAJA1* could constitute a new marker of beef sensory quality.

KEYWORDS: Gene expression; microarray; muscle; bovine; tenderness; juiciness; flavor

INTRODUCTION

Control of beef quality, and more particularly its sensory characteristics (tenderness, flavor, juiciness, and color), is important for beef producers and retailers to satisfy consumers' preferences. Multiple factors are involved in controlling beef sensory quality so great variation can be induced. This is why an increasing amount of research is being conducted to improve understanding of the impact of these factors on beef quality, especially on tenderness and flavor. These studies have shown that meat sensory quality depends not only on production factors such as breed, genotype, age, diet, growth path, or slaughter weight but also on technological factors (slaughtering conditions, aging time, cooking process) (for reviews, see refs *1*−*3*).

Genetic and rearing factors are known to affect the biological characteristics of muscles (fiber type, collagen, intramuscular adipose tissue, protease activities), which in turn regulate tenderness and flavor. Tenderness has two major components: the background toughness, which results from connective tissue characteristics (mainly collagen content and solubility), and the myofibrillar component. The latter is closely related to muscle fiber characteristics that control the tenderization phase characterized by post-mortem proteolysis, a major biological process involved in the conversion of muscle into meat (*2*). The intramuscular fat and its fatty acid composition determine meat flavor, and lipid oxidation is responsible for odors usually described as rancid. Juiciness is more difficult to evaluate, but it can be influenced by the structure of the meat and its water-binding capacity (for a review, see ref *3*). However, Renand et al. (*4*) have shown that less than one-third to a fourth of the variability in tenderness and flavor can be explained by variability in the muscle characteristics of live animals. This suggests that other muscle characteristics, so far unidentified, contribute to variability in tenderness and flavor.

The biological characteristics of muscle are controlled by gene expression. Knowledge of these genes and their expression profiles would provide a better understanding of muscle physiological processes and their influence on meat quality (*5*). Functional genomics (which includes analysis of the transcriptome and proteome) provides new opportunities for determining the molecular processes related to meat quality (*6*, *7*). Microarray technology enables multiple genes associated with variation in different sensory traits to be identified. To date, however, only a few studies have been conducted in this connection in cattle (*8*, *9*), mainly because of the lack of appropriate and specific tools for livestock species. Therefore, a great deal of effort has been put into preparing cDNA arrays specific for bovines (for instance, see refs *10*−*12*). In parallel, new technologies based on oligonucleotide arrays have been developed and an accurate

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selection of muscle-specific probes has become available from studies on mammals (*13*).

Taking advantage of progress in genomics, the aim of the present study was to identify new bovine muscle biological markers that are differentially expressed between beef meat cuts differing in tenderness, juiciness, and flavor. Long-oligonucleotide microarrays representing genes previously selected for their involvement in skeletal and cardiac muscle physiology were used to compare gene expression profiles in Longissimus thoracis muscle of 15- and 19-month-old Charolais bulls. The Charolais breed was chosen as it is the main beef breed in France and is widely distributed throughout the world.

MATERIALS AND METHODS

Animal and Muscle Samples. This study was conducted with 13 and 12 Charolais bull calves from an INRA experimental herd, 15 and 19 months of age, respectively, weaned at 32 weeks and then kept in an open shed. The calves were randomly allocated to the two slaughter age groups. They were fed a complete pelleted diet distributed ad libitum with a limited amount of straw until slaughter (*4*). The warm carcass and the internal fat deposit weights were recorded. Longissimus thoracis muscle (red oxidative muscle, LT) was excised from each animal within 10 min post-mortem. The samples were immediately frozen in liquid nitrogen and stored at −80 °C until they were analyzed. On the following day the sixth rib was dissected and the carcass composition (muscle and fat contents) was estimated using the Robelin and Geay prediction equation (*14*).

Biochemical and Mechanical Studies. All methods used here were described by Cassar-Malek et al. (*15*) and/or Sudre et al. (*8*). Glycolytic metabolism was evaluated by measuring lactate dehydrogenase (LDH, EC 1.1.1.27) activity and oxidative metabolism by isocitrate dehydrogenase (ICDH, EC 1.1.1.42), citrate synthase (CS, EC 2.3.3.1), and cytochrome *c* oxidase (COX, EC 1.9.3.1) activities. The enzyme activities were expressed in micromoles per minute and per gram of muscle. Total collagen content was measured in lyophilized muscle powder, and the data (means of triplicates) were expressed as micrograms of hydroxyproline per milligram of dry matter. The histological architecture of the muscle fibers was revealed by azorubin staining of 10 μm thick serial cross sections, prepared using a cryotome at −20 °C. The stained sections were examined under a microscope, and two randomly selected images were captured. Mean fiber area was determined using an image analysis system (Visilog, Noesis, France). Total lipids were extracted for 3 × 1 min with chloroform/methanol (2:1, v/v) at room temperature and determined gravimetrically. Triglycerides were determined in the total lipid extracts. Phospholipids in the total lipid extracts were analyzed by colorimetry after mineralization of the organic phosphorus.

Sensory Assessment. LT steaks were vacuum packaged, aged at 4 °C for 14 days, and frozen. They were thawed rapidly under flowing water. On the following day they were grilled to a core temperature of 55 °C and immediately served to panelists. A total of 10−12 trained panelists were used in each session. They underwent 8−10 test sessions for training, and during these they evaluated meats from different muscles, type of animals, and cooking processes. Once trained, they evaluated a maximum of eight meat samples, presented sequentially in each session. They scored initial tenderness and overall tenderness, juiciness, and flavor intensity on 10-point scales: from 1 (tough; dry; less tasty) to 10 (tender; juicy; tasty). Meat samples from different bull calves in the same slaughter age group were randomly presented to the panelists. Each sample was tested once independently by each panelist. Scores were averaged over the different panelists for each animal.

Animal Ranking According to Meat Sensory Traits. Each animal was ranked according to the score for each sensory attribute [tenderness (T), juiciness (J), and flavor (F)]. For each criterion, eight animals (four per age group) were chosen from the extremes of the distribution so that high (+) and low (−) meat quality scores in tenderness, flavor, and juiciness could be compared. **Table 1** shows the animals selected for different traits.

Table 1. Beef Quality Scores (T, Tenderness; J, Juiciness; F, Flavor) of the 25 Animals at 15 Months (n = 13) or 19 Months (n = 12).

animal[a]	tenderness[b]	juiciness[b]	flavor[b]
15-month-old			
2538	6.25	5.36	5.23
2575	4.83	5.30	5.30
2576	4.83	4.76	5.42
2555	6.10	5.03	5.45
2566	6.08	5.54	5.70
2595	5.94	5.30	5.80
2551	6.01	6.59	5.92
2548	6.96 (T+)	6.76 (J+)	6.16 (F+)
2556	7.10 (T+)	4.62 (J–)	5.29
2582	3.26 (T–)	4.92	4.88 (F–)
2585	3.11 (T–)	3.16 (J–)	4.83 (F–)
2587	5.43	5.98	5.96 (F+)
2578	5.53	6.69 (J+)	5.69
19-month-old			
2544	6.12 (T+)	6.49 (J+)	6.98 (F+)
2588	5.62 (T+)	4.13	5.33
2594	3.73 (T–)	5.10	4.81 (F–)
2531	3.49 (T–)	5.30	5.51
2567	4.61	2.63 (J–)	4.23 (F–)
2547	5.23	5.81	6.21 (F+)
2533	3.85	5.87 (J+)	6.07
2560	5.20	4.12 (J–)	5.05
2597	4.89	4.69	4.99
2540	4.23	4.86	5.04
2604	4.13	5.10	5.17
2562	3.92	4.71	5.23

[a] The 14 animals selected on the basis of extreme scores for tenderness, juiciness, and flavor are indicated in bold. These 14 animals were used to performed comparative analyses. [b] + and −: high and low meat quality scores, respectively.

Microarray Experiments. Transcriptome analysis was performed using microarrays, prepared at the West Genopole transcriptome platform, on which 50-mer oligonucleotides probes (MWG Biotech) were spotted. The oligonucleotides were designed from 3861 human and 1557 murine genes implicated in normal and pathological skeletal and cardiac muscle. The 5418 genes represented on the microarray were spotted in triplicate with 2898 control spots (buffer and empty). These genes encode proteins involved in different biological process according to Gene Ontology (http://cardioserve.nantes.inserm.fr/ptf-puce/myochips_fr.php).

Total RNA was extracted from LT muscle using Trizol reagent (Life Technologies). RNA quality was assessed using an Agilent 2100 bioanalyzer (Agilent Technologies, Palo Alto, CA). To compare animal groups in the microarray experiments, each sample was compared to a reference pool composed of LT muscle transcripts isolated from 19-month-old animals. Reverse transcription and indirect labeling of 4 × 15 μg of total RNA from each sample were performed using the CyScribe cDNA Post Labeling Kit (Amersham Pharmacia Biotech) as described in the manufacturer's protocol. Samples from each animal were labeled individually with Cy3 and mixed with an equal amount of the reference pool labeled with Cy5. The slides were then preincubated with 40 μL of a hybridization solution containing Denhardt 5×, yeast tRNA (0.5 μg/μL), polyA RNA (0.5 μg/μL), SSC 3.5×, SDS 0.3%, and formamide and hybridized to the microarrays. The incubation was performed at 42 °C for 17 h. Four independent hybridizations were performed for each animal.

After washing, the hybridized arrays were scanned by fluorescence confocal microscopy (Affymetrix 428 Array Scanner). Measurements were obtained separately for each fluorochrome at 10 μm/pixel resolution.

Data Analyses and Statistics. The hybridization signals were quantified using Genepix Pro 6.0 image analysis software (Axon Instruments, Inc., Union City, CA). Expression values were normalized using MADSCAN (http://cardioserve.nantes.inserm.fr/mad/madscan/; *16*), which combines the rank invariant and lowess fitness methods with spatial normalization. Outliers detected by MADSCAN within

Table 2. Primer Sequences Used in Quantitative Real-Time PCR

gene symbol	forward primer (5′)[a]	reverse primer (3′)[a]
CPT1B	CTTCCACGTCTCCAGCAAGT	TTCCGGAGATGTTCTTGGAG
Xlkd1	GCCGATGATAGCAACCCTAA	GCTTGCTTGGTTCTCTGGTC
NDUFB4	CAAGATGTCGTTCCCCAAGT	CCAAGGCAGGATCTTCGATA
JMJD1B	GTTGCATCAAAGTCGCAGAA	GCTTCACAGGGGAGTTTGAA
MYH7	CACCAACCTGTCCAAGTTCC	ACTGGGAGCTTCAGTTGCAC
TPM3	CTGGAGGAGGAGCTGAAGAA	CAGCTTGGCTACCGATCTCT
PLN	ACTTGGCTGGCAGCTTTTTA	ACTGGGATTGCAGCAGAACT
ATP2A2	TCTGCCTGTCGATGTCACTC	GTTGCGGGCCACAAACTT
DNAJA1	AGGGTCGCCTAATCATTGAA	TCCTCGTATGCTTCTCCATTG
CSRP3	ATGCGGAAAGTCGGTCTATG	ACCTGTAGGGCCGAAGTTTT
CRYAB	CGCCATTACTTCATCCCTGT	TCACTGGTGGGGAACTTTTC
HSPB1	CGTTGCTTCACTCGCAAATA	TACTTGTTTCCGGCTGTTCG

[a] All primer sequences were designed using Primer3 software. The primer annealing temperature was 60 °C.

arrays (based on triplicate spot replicates) and between arrays (based on replicate hybridizations × triplicate spots) were eliminated from further analysis. MADSCAN then attributed a score to each spot according to quality. The main criteria were background level, signal-to-noise ratio, diameter, and saturation level. Genes with lower than 50% expression values were also excluded from analyses. Differentially expressed genes were identified using Significance Analysis of Microarrays (SAM) (*17*). The results were expressed according to the fold change value (FC), which represents the expression ratio of the plus (+) condition to the minus (−) condition, for instance, the ratio of a value in tender beef to that in tough beef. A gene was declared to be up-regulated (FC > 1) or down-regulated (FC < 1) when its expression was respectively higher or lower in animals giving high meat quality than in animals giving low meat quality. Ontologies of differentially expressed genes, notably biological process and metabolic pathway, were determined using the PANTHER classification system (*18*). Hierarchical clustering (average linkage and Pearson correlation) was performed using Genesis software (*19*) to identify similar expression profiles between differentially expressed genes. These genes were used for subsequent analysis using the BiblioSphere Pathway Edition tool from the Genomatix Suite (http://www.genomatix.de/), a data-mining solution for extracting and analyzing gene relationships from literature databases and genome-wide promoter analyses.

The study was performed according to MIAME standards (Minimum Information About a Microarray Experiment; *20*). The data discussed in this publication have been deposited in the NCBI Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/gep/) and are accessible through GEO Series accession no. GSE5561.

To calculate the percentage variability in meat sensory traits explained by muscle characteristics or gene expression levels, correlations were determined using Statistica software (StatSoft, France).

Real-Time RT-PCR. Some of the differentially expressed genes were validated by real time RT-PCR. mRNA levels were assessed using a LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics GmbH, Mannheim, Germany) and selected gene-specific primer pairs (**Table 2**) according to the manufacturer's instructions. A purified cDNA dilution series was created for each gene to establish a standard curve to which results expressed in picograms per micromole referred. Each reaction was subjected to melting curve analysis to confirm single amplified products.

RESULTS

Meat Sensory Traits and Animal or Muscle Characteristics. The correlation coefficients between the 11 characteristics measured and the sensory scores are reported in **Table 3**. These correlations were computed for all 25 animals or in the sample of 14 animals selected on the basis of their extreme scores for the meat (**Table 1**). They show that variability in tenderness was predominantly related to muscle mass in the carcass and to ICDH and COX activities (oxidative metabolism), particularly within the subset of 14 animals. Variation in juiciness tended to be explained by fat mass in the carcass, total lipid and triglyceride contents, and COX activity. The latter, as well as collagen content, appeared to be correlated with flavor. Each carcass or muscle characteristic alone explained <33% of flavor variability and <25% of tenderness and juiciness variability. Together, these characteristics accounted for up to 50% of sensory trait variability.

Gene Expression Changes Associated with Meat Sensory Quality. *Data Analysis.* The hybridization results showed that about 83% of the genes gave valid expression values. Moreover, technical variability among the valid hybridization signals for four different arrays for each sample (CV mean = 11.3%) was low, showing good experimental reproducibility.

Two groups of SAM analyses [false discovery rate (FDR) ≤ 1%] were performed to determine gene expression changes associated with tenderness, juiciness, or flavor scores. The results are shown in **Table 4**. Analyses were performed for each sensory trait for the 15- and 19-month age groups separately and in combination. Analyses based on tenderness scores (T+ vs T−) identified 615 differentially expressed genes. Analyses based on meat juiciness (J+ vs J−) led to the identification of 1005 genes that differed in expression between the two evaluations. Last, 799 genes showed significant differences in expression between the tastiest and the least tasty meats (F+ vs F−). Gathering all of the differentially expressed genes (**Table 4**), only 1772 were associated with tenderness and/or juiciness and/or flavor; some were common to at least two sensory traits. Two hundred and fifteen of the 1772 genes were up-regulated (FC > 1.4) or down-regulated (FC < 0.71) in at least one condition.

Homology and Gene Ontology Analysis. These 215 differentially expressed genes were represented by 159 human and 56 murine oligonucleotides. However, not all of these genes have yet been identified in the bovine genome. Some are known (37%), whereas others correspond to EST sequences (52%). One hundred and eighty-five of the oligonucleotides showed sequence homologies with the bovine genome, and 84% of these had high homology (>80%).

The differentially expressed genes were mostly involved in signal transduction and various metabolic pathways and their regulation including protein and nucleic acid metabolism, cell structure and motility, developmental processes, muscle contraction, immunity and defense, and transport (data not shown). However, the ontology of 20% of these genes was unknown and could not be associated with any main pathway.

Differentially Expressed Genes Related to the Three Sensory Traits. Taking all of the different analyses into account, differential expression of 58 of the 215 genes corresponded to all three sensory traits: tenderness, juiciness, and flavor (**Figure 1**). Some genes were common to only two sensory traits: 10 to tenderness and juiciness, 33 to flavor and juiciness, and 49 to tenderness and flavor. Finally, 29, 21, and 15 differentially expressed genes were specific for one trait only: tenderness, juiciness, or flavor, respectively.

The 58 genes that corresponded to all three traits were distributed as follows: 33 were up-regulated in muscles from which the tenderest, juiciest, and tastiest meats were obtained, and 9 were down-regulated in the same samples (**Table 5**). The remaining 16 genes had different expression profiles between tenderness, juiciness, and flavor. Ten of the 33 up-regulated genes appeared to be specific to the 15-month-old animals, whereas the remaining 23 were differentially expressed at both 15 and 19 months of age. Hierarchical classification of the 215 differentially expressed genes grouped 9 of those that were

Table 3. Correlation Coefficients[a] of Sensory Scores (Tenderness, Juiciness, and Flavor) with Carcass Traits (Muscle and Fat Weight) and Muscle Characteristics

	muscle	fat	COX	CS	ICDH	LDH	lipids	phospholipids	triglycerides	collagen	fiber area
tenderness	**–0.41***	**–0.06**	**0.08**	**0.13**	**0.11**	**–0.08**	**0.07**	**0.15**	**0.11**	**0.06**	**–0.18**
	–0.36	0.06	0.37	0.05	0.50	–0.20	0.15	0.15	0.23	0.15	–0.03
juiciness	**–0.29**	**–0.34**	**0.18**	**0.06**	**–0.14**	**0.12**	**–0.35**	**0**	**–0.31**	**0.19**	**–0.15**
	–0.22	–0.45	0.30	–0.03	–0.15	0.14	–0.50	–0.03	–0.45	0.28	–0.04
flavor	**–0.28**	**–0.06**	**0.43***	**0.06**	**–0.15**	**0.07**	**–0.21**	**–0.28**	**–0.18**	**0.30**	**–0.24**
	–0.18	–0.03	0.58*	–0.09	–0.16	0.01	–0.31	–0.35	–0.26	0.44	–0.07

[a] These correlations were computed for the 25 animals (bold type) in the study and the subset of 14 animals (not bold) selected on the basis of extreme score values of meat quality. *, the correlation is significant at $P < 0.05$ if $|r| \geq 0.40$ or $|r| \geq 0.53$ for the 25 and 14 animals, respectively. Only the correlations between tenderness and muscle mass and between flavor and COX activity are significant. The following animal and muscle characteristics were used (for each variable, the mean and standard deviation from all 25 animals are indicated in parentheses): muscle mass in the carcass (muscle) (279.1 ± 44.3 kg); fat mass in the carcass (fat) (58.4 ± 10.6 kg); cytochrome *c* oxidase activity (COX) (10.8 ± 3.2 μmol min^{-1} g^{-1}); citrate synthase activity (CS) (4.1 ± 0.8 μmol min^{-1} g^{-1}); isocitrate dehydrogenase activity (ICDH) (1.1 ± 0.2 μmol min^{-1} g^{-1}); lactate dehydrogenase activity (LDH) (1001.2 ± 111.1 μmol min^{-1} g^{-1}); lipid content (lipids) (23.2 ± 6.7 mg g^{-1}); phospholipid content (phospholipids) (7.0 ± 0.5 mg g^{-1}); triglyceride content (triglycerides) (13.9 ± 6.1 mg g^{-1}); total collagen content (collagen) (3.6 ± 0.8 μg g^{-1} of dry matter); muscle fiber area (fiber area) (2507.6 ± 293.1 μm^2).

Table 4. Number of Significantly Differentially Expressed Genes Related to Meat Sensory Quality by SAM Analysis (FDR ≤ 1%)

tenderness (T+ vs T–)[a]	juiciness (J+ vs J–)[a]	flavor (F+ vs F–)[a]	total[b]
differentially expressed genes			
615	1005	799	1772
differentially expressed genes with fold change[c] >1.4 or <0.71 in at least one condition			
146	122	155	215

[a] T+ vs T–, J+ vs J–, and F+ vs F– analyses compared, respectively, the tenderest and the least tender meats, the juiciest and the least juicy meats, and the tastiest and the least tasty meats. [b] The total number of genes does not correspond to the sum of the numbers above because some genes are common to several analyses. [c] The fold change value represents the ± expression ratio.

Figure 1. Distribution of the 215 differentially expressed genes in relation to tenderness, juiciness, and flavor.

specific to 15-month calves and 19 of the other 23 genes into two clusters (**Table 5**). The 9 down-regulated genes were classified into other and different clusters. However, 5 of these genes showed high differential expression (FC < 0.71) for the three criteria simultaneously (tenderness, juiciness, and flavor) (**Table 5**). Real-time RT-PCR experiments on 12 selected up- or down-regulated genes confirmed the differential expression according to tenderness, juiciness, and flavor for 10 of them; for 2 (*CPT1B* and *NDUFB4*), only differential expression specific to the 15-month animals was validated (**Table 6**).

Meat Sensory Traits and Microarray Data. A correlation matrix between the sensory scores and the differentially expressed genes was constructed to show the percentage of the variability in meat sensory traits that was explained by gene expression levels. Specific attention was paid to up-regulated genes belonging to cluster 1 and to the five down-regulated genes with FC < 0.71. The correlation coefficients are shown in **Table 7**. Eighteen of the 19 up-regulated genes were highly correlated with both meat juiciness and flavor, and they explained up to 50 and 60% of juiciness and flavor variability, respectively. Four of them were also related to tenderness (*FLJ12193*, *Npm3*, *TRIM55*, and *Cbr2*) and accounted for 30–42% of its variability. One down-regulated gene (*DNAJA1*) showed a strong negative correlation with tenderness and explained 63% of its variability. Moreover, this high negative correlation was conserved when data from all 25 animals were analyzed ($|r| = -0.66$, $p < 0.01$; data not shown). These two correlation analyses gave comparable results and showed higher correlations between sensory traits and gene expression levels than between sensory traits and muscle biochemical characteristics.

Regulatory Networks. Differentially expressed genes belonging to cluster 1 were used as input data for the BiblioSphere Pathway Edition tool to identify one or several putative regulatory networks. This approach revealed the relationships between 12 genes from cluster 1 and other genes/transcription factors co-cited with them in human databases. These 12 genes seem to be regulated by different transcription factors, especially the androgen receptor (*AR*), the transcription factor Sp1, and the upstream transcription factor 2, c-fos interacting (*USF2*) (**Figure 2**). They contain specific transcription factor binding sites in their promoters. AR can regulate *NDUFB4*, *Xlkd1*, and *CACNA1C* by binding to the glucocorticoid responsive and related elements (V$GREF) in their promoters (**Figure 2A**). *PRRX2* and *NID1* bind Sp1, a zing-finger protein on the site V$SP1F in their promoters (**Figure 2B**), whereas *CPT1B* and *Ireb2* are predicted to be regulated by another transcription factor, *USF2* (**Figure 2C**), a member of the basic helix–loop–helix leucine zipper family.

DISCUSSION

One major original aspect of this work is the identification of new biological indicators of beef quality (tenderness, flavor) using transcriptomic approaches. These novel results first underscore the usefulness of functional genomics in farm animals. However, the method depends on the availability and quality of genomic tools and their utilization in livestock. Second, the novel results of this study are of prime importance for a better physiological understanding of the relationship between muscle biology and beef quality (*5*).

Transcriptome Analysis in Farm Animals. Microarray technology is a powerful tool because it allows multiple gene

Table 5. Up- and Down-Regulated Genes According to Tenderness, Juiciness, and Flavor

symbol	gene name	tenderness[a]	juiciness[a]	flavor[a]	hierarchical clustering[b]
	Up-Regulated Genes				
	15- and 19-Month-Old Bulls				
C:6970	*Homo sapiens* chromosome 5 clone CTD-2151N11	*	**	**	cluster 1
CACNA1C	calcium channel, voltage-dependent, L type, α 1C subunit	*	**	**	cluster 1
Cbr2	carbonyl reductase 2	**	**	**	cluster 1
CCNA1	cyclin A1	*	**	*	
CGREF1	cell growth regulator with EF-hand domain 1	*	*	**	cluster 1
CPT1B	carnitine palmitoyltransferase 1B (muscle)	**	**	**	cluster 1
Cyp2c50	cytochrome P450, family 2, subfamily c, polypeptide 50	**	**	**	cluster 1
FLJ12193	hypothetical protein FLJ12193	**	**	**	cluster 1
Ireb2	iron-responsive element binding protein 2	*	**	**	cluster 1
JMJD1B	jumonji domain containing 1B	**	**	**	cluster 1
LAMA3	laminin, α 3	**	**	**	cluster 1
LGALS3BP	lectin, galactoside-binding, soluble, 3 binding protein	*	*	**	
MPDZ	multiple PDZ domain protein	*	**	*	cluster 1
NDUFB4	NADH dehydrogenase (ubiquinone) 1 β subcomplex, 4, 15 kDa	**	**	**	cluster 1
NID1	nidogen 1	*	**	*	cluster 1
Npm3	nucleophosmin/nucleoplasmin, 3	**	**	**	cluster 1
OTOR	otoraplin	*	**	**	cluster 1
PHF13	PHD finger protein 13	**	*	**	
PRKAG1	protein kinase, AMP-activated, γ 1 noncatalytic subunit	*	**	**	cluster 1
PRRX2	paired related homeobox 2	*	**	**	cluster 1
SLC25A12	solute carrier family 25 (mitochondrial carrier, Aralar), member 12	*	**	*	
TRIM55	tripartite motif-containing 55	**	**	**	cluster 1
Xlkd1	extracellular link domain-containing 1	**	**	**	cluster 1
	15-Month-Old Bulls				
ANXA10	annexin A10	*	**	*	cluster 2
ATP2A2	ATPase, Ca^{2+} transporting, cardiac muscle, slow twitch 2	*	**	*	cluster 2
C:3400	*Homo sapiens* genomic DNA, chromosome 11 clone:RP11-867G2	**	**	**	cluster 2
CASP3	caspase 3, apoptosis-related cysteine peptidase	*	*	**	cluster 2
CCR5	chemokine (C—C motif) receptor 5	**	*	**	cluster 2
MYH7	myosin, heavy polypeptide 7, cardiac muscle, β	**	**	**	cluster 2
PLN	phospholamban	**	**	**	cluster 2
RTN1	reticulon 1	**	**	**	cluster 2
STK1	stem cell tyrosine kinase 1 (STK-1) gene, exons 9–11 and partial cds	*	*	**	cluster 2
Tpm3	tropomyosin 3	**	**	**	
	Down-Regulated Genes				
CRYAB	crystallin, αB	**	**	**	
CSRP3	cysteine- and glycine-rich protein 3 (cardiac LIM protein)	**	**	**	
DNAJA1	DnaJ (Hsp40) homologue, subfamily A, member 1	**	**	**	
FLNC	filamin C, γ (actin binding protein 280)	*	**	**	
HSPB1	heat shock 27kDa protein 1	**	*	*	
Pbef1	pre-B-cell colony enhancing factor 1	*	**	*	
PDK4	pyruvate dehydrogenase kinase, isozyme 4	**	**	**	
RGS2	regulator of G-protein signaling 2, 24 kDa	*	**	**	
THOC3	THO complex 3	**	**	**	

[a] The fold change value (FC) represents the ± expression ratio: FC > 1 represents up-regulated genes, and FC < 1 represents down-regulated genes. *, FC < 1.4 or FC > 0.71; **, FC > 1.4 or FC < 0.71. [b] Hierarchical clustering (average linkage and Pearson correlation) was performed using Genesis software and allowed two clusters to be identified.

expression profiles to be studied. Nowadays, transcriptome analyses are expanding more and more in major livestock species such as cattle (*7*). To date, some gene expression-based research related to beef quality has focused on identifying molecular processes involved in meat quality traits such as toughness and marbling (*6*). Different studies have dealt with fetal muscle development (*21*), muscle growth potential (*8, 22*), and diet effects (*23*), all of which influence the composition of muscle tissue. Other studies have focused on intramuscular fat development, which influences marbling and thus juiciness and flavor (*9*). However, none of these studies has sought to identify differentially expressed genes related to beef sensory quality, especially tenderness, juiciness, and flavor. Therefore, taking advantages of the continuous progress in microarray technology, our study was designed to find genes of which the expression was altered in Longissimus thoracis muscles of Charolais bull calves that give meats of different tenderness, juiciness, and flavor.

Indicators of Beef Sensory Quality. The LT muscle characteristics studied here individually explained a fourth to one-third (maximum) of the variability in tenderness, juiciness, or flavor, as previously shown by Renand et al. using a similar experimental design (*4*). To our knowledge, the muscle characteristics of live animals, including calpain and calpastatin activities, have not explained more than 40% of the variability in tenderness, even in combination (*24*). Our transcriptome study allowed many differentially expressed genes to be identified in relation to meat sensory qualities, although the expression differences between groups were low. We chose to retain genes that were differentially expressed between the different sensory

Table 6. Examples of Validation by Real Time RT-PCR (Boldface Type) of Differential Expressions[a] Obtained by Microarray Experiment (Lightface Type) among 15- and 19-Month-Old Animals

	tenderness				juiciness				flavor			
	15 months		19 months		15 months		19 months		15 months		19 months	
gene	microarray	RT-PCR	microarray	RT-PCR	microarray	RT-PCR	microarray	RT-PCR	microarray	RT-PCR	microarray	RT-PCR
up-regulated												
CPT1B	1.72	**0.95**	1.50	**0.78**	1.53	**1.95**	1.90	**0.74**	1.55	**1.45**	1.60	**0.76**
Xlkd1	1.72	**1.49**			1.51	**2.35**	1.70	**1.15**	1.57	**1.08**	1.52	**1.24**
NDUFB4	1.66	**1.60**			1.67	**1.34**	1.77	**0.64**	1.58	**1.51**	1.65	**0.83**
JMJD1B	1.41	**1.20**			1.36	**1.63**	1.67	**1.68**	1.55	**1.42**	1.80	**1.28**
MYH7	1.68	**2.78**			1.47	**1.25**			2	**2.66**		
Tpm3	1.84	**2.44**			1.54	**1.62**			1.87	**2.87**		
PLN	2.06	**3.47**			1.56	**2.32**			2.13	**3.75**		
ATP2A2	1.33	**1.21**			1.42	**1.57**	1.30	**1.44**	1.36	**1.33**		
down-regulated												
DNAJA1	0.62	**0.62**	0.71	**0.50**	0.71	**0.91**			0.66	**0.71**		
CSRP3			0.62	**0.46**			0.16	**0.14**			0.29	**0.25**
CRYAB	0.58	**0.61**					0.46	**0.21**			0.55	**0.23**
HSPB1	0.45	**0.55**					0.77	**0.34**			0.72	**0.36**

[a] The fold change value (FC) is indicated for each gene. It represents the ± expression ratio: FC > 1 represents up-regulated genes and FC < 1 represents down-regulated genes.

Table 7. Correlation Coefficients[a] of Up- and Down-Regulated Genes with Sensory Scores (Tenderness, Juiciness, and Flavor) within the Subset of 14 Animals Selected on the Basis of Extreme Score Values of Meat

gene	tenderness	juiciness	flavor
up-regulated			
CPT1B	0.48	0.61*	0.65*
Xlkd1	0.50	0.64*	0.65*
NDUFB4	0.40	0.62*	0.65*
JMJD1B	0.44	0.67**	0.77**
LAMA3	0.48	0.59*	0.67**
FLJ12193	0.58*	0.66**	0.74**
Npm3	0.59*	0.59*	0.65*
Cyp2c50	–0.31	0.05	0.01
TRIM55	0.54*	0.68**	0.76**
Cbr2	0.65*	0.64*	0.70**
C:6970	0.45	0.68**	0.73**
PRRX2	0.36	0.59*	0.67**
OTOR	0.49	0.70**	0.73**
CACNA1C	0.41	0.67**	0.69**
Ireb2	0.49	0.60*	0.73**
PRKAG1	0.43	0.62*	0.77**
NID1	0.31	0.50	0.59*
MPDZ	0.36	0.67**	0.70**
CGREF1	0.44	0.65*	0.72**
down-regulated			
PDK4	–0.27	–0.17	–0.40
DNAJA1	–0.80**	–0.40	–0.52
CSRP3	–0.14	–0.54*	–0.65*
CRYAB	–0.30	–0.37	–0.47
THOC3	–0.16	–0.38	–0.31

[a] *$|r| \geq 0.53$, $p < 0.05$; ** $|r| \geq 0.66$, $p < 0.01$.

conditions with FC > 1.4 or FC < 0.71 for up-regulated or down-regulated genes, respectively. Thus, only 12% of the differential genes seemed to be interesting for sensory quality, and 42 genes in particular were differentially expressed in relation to all three criteria (tenderness, juiciness, and flavor). Thirty-three of these appeared to be associated with high sensory quality either in an age-specific manner (e.g., *MYH7*, *PLN*, and *Tpm3*) or at both ages studied (e.g., *Xlkd1* and *NDUFB4*), and 9 were classified as unfavorable for beef quality (e.g., *DNAJA1*, *CSRP3*, and *CRYAB*). However, neither a clear biochemical pathway nor a biological process was identified as highly involved in beef quality. These genes indeed showed different ontologies, such as protein and nucleic acid metabolism or signal transduction. Therefore, beef sensory quality seems not to be related to a specific metabolic pathway or a biological process (e.g., proteolysis potential at slaughter, which would explain most of the variability in meat tenderness, or lipid and fatty acid metabolism, which could play a major role in flavor). This supports the hypothesis that each beef quality score results from a combination of different biological processes (for instance, toughness of connective tissue, meat proteolysis, etc., for tenderness) (*2*, *3*).

Specific Indicators of Tenderness. Interestingly, among the five most highly down-regulated genes for the three sensory traits, only one was strongly and negatively related to tenderness and explained up to 63% of its variability. This gene, *DNAJA1*, encodes a member of the large 40 kDa heat shock protein family (Hsp40). This protein is a co-chaperone of the 70 kDa heat shock protein (Hsp70) and is believed to play a role in protein folding and mitochondrial protein import. The DNAJA1/Hsp70 complex directly inhibits apoptosis by preventing mitochondrial translocation of the pro-apoptotic Bax protein (*25*). Interestingly, a novel hypothesis recently proposed that apoptosis is the first stage of meat aging (*26*). If this is so, DNAJA1 anti-apoptotic activity could contribute to retarding cellular death during the conversion of muscle into meat and consequently to lowering meat tenderization. This provides an explanation for the finding that the LT muscles of bulls with the lowest *DNAJA1* expression give the tenderest meats.

Another gene (*HSPB1*) encoding a 27 kDa heat shock protein (Hsp27) was also down-regulated. Hsp27 belongs to the small heat shock protein (hsp20) family. It is involved in stress resistance and actin organization. It associates with α- and β-tubulin and microtubules and interacts with hspb8. Guay et al. (*27*) showed that down-regulation of *Hsp27* led to actin polymerization and thus enhanced the stability of actin microfilaments. We therefore hypothesize that its down-regulation could favor actin disorganization or degradation. The down-regulation observed in T+ muscles could induce reduced toughness or higher degradation of actin microfilaments during meat aging and thus explain the highest tenderness scores attributed to the meat. The post-mortem degradation of actin has indeed been shown to contribute to the tenderization process in pork (*28*). Recent studies have shown that Hsp27 is an anti-apoptotic protein able to interact with key components of the

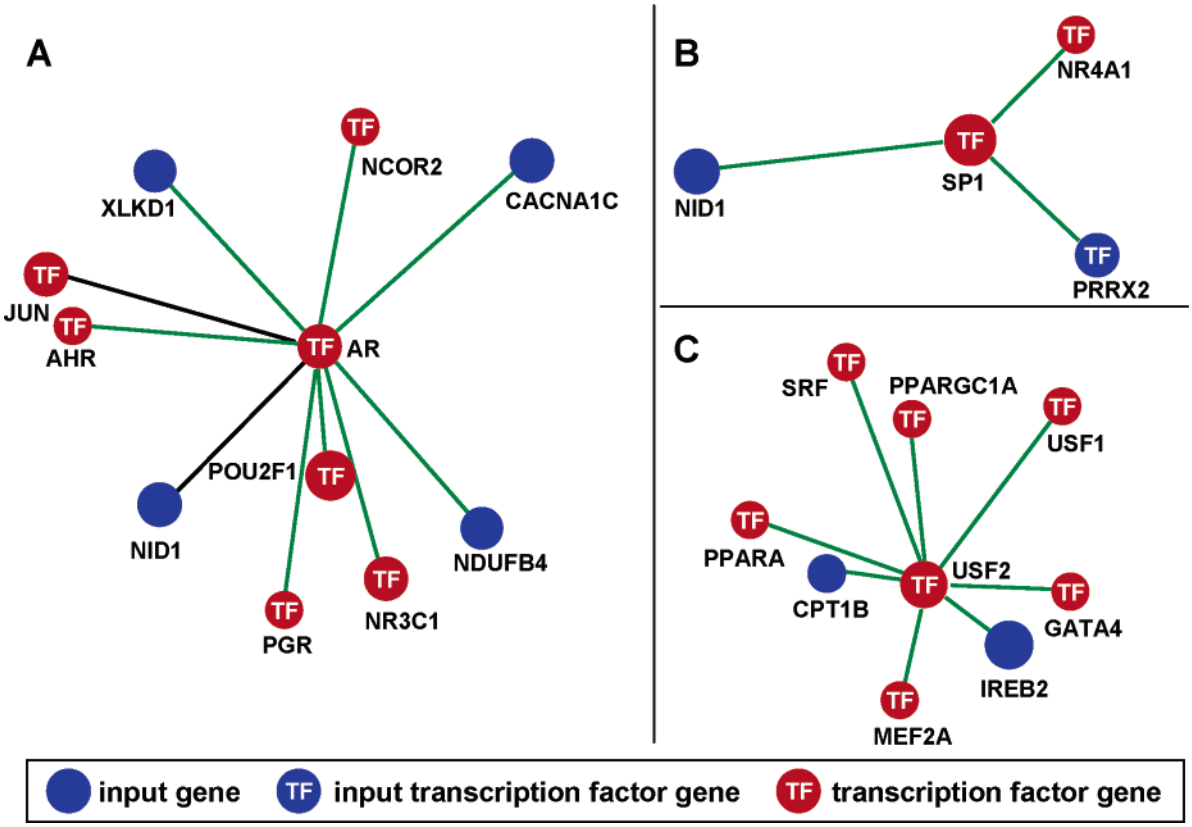

Figure 2. Putative regulatory networks of the genes in cluster 1 by co-cited transcription factors using the BiblioSphere Pathway Edition tool from the Genomatix Suite: (**A**) androgen receptor AR; (**B**) transcription factor Sp1; (**C**) upstream transcription factor 2, c-fos interacting USF2.

apoptotic signaling pathway, particularly those involved in caspase activation and apoptosis (for a review, see ref *29*).

Another gene, *CRYAB*, which encodes αB-crystallin and shares homology with Hsp27, was also down-regulated in the same samples. This protein is also a member of the small heat shock protein family, and its stability was shown to be promoted by Hsp27 (*30*). Interestingly, expression of these genes was strongly intercorrelated ($|r| = 0.725$, data not shown). αB-crystallin plays an important role in protecting intermediate filaments, because it stabilizes and protects target proteins including desmin by preventing their irreversible aggregation (*31*). We hypothesize that down-regulation of *CRYAB* could promote degradation of intermediate filaments and, consequently, good meat tenderness.

Specific Indicators of Flavor and Juiciness. The up-regulated genes in cluster 1, except *Cyp2c50*, all correlated positively with the juiciness and flavor scores (**Table 7**). Among these genes, *PRKAG1* encodes a protein involved in fatty acid metabolism. This protein is one of three isoforms of the AMP-activated protein kinase γ subunit. It is responsible for regulating fatty acid synthesis by phosphorylating the lipogenic enzyme acetyl-CoA carboxylase and plays an important role in regulating the expression of genes involved in glucose metabolism. Characterization of bovine *PRKAG1* has just begun (*32*), and its involvement in beef quality is still unknown. However, studies on pigs have shown that a dominant mutation (denoted RN−) in *PRKAG3*, encoding another isoform of the AMPK γ subunit, induces constitutive activation of AMPK activity, leading to a high glycogen content in skeletal muscle, a low ultimate pH, reduced water-holding capacity, and reduced yield of cured cooked ham, which has a negative impact on meat quality (*33*). Hamilton et al. (*34*) reported that a mutation in human *PRKAG1*, structurally equivalent to RN−, also resulted in increased AMPK activity, but the possible involvement of *PRKAG1* in the regulation of fatness traits remains to be demonstrated.

Regarding the other genes up-regulated in muscles that produce the juiciest and tastiest meats, their relationships to juiciness and flavor have to be clarified. They are involved in various biological processes. However, data mining of the literature using the Genomatix Bibliosphere Pathway Edition tool provided elements suggesting that some of these genes were regulated by transcription factors (**Figure 2**), especially involved in regulating the expression of adipocyte-specific genes. For example, AR has been demonstrated in human preadipocytes and adipocytes, suggesting that androgens may contribute to the control of adipose tissue development through regulation by their own receptors (*35*). Another transcription factor, Sp1, is implicated in the transcriptional regulation of the adipocyte amino acid transporter gene (*36*). Further analyses have shown that glucose activation of the acetyl-CoA carboxylase (ACC) promoter is Sp1-mediated (*37*). ACC is the rate-limiting enzyme in fatty acid biosynthesis. We can suppose that these transcription factors are major regulators, but further analyses are needed because these genes were not represented on the chip.

The transcription factor *USF2* can activate transcription of the hormone-sensitive lipase (HSL) promoter through E-box motifs in the glucose-responsive region (*38*). This enzyme catalyzes the rate-limiting step in the mobilization of fatty acids from adipose tissue. The oligonucleotide specific for *USF2* was represented on the myochip, but its expression was not modified in relation to meat quality. Nevertheless, this could be due to the heterologous system used and a low homology between the oligonucleotide sequence and the corresponding bovine gene.

Although the up-regulated genes were not biologically related to flavor and juiciness, their regulation by certain transcription factors is a promising topic for future research.

In conclusion, our results allowed us to identify differentially expressed genes that are associated with variability in beef tenderness, juiciness, and flavor and may be new indicators of beef quality. However, further studies are needed to complete this research and to understand better the relationships between the genes identified and the quality of the meat.

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