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TRANSCRIPTOMIC INDEX OF SKELETAL MUSCLE OF BEEF BREEDS BULLS

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In the present study cDNA microarray (18263 probes) were used for analysis of bovine skeletal muscle (*m.semitendinosus*) transcriptome in 12-month-old bulls of four cattle breeds: Holstein-Friesian (HF), Limousine (LIM), Hereford (HER) and Polish Red (PR), aiming to identify the genes, which expression is common for beef breed bulls. The number of transcripts significantly different from HF bulls muscle amounted to 393, 462 and 638 for LIM, HER and PR, respectively. As a result of this study the transcriptomic index was proposed, being the set of 48 genes expressed similarly in beef breed bulls in comparison to HF bulls. Classification of genes according to molecular function of their protein products has shown the highest number of genes encoding proteins involved in nucleic acid binding (10), regulatory proteins (6), kinases (4) and signaling molecules (3). Classification according to biological processes revealed the highest number of genes involved in protein metabolism i modification (14), signal transduction (5), cell cycle (4), intracellular protein traffic (4), nucleoside, nucleotide and nucleic acid metabolism (4), apoptosis (3), cell structure and motility (3), and cellular transport (3). Since the role of the most genes included to the transcriptomic index has not been described yet in bovine skeletal muscle, obtained results may be very useful in revealing new candidate genes to search a new criteria of animal selection in beef production.

Keywords: *microarray, transcriptome, muscle, cattle breeds, gene expression*

INTRODUCTION

During the past few decades, advances in molecular genetics have led to the identification of genes that affect meat quality in farm animals (1-3). In cattle, important genes affecting beef meat quality have been developed (4-6). Validation of DNA tests for quantitative beef quality traits have been performed for their use in cattle breeding practice (7, 8). For meat tenderness several gene markers have been found, *e.g.* the calpain I and the inhibitor of calpain – the calpastatin gene (9-16). Furthermore, the genes encoding leptin (17-19), thyroglobulin (20), DGAT1 (21), growth hormone (22, 23), growth hormone receptor (24-26), STAT5A (27); myostatin (28), DNAJA1 (29), were associated with beef quality traits. Another group of important candidate genes for muscle growth, not yet exploited in cattle, are those encoding myogenic regulatory factors (MRFs).

As a working hypothesis to the present study it was assumed that animals of significantly different production type, *e.g.* milk or meat production, should also differ in expression profiles of genes determining these traits. Such differing expression levels might result from nucleotide sequence polymorphisms of gene regulatory sequences. In the present experiments cDNA microarray were applied for transcriptomic analysis and to identify the genes which expression in skeletal muscle was significantly different in typical beef breed bulls

(LIM and HER) in comparison with bulls of diary breed (HF) and dual-purpose breed (PR). As a result of this study the transcriptomic index was described, being the set of 48 genes which expression was common for the muscle of beef breeds bulls.

MATERIALS AND METHODS

Animals and sampling

The cattle's experimental group was formed of four breeds differing in meat production and utility type: Holstein-Friesian (HF; type Polish Black-and-White) – dairy cattle, Polish Red (PR) – dual purpose breed (combined milk and meat), Limousine (LIM) – beef cattle of high-meat low-fat production, maturing lately, and Hereford (HER) – beef cattle, high meat high-fat production, maturating very early. Bulls were slaughtered at the age of 12 months, after 24 hours fasting. The carcasses were chilled for 24 hours at 4°C, and dissected into lean, fat and bone, 15 males in each breed group. Mean body weight and major carcass traits of bulls of four breeds slaughtered at the age of 12 months are shown in *Table 1.*

Based on the methodology described in our previous work (31) we identified animals with genotypes CC, GG and GC for the polymorphism in 5'flanking region of the *mstn* gene (G/C substitution at position -7828 relative to ATG), which influences transcript and protein level of myostatin – a key factor involved in regulation of skeletal muscle growth. Samples for RNA isolation were taken from three bulls of CC genotype in each breed (total 12 bulls were sampled), but from each animal two independent samples (from separate portions of *m.semitendinosus*) were taken. Sampling of larger group of animals was limited by the rare occurrence of CC genotype in HF bulls. The selection of animals with *mstn* genotype CC was due to its sole occurrence in LIM bulls. Samples of *m.semitendinosus* were taken immediately after slaughtering and frozen in liquid nitrogen. All of the procedures carried out with the use of animals were approved by the Local Ethics Commission, permission No. 3/2005.

DNA microarrays, RNA isolation and validation, labelling of probes, hybridization, signal detection, quantification, analysis and selection of the most differently expressed genes were performed according to the previously described methodology (32, 33)

Real-time RT-PCR

Expression of MRPS30, TRIP12, PYCRL and c-erbB3 gene was checked by Real-time RT-PCR using following primers:
(MRPS30-F: CGAGTTGATGCTGTGCGATAC,
MRPS30-R: ATGGGAGTTTGTCTGGCTTAC;
TRIP12-F: TACCCAAAGGCTAACCCACC,
TRIP12-R: CACAGGAGAAAGTGAAGCGTT;
PYCRL-F: GTCTGTGAAGGGACCAACAAG,
PYCRL-R: AGGTGAAGAAATGGACTCTGG;
c-erbB3-F: ACGCCTGGCATCAGAATCATCG,
c-erbB3-R: ACCATTGACATCCTCTTCCTCTAACC) using a LightCycler FastStart DNA Master SYBR Green I kit (Roche Diagnostics GmBH, Germany) according to the following procedure: Mg^{2+} added at a final concentration of 1,5 mM; pre-incubation step at 95°C for 10 min; amplification step (40 cycles) including denaturation at 95°C for 10 s, annealing for MRPS30, TRIP12 and PYCRL, c-erbB3 at 63°C and 57°C for 10s, respectively, extension at 72°C for 10 s; melting curve including denaturation at 95°C for 0 s, annealing at 65°C for 15 s, continuous melting at 95°C for 0 s (slope = 0.1°C/s); cooling step at 40°C for 30 s. For *gapdh*, annealing was at 57°C. Results are presented as the ratio of *mrps30, trip12, pycrl, c-erbB3* to *gapdh* expression. Data obtained were statistically analyzed using ANOVA with Tukey test analysis of variance using GraphPad Prism 5.0 software.

RESULTS

Identification of differentially expressed genes between particular cattle breeds

Comparisons of HF vs. LIM, HER and PR

The comparison of HF *vs.* LIM, HER and PR bulls revealed significant ($P < 0.05$) changes in expression of 393, 462 and 638 genes, respectively (*Fig. 1*)

Among the genes differing significantly between HF and three remaining cattle breeds (LIM, HER and PR) we identified 50 common genes, however 48 of them were the genes common for two beef cattle breeds (LIM and HER*)*. Only two genes differing in expression to HF were genes common for PR, LIM and HER. That is why we accepted 48 genes differing significantly in both LIM and HER vs. HF as a transcriptomic index of beef bulls muscle and only these genes were further investigated (*Fig 1*, *Table 2*).

Since the expression of genes belonging to transcriptomic index in LIM and HER differed significantly from their expression in HF we calculated the average fold change of these genes. Average fold change is a mean of two values describing fold change between LIM vs. HF and HER vs. HF. Both fold-change values were at least 1.5 which was necessary to consider differences in fold change as significant (31). Among 48 genes in transcriptomic index we identified 23 with higher

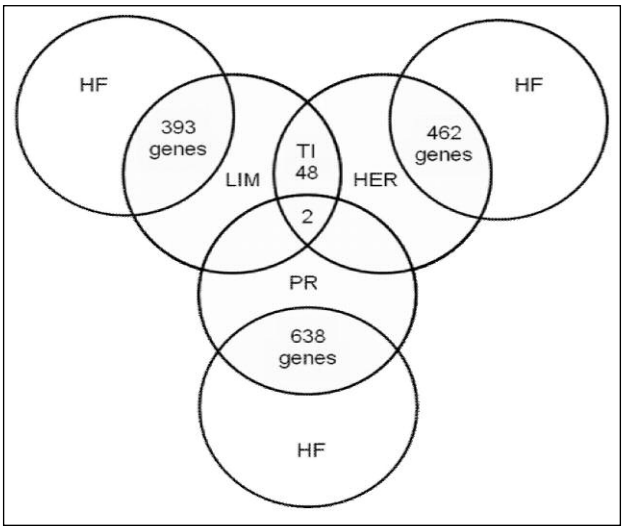

Fig. 1. The number of genes which were significantly (P < 0.05) different between examined cattle breeds. HF – Holstein-Friesian (type Polish Black-and-White), LIM – Limousine, HER – Hereford, PR – Polish Red, TI – Transcriptomic Index

Table 1. Major carcass traits of bulls of four breeds slaughtered at the age of 12 months (n=15).

Breed	Carcass dressing percentage (%)		Percentage of lean in the valuable cuts (%)	
	LSM	SE	LSM	SE
PR	54,45 ^{B}	0,81	71.91 ^{B}	0.60
HF	50,94 ^{C}	0,90	68.99 ^{B}	0.60
HER	54,92 ^{B}	1,20	69.01 ^{B}	0.80
LIM	59,25^{A}	1,30	78.45 ^{A}	0.90

Values with different superscript differ significantly at p≤0,01. LSM – Least Squares Means, SE – Standard Error.

Table 3. Validation of the expression of selected genes in the transcriptomic index with real-time RT-PCR method. cDNA microarray – n=6 (3 individuals x 2 different tissue samples); real-time RT-PCR – n=9 for each breed (3 individuals x 3 different tissue samples).

Gene Name	Method used	
	cDNA microarray	real-time RT-PCR
MRPS30	+3.48	+3.53
TRIP12	+2.35	+7.58
PYCRL	-2.48	-1.52
c-erbB3	-2.04	-2.33

expression in beef breeds (LIM and HER) and 25 genes with higher expression in typical dairy breed (HF) (*Table 2*).

Validation of microarray results by using real-time RT-PCR

For the validation of microarray results we have selected four genes: MRPS30 (position 1 in *Table 2*), TRIP12 (position 5), PYCRL (position 47), c-erbB3 (position 44). Real-time RT-PCR results showed similar trends in gene expression changes as we observed in microarray studies, namely expression of MRPS30 and TRIP12 was significantly higher ($p<0.05$) in LIM and HER than in HF (*Fig. 2*). Conversely, the expression of PYCRL and c-erbB3 was significantly lower ($p<0.05$) in LIM and HER than in HF. The comparison of the results from real-time RT-PCR and microarray is presented in *Table 3*.

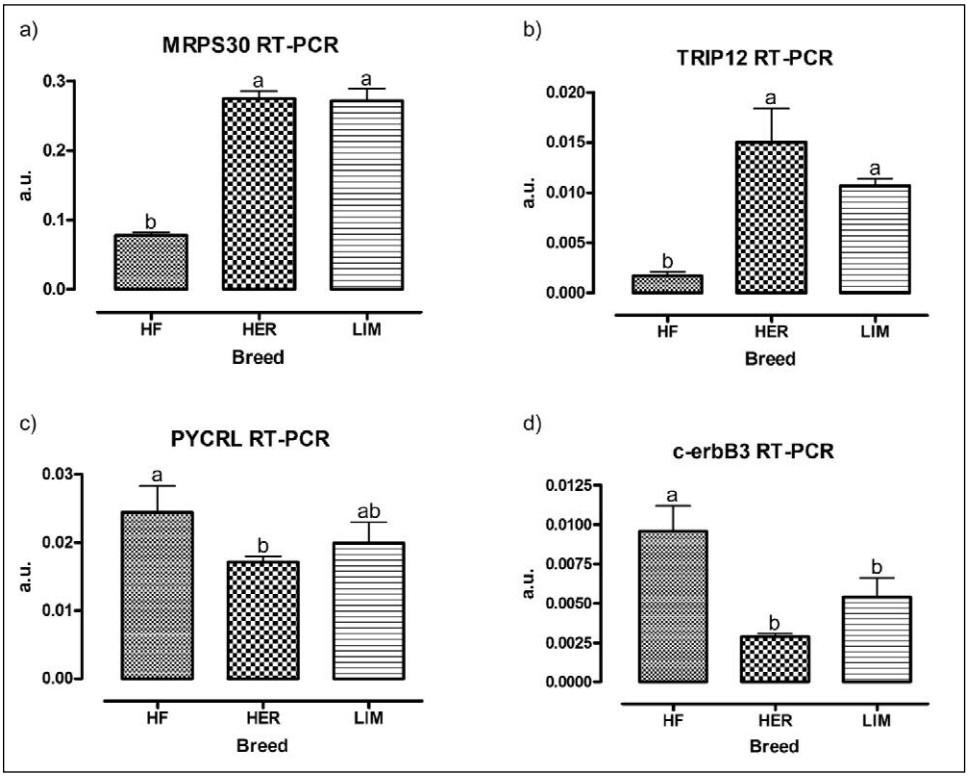

Fig. 2. Expression of *MRPS30* (a), *TRIP12* (b), *PYCRL* (c) and *c-erbB3*(d) genes in *m. semitendinosus* of Holstein-Friesian (HF), Limousine (LIM) and Hereford (HER) bulls analyzed by real-time RT-PCR. The results were obtained by dividing expression of genes mentioned above by *gapdh* expression and are presented in arbitrary units. Bars with different superscripts differ significantly ($p<0.05$). n=9 for each breed (3 individuals x 3 different tissue samples).

Table 2. Transcriptomic index of beef breeds bulls.

No.	GenBank ID	Change Fold	Gene Name	E Value	P Value	Biological function
1	XM_001251581	+3.48	PREDICTED: Bos taurus similar to Mitochondrial 28S ribosomal protein S30 (S30mt) (Programmed cell death protein 9) (MRP-S30)	0.00E+00	0.004	Protein biosynthesis. Apoptosis, other metabolic processes
2	XM_534172	+2.77	PREDICTED: Canis familiaris similar to Transmembrane 9 superfamily protein member 2 precursor (p76)	0.00E+00	0.004	Phagocytosis, small molecule transport
3	NM_001015573	+2.62	Bos taurus ubiquitin-like 7 (bone marrow stromal cell-derived) (UBL7)	0.00E+00	0.010	Proteolysis
4	XM_593711	+2.59	PREDICTED: Bos taurus hypothetical LOC540389. transcript variant 1	0.00E+00	0.010	Biological processes unclassified
5	XM_592231	+2.35	PREDICTED: Bos taurus similar to thyroid hormone receptor interactor 12. transcript variant 1 (TRIP12)	0.00E+00	0.010	Proteolysis
6	AB015947	+2.26	Bos taurus AQP-4 mRNA for aquaporin-4-A (AQP4)	0.00E+00	0.016	Other transport, Other homeostasis activities
7	XM_611826	+2.22	PREDICTED: Bos taurus similar to protease M (KLK6)	1.00E-119	0.016	Proteolysis
8	AF058700	+2.11	Bos taurus ubiquitin-S27a fusion protein (RPS27A)	0.00E+00	0.016	Proteolysis
9	NM_178109	+2.05	Bos taurus protein kinase. interferon-inducible double stranded RNA dependent (PRKR)	0.00E+00	0.025	Protein biosynthesis, protein phosphorylation, translation regulation
10	NM_001098909	+2.05	PREDICTED: Bos taurus hypothetical LOC508503. transcript variant 1	0.00E+00	0.025	Biological processes unclassified
11	NM_001046225	+2.03	Bos taurus progestin and adipoQ receptor family member VI (PAQR6)	0.00E+00	0.025	Fatty acids metabolism, lipid metabolism, Cell surface receptor mediated signal transduction
12	NM_001038187	+2.02	Bos taurus similar to interleukin enhancer binding factor 2 (ILF2)	0.00E+00	0.025	Immunity and defence
13	AK236223	+1.99	Sus scrofa mRNA. clone:OVRM10165C01. expressed in ovary	0.00E+00	0.025	Biological processes unclassified
14	XR_027597	+1.87	PREDICTED: Bos taurus similar to carboxypeptidase D (CPD)	8.00E-166	0.025	Proteolysis
15	NM_001083647	+1.84	Bos taurus programmed cell death 4 (PDCD4)	0.00E+00	0.037	Protein biosynthesis, apoptosis induction
16	NM_001046321	+1.82	Bos taurus mitochondrial ribosomal protein L44 (MRPL44)	0.00E+00	0.037	Biological processes unclassified
17	XM_592085	+1.81	PREDICTED: Bos taurus kinesin family member 5A. transcript variant 1 (KIF5A)	0.00E+00	0.037	Intracellular protein traffic, cell structure
18	XM_862382	+1.74	PREDICTED: Canis familiaris similar to transmembrane protein 30A. transcript variant 4 (TMEM30A)	6.00E-139	0.037	Biological processes unclassified
19	BC126664	+1.73	Bos taurus similar to phosphatase and actin regulator 1 (PHACTR1)	1.00E-147	0.037	Neuronal activities, cell structure
20	XM_863156	+1.67	PREDICTED: Bos taurus similar to Ras-related protein Rab-34 (Rab-39) (Ras-related protein Rah). transcript variant 4 (RAB34)	0.00E+00	0.037	Intracellular signaling cascade, Receptor mediated endocytosis, General vesicle transport
21	NM_001025339	+1.55	Bos taurus ribosomal protein S24 (RPS24)	0.00E+00	0.037	Protein biosynthesis
22	NM_001076460	+1.55	Bos taurus similar to claudin 5 (cldn5)	0.00E+00	0.037	Cell structure
23	XM_866188	+1.52	PREDICTED: Bos taurus similar to RalA-binding protein 1 (Ral interacting protein 1) (76-kDa Ral-interacting protein) (Dinitrophenyl S-glutathione ATPase) (DNP-SG ATPase). transcript variant 2 (RalBP1)	0.00E+00	0.037	Mitosis
24	AC087623	-1.56	Homo sapiens chromosome 8. clone RP11-350N15	9.00E-21	0.037	Biological processes unclassified
25	NM_001046532	-1.60	Bos taurus RAB3A interacting protein (rabin3)-like 1 (RAB3IL1)	0.00E+00	0.037	Signal transduction, transsynaptic signal transduction
26	XM_617846	-1.60	PREDICTED: Bos taurus similar to Biotinidase (BTD)	0.00E+00	0.037	Vitamin metabolism
27	NM_001083414	-1.64	Bos taurus similar to WW domain containing E3 ubiquitin protein ligase 2 (Wwp2)	0.00E+00	0.037	Proteolysis

28	AK236193	-1.68	Sus scrofa mRNA. clone:OVRM10162C07. expressed in ovary	8.00E-73	0.037	Biological processes unclassified
29	XM_865141	-1.72	PREDICTED: Bos taurus similar to nucleolar protein 1. 120kDa (LOT3)	7.00E-28	0.037	rRNA metabolism
30	XM_604945	-1.72	PREDICTED: Bos taurus similar to APCL protein	0.00E+00	0.037	Other receptor mediated signaling pathway, other intracellular cascade, cell adhesion, Apoptosis, cell cycle control, tumor suppressor
31	XM_001249787	-1.72	PREDICTED: Bos taurus similar to topoisomerase (DNA) III alpha (Top3a)	0.00E+00	0.025	DNA replication
32	NM_001034214	-1.72	Bos taurus ubiquitin-like 4A (UBL4A)	3.00E-73	0.025	Proteolysis
33	NM_001075649	-1.73	Bos taurus similar to CG18177-PB	2.00E-108	0.025	Biological processes unclassified
34	XM_001113758	-1.75	PREDICTED: Macaca mulatta similar to galactokinase 2 isoform 2. transcript variant 4 (GALK2)	6.00E-14	0.025	Monosaccharide metabolism
35	NM_001075959	-1.79	Bos taurus similar to synaptosomal-associated protein 29 (SNAP-29)	0.00E+00	0.025	General vesicle transport, transport
36	XM_583735	-1.79	PREDICTED: Bos taurus similar to TRRAP protein	0.00E+00	0.025	Chromatin packaging and remodeling
37	XM_864237	-1.80	PREDICTED: Bos taurus similar to KIAA0369 doublecortin-like kinase 1 (DCLK1)	0.00E+00	0.016	Protein phosphorylation, neuronal activities, cell structure
38	NM_001034274	-1.85	Bos taurus similar to mitochondrial ribosomal protein L17 (MRPL17)	3.00E-88	0.016	Protein biosynthesis
39	AC069226	-1.86	Homo sapiens 3 BAC RP11-426J22 (Roswell Park Cancer Institute Human BAC Library)	4.00E-11	0.016	Biological processes unclassified
40	XM_874885	-1.86	PREDICTED: Bos taurus similar to TBC1 domain family. member 4. transcript variant 3 (TBC1D4)	0.00E+00	0.016	Biological processes unclassified
41	XM_582759	-1.89	PREDICTED: Bos taurus similar to Aldehyde dehydrogenase 16 family. member A1 (aldh16a1)	0.00E+00	0.010	Biological processes unclassified
42	BC042752	-1.92	Homo sapiens mitochondrial ribosomal protein S6 (MRP-S6)	1.00E-22	0.010	Protein biosynthesis
43	XR_028825	-2.00	PREDICTED: Bos taurus similar to KIAA0342 protein	0.00E+00	0.010	Biological processes unclassified
44	XR_028216	-2.04	PREDICTED: Bos taurus similar to Receptor tyrosine-protein kinase erbB-3 precursor (Tyrosine kinase-type cell surface receptor HER3) (c-erbB3)	0.00E+00	0.010	Protein phosphorylation, Receptor protein tyrosine kinase signaling pathway, Cell cycle control, Cell proliferation and differentiation, Oncogenesis
45	AC189717	-2.06	Canis familiaris. clone XX-115F18	5.00E-74	0.010	Biological processes unclassified
46	XM_593566	-2.16	PREDICTED: Bos taurus similar to Family with sequence similarity 113. member B. transcript variant 1 (FAM113B)	9.00E-101	0.006	Biological processes unclassified
47	NM_001014906	-2.48	Bos taurus pyrroline-5-carboxylate reductase-like (PYCRL)	0.00E+00	0.006	Amino acids biosynthesis
48	NM_001034728	-2.55	Bos taurus similar to thrombospondin 4 precursor (Thbs4)	0.00E+00	0.006	Blood clotting, other developmental processes

E value – expectation value. E-value indicates the probability of generation of identical score for the sequence chosen (presumed to be the basis of query identification) resulting from random matching of our query with other sequences available in GenBank. The lower E value (closer or equal 0 – so the lower probability of random score generation), the higher reliability of BLAST score and the identification of sequence is closer to the bone.

Fold change – value describing level of increase or decrease of gene expression in sample investigated when compared to the control sample, which expression is arbitrary accepted as 1.0. As a threshold value (lowest change of expression) for the difference between genes expression to be considered a significant 1.50 value has been accepted. Positive values correspond to higher expression in beef cattle, whereas negative values correspond to lower expression in beef cattle, when compared to dairy cattle.

P value – Level of significance. Probability of making a mistake of first order consisting in acceptance of false hypothesis as true one. In presented studies a level of significance at $p<0.05$ has been accepted, based on the results of Wilcoxon non-parametric rank test.

TI – transcriptomic index. Transcriptomic index consists on genes which average expression in HER and LIM was at least 1.5 times different (higher or lower) than in HF cattle. Values presented are the difference between average gene expression in HER and LIM and expression in HF, however the individual differences between HER and HF and between LIM and HF was also at least 1.5. For each direct comparison (HER vs. HF; LIM vs. HF) total n=6 microarrays were performed (3 individuals x 2 different tissue samples).

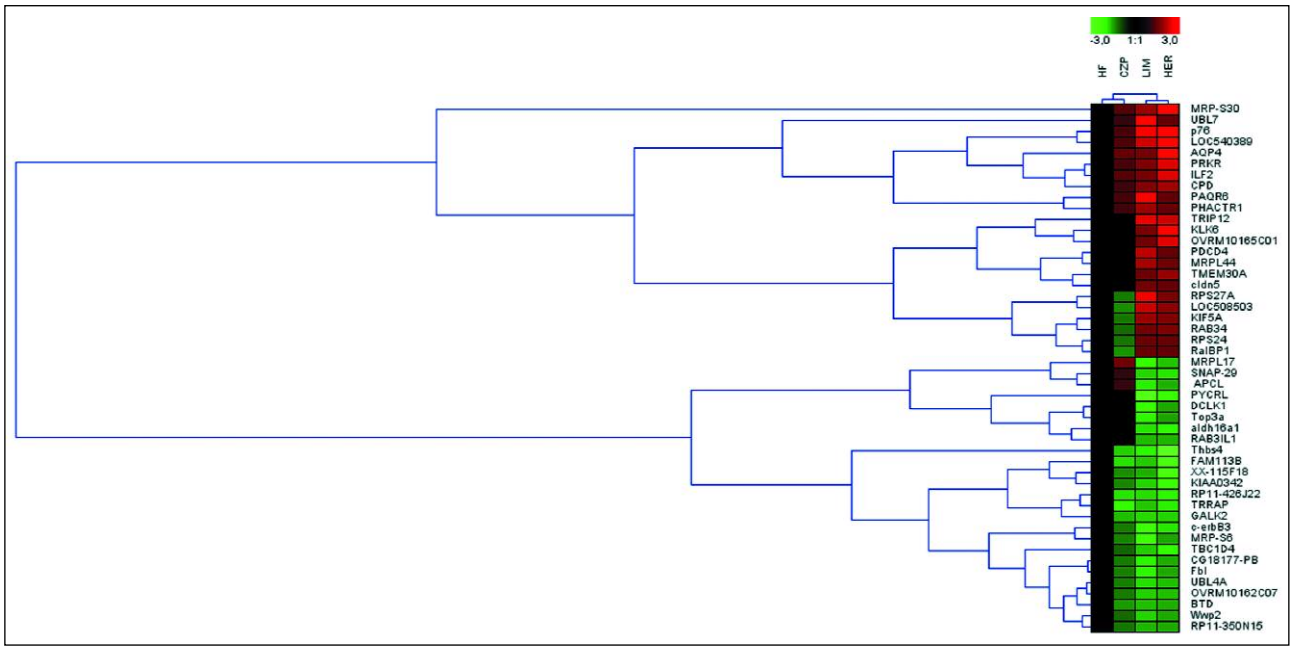

Fig. 3. Hierarchical group analysis (clustering) of expression of genes belonging to the transcriptomic index in Holstein-Friesian (HF), Limusine (LIM), Hereford (HER) and Polish Red (PR) bulls.

The change fold for particular genes was comparable using cDNA microarray and real-time RT-PCR methods.

Hierarchical analysis of transcriptomic index

Clustering of genes belonging to the transcriptomic index was performed using *Genesis* software (34) and revealed similar patterns of transcriptomes in LIM and HER breeds (*Fig. 3*). It should be also pointed out that in HF breed, which is a typical dairy breed, the expression pattern of genes belonging to the transcriptomic index was completely different from that in both beef breeds, whereas PR breed posses some features of meat and dairy cattle breeds. Hierarchical analysis revealed also that changes in expression of all genes belonging to the transcriptomic index in both LIM and HER were similar and the groups of genes with lower and higher expression form two separate groups of genes with quite small interrelationship (*Fig. 3*). In case of PR breed we observed two groups of genes with clearly different expression when compared to HF breed and to both beef breeds. One of them consists of six genes with quite close dependence of expression (RPS27A, OVRM10165001, KIF5A, RAB34, RPS24, RalBP1) and has lower expression in PR when compared to all the remaining cattle breeds. The second group consists of three genes (MRPL17, SNAP29, APCL) and has higher expression in PR when compared to all the remaining cattle breeds. There are also some genes in PR not differing in expression when compared to HF (TRIP12, KLK6, OVRM10165001, PDCD4, MRPL44, TMEM30A, cldn5, PYCRL, DCLK1, Top3a, aldh16a1, RAB3Il1) but with significant differences (increase or decrease) when compared to LIM and HER. These results strongly confirm dual: meat and dairy type of PR cattle breed.

Functional characteristics of genes belonging to transcriptomic index

Using *Panther* software we performed functional analysis of genes belonging to transcriptomic index in order to establish their role in particular biological processes and molecular function (35). With respect to molecular function we identified three predominating groups of genes: one group of 10 genes which protein products bind to nucleic acids (RPS27A, ILF2, PDCD4, RPS24, KIAA0342, MRPS6, MRPL17, Fbl, Top3a, UBL4A), second group of 6 genes encoding regulatory proteins (RAB3IL1, RalBP1, Thbs4, Top3a, RAB34, PHACTR1) and third group of 4 genes for kinases (TRRAP, PRKR, GALK2, c-erbB3). Five genes had molecular function unclassified and the remaining genes belonged to following classes: signaling molecule, cytoskeletal protein, ligase, miscellaneous function, oxidoreductase, protease and receptor (*Fig. 4*). With respect to biological processes total 14 out of 48 genes were involved in protein metabolism and modification (*Fig. 5*). Among them 6 were involved in protein synthesis (MRP-S30, PRKR, PDCD4, RPS24, MRP-

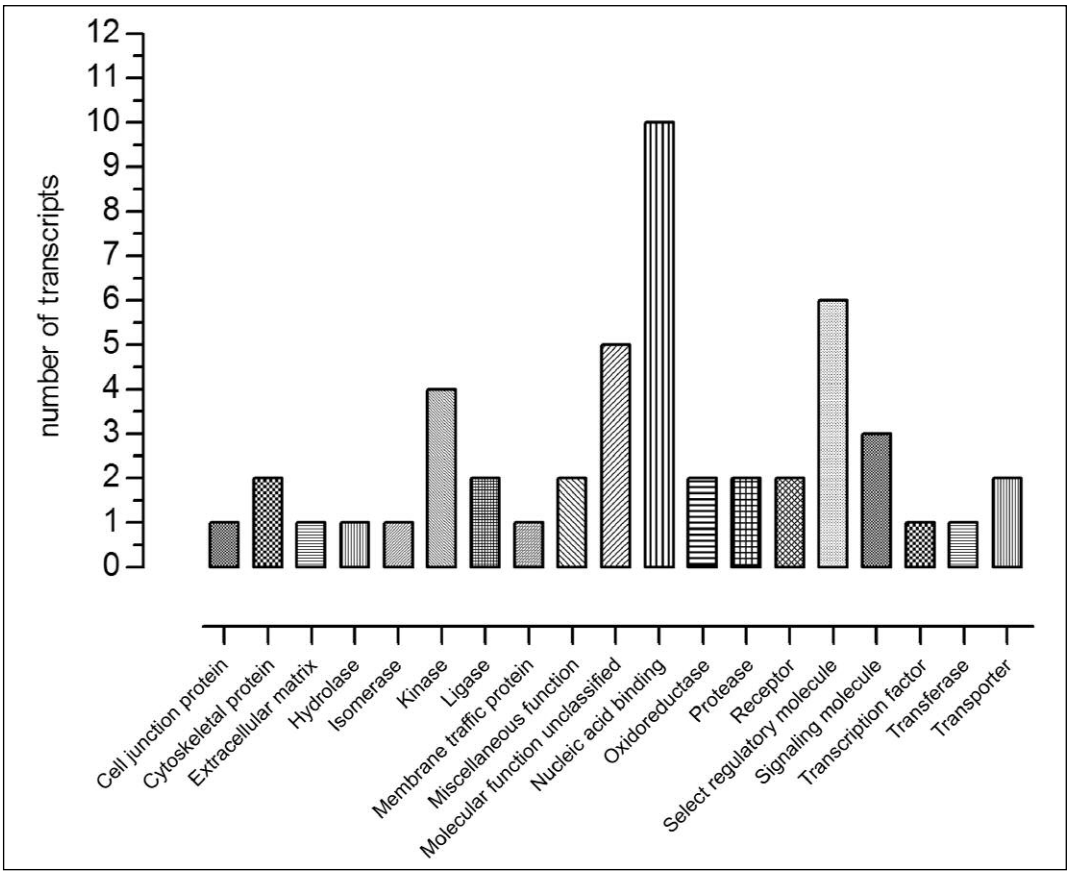

Fig. 4. Molecular functions of genes belonging to the transcriptomic index characteristic for muscle of beef breed bulls.

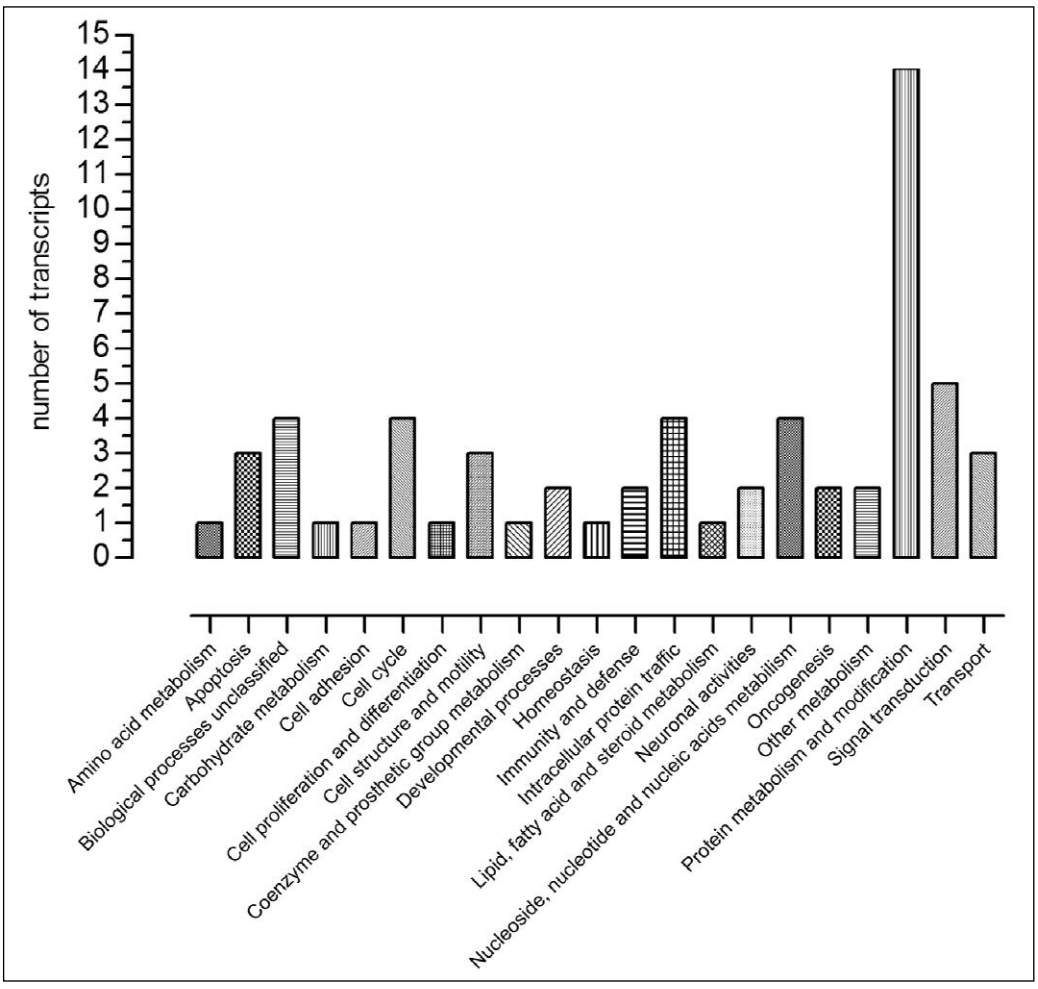

Fig. 5. Classification of genes included to the transcriptomic index characteristic for muscle of beef breeds bulls according to biological processes.

S6, MRPL17), 2 in protein modification (PRKR, c-erbB3), and 7 in proteolysis (UBL7, TRIP12, KLK6, RPS27A, CPD, UBL4A, Wwp2). According to *Panther* analysis PRKR was classified both in the group of genes involved in protein synthesis and protein modification. The remaining genes were involved in following biological processes: signal transduction (5 genes) cell cycle (4 genes), intracellular protein traffic (4 genes), nucleoside, nucleotide and nucleic acid metabolism (4 genes), biological processes unclassified (4 genes), apoptosis (3 genes), cell structure and motility (3 genes), transport (3 genes) and few other biological processes (1 or 2 genes involved).

DISCUSSION

DNA microarray technique is a new and very promising tool in functional genomics, which permits to monitor global gene expression pattern and characterization of the gene networks that regulate various physiological processes. There is an increasing number of studies on growth and metabolism of skeletal muscle in cattle where this method was applied. Among them there are studies concerning age-dependent changes in bovine skeletal muscle transcriptomic profile (32), identification of differentially expressed genes in distinct skeletal muscles in cattle (36, 37), effect of double muscled phenotype (mutation nt821 (del11)) on gene expression pattern in bovine embryos (38) and fetuses (39), transcriptome analysis of two bovine muscles (oxidative and glycolytic) during ontogenesis (40), gene expression profiling of muscle tissue in Brahman steers during nutritional restriction (41) and gene expression in developing bovine longissimus muscle from two different beef cattle breeds (42). However, there is a limited number of data on transcriptomic profiling in skeletal muscle of different cattle breeds. They concern the transcriptional profiling of skeletal muscle tissue from two breeds of cattle (43) and development of bovine longissimus muscle from two different beef cattle breeds (42). In the present study cDNA microarrays were used for analysis of bovine skeletal muscle transcriptome in 12-month-old bulls of four cattle breeds aiming to identify the genes, which expression was common for beef breed bulls. Comparison of the transcriptomic profile between typical dairy breed bulls (Holstein-Friesian) used as a reference, and beef breed bulls (Limousine, Hereford) as well as dual: meat and dairy type breed bulls (Polish Red) revealed significant differences in gene expression pattern (*Fig. 1*). To establish transcriptomic responses which are typical for beef breeds of bulls, we selected these transcripts which responded similarly in LIM and HER with exclusion of 2 similar responses in PR (*Fig. 1*). As a result we obtained the transcriptomic index being the set of genes expressed similarly in beef breeds of bulls in comparison to dairy and dual-purpose breed of bulls. We postulate that genes belonging to the transcriptomic index may have a real influence on skeletal muscle growth and development in postnatal period of life. Identification of transcriptomic index revealed the genes which were not earlier described as those rate-limiting skeletal muscle growth and development. Transcriptomic index consists of 48 transcripts, which level was higher (23 transcripts) or lower (25 transcripts) in beef breeds than dairy breed muscle (*Table 2*). However, it should be pointed out that as many as 343 genes in the case of LIM and 412 genes in the case of HER, which expression was significantly different than in HF bulls, have not been included in transcriptomic index. It suggests breed-dependent specificity in the regulation of muscle growth in LIM and HER bulls. Although LIM and HER are typical beef breeds, they differ in time of maturity, percentage of carcass dressing and lean meat in valuable cuts (*Table 1*) and intramuscular fat content. It may explain why transcriptomic index contains only 48 genes which are similarly expressed in both beef breeds in comparison with HF as a typical dairy breed. To transcriptomic index have not been included well known genes which expression was significantly different for separate comparison of transcriptomes between HF and LIM as well as HF and HER. For example in HF-LIM comparison the significant difference in expression has been found in the case of: ornithine dexarboxylase, GH receptor, becklin1, early growth response 4 (EGR4), MAP4kinase4, kallikrein 10 precursor and tubulin beta4. For HF-HER comparison we found significantly different: TGFα, IP6, creatine kinase, carbamylophosphate synthetase2, fibronectin III, myosin1C, collagen VII, Bcl-2, SMAD4, ATG9, MyoD1, PDGF-A, JAK3 and apoptosis inhibiting factor 5 (API5). Above results indicate that regulation of muscle growth and metabolism in beef breeds is not universal and their meat pheonotype is attained by interactions of common and breed-specific genes. Breed-dependent similarities and differences in transcriptomic responses were very well visible when hierarchical group analysis of transcripts belonging to transcriptomic index was performed (*Fig. 3*). It has been shown that clustering of transcripts was very close in LIM and HER, suggesting the similarity in genomic conditioning of muscle growth in these breeds. Classification of genes according to molecular function of their protein products has

shown the highest number of genes encoding proteins involved in nucleic acid binding (10), regulatory proteins (6) and kinases (4) (*Fig. 4*). Classification according to biological processes revealed the highest number of genes involved in protein metabolism and modification (14 genes) (*Fig. 5*), thus confirming the crucial role of protein turnover in skeletal muscle growth and development. Among them 6 were involved in protein synthesis, 2 in protein modification and 7 in proteolysis (*Table*

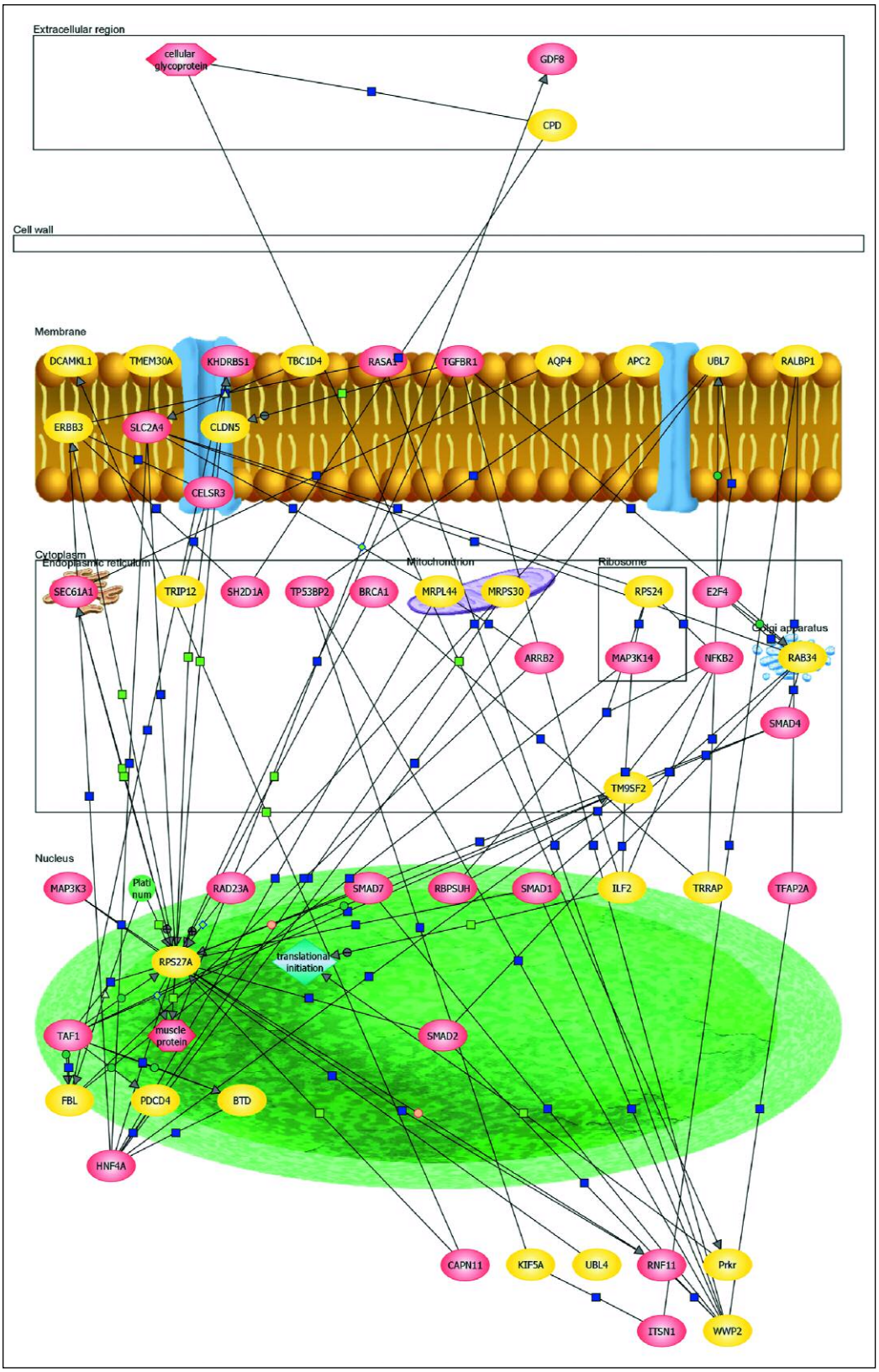

Fig. 6. The network of genes belonging to the transcriptomic index (yellow) characteristic for muscle of beef breeds bulls performed using *Pathway Architect* software.

2). Of the genes 9 were up-regulated and 5 down-regulated in beef breeds of bulls. A higher expression of genes involved in protein metabolism and modification in muscle of LIM and HER bulls is in concordance with better feed conversion in these breeds than in HF crosses. It has been shown that LIM and HER bulls required less DM, CP, UFV, and PDI to gain 1 kg live weight than the other breed bulls, including HF (44).

To localize the protein products of gene expression belonging to the transcriptomic index on metabolic pathways in skeletal muscle fibers we used *Pathway Architect* (Stratagene, USA) software (*Fig. 6*). In the network there are proteins of special significance, which form junctions converging with other pathways, *e.g. Bos taurus* ubiquitin-S27a fusion protein (RPS27A), *Bos taurus* similar to WW domain containing E3 ubiquitin protein ligase 2 (Wwp2), *Bos taurus* similar to receptor tyrosine-protein kinase erbB-3 precursor (Tyrosine kinase-type cell surface receptor HER3) (c-erbB3).

The best known function of ubiquitination is its role in protein degradation, where polyubiquitinated proteins are recognized by the 26S proteasome and are rapidly degraded. RPS27A, a highly conserved protein has a major role of targeting of cellular proteins for degradation by the 26S proteasome. The gene encodes a fusion protein consisting of ubiquitin at the N terminus and ribosomal protein S27a at the C terminus. When expressed in yeast, the protein is post-translationally processed, generating free ubiquitin monomer and ribosomal protein S27a. Ribosomal protein S27a is a component of the 40S subunit of the ribosome and belongs to the S27AE family of ribosomal proteins. In humans, the two human ubiquitin-CEP (carboxyl extension protein) genes are Uba80 (RPS27A) and Uba52, which code for ubiquitin fused to ribosomal protein S27a and L40, respectively (45). The role of RPS27A gene in bovine skeletal muscle has not been described up to now. It has been suggested that overexpression of this gene in colon cancer (46) and prostate cancer (47) may promote tumor development by increasing the degradation of proteins, which are involved in growth inhibition or apoptosis *via* the proteasome pathway. It cannot be excluded that a higher expression of this gene in muscle of beef breeds of bulls may promote muscle growth in similar manner.

Although the best-known function of ubiquitination is its role in protein degradation, other studies showed that under certain circumstances ubiquitination of transcription factors, independent of proteolysis, is required for the function of transcription factors (48). It is generally accepted that mono-Ub is a regulator of the location and activity of diverse cellular proteins, whereas multi-Ub chains mediate protein destruction by the proteasome (49). It has been demonstrated that Wwp2-anE3 ubiquitin ligase interacts with transcription factor Oct-4 and promotes its ubiquitination both *in vivo* and *in vitro* (48). This modification dramatically suppresses the transcriptional activity of Oct-4. Mentioned authors suggest that other cellular regulatory proteins can be modified and functionally regulated in a similar manner. It is possible that the down-regulation of *wwp2* gene in beef breeds muscle may release transcription factors involved in myogenesis from inhibitory influence of monoubiquitination.

Another gene which may be important in the regulation of muscle growth and development is *c-erbb3* encoding tyrosine kinase-type cell surface receptor for neuregulin (NRG). Apart of ErbB3 (HER3) to the type I subfamily of receptor tyrosine kinases for NRG belong: ErbB2 (HER2/Neu) and ErbB4. Heteromeric complexes of ErbB2, ErbB3 and ErbB4 and homomeric ErbB4 are activated by NRG binding and lead to phosphorylation of cytoplasmic tyrosine residues that initiate a diverse array of downstream signaling events (50). Nerve- and muscle-derived members of the NRG family have been observed to stimulate myotube formation and muscle specific gene expression (51, 52), induce acetylcholine receptor expression (53), regulate formation of synapses (54) and facilitate glucose uptake (55). Activation of NRG/ErbB signaling may also mediate one or more adaptive growth and metabolic responses of skeletal muscle to exercise (50). Above data indicate that ErbB3 receptor is involved in NGR signaling which is favourable in myogenesis. Therefore it is difficult to explain why *c-erbb3* gene is down-regulated in LIM and HER bulls in comparison to HF (*Table 2*).This result was validated with real-time RT-PCR method and showed similar to cDNA microarray values, *i.e.* a higher expression of erbB3 in muscle of HF than in LIM and HER (*Fig. 2*, *Table 3*). In the next step it would be interesting to compare the level of ErbB3 protein in muscles of examined breeds, as in some cases there is auto-regulation of gene expression by its protein product. Simplified scheme of interactions of RPS27A, Wwp2 and c-erbB3 with well known genes involved in the regulation of skeletal muscle growth and metabolism was shown on *Fig. 7*. According to this scheme identified key genes are functionally linked with signaling pathway of TGF-beta super family throughout receptor TGFBR1, transcription factors : Smad1, Smad2, Smad3 and Smad7 as well as bone morphogenic protein 6 (BMP6). Moreover they can be regulated by insulin and epidermal growth factor (EGF).

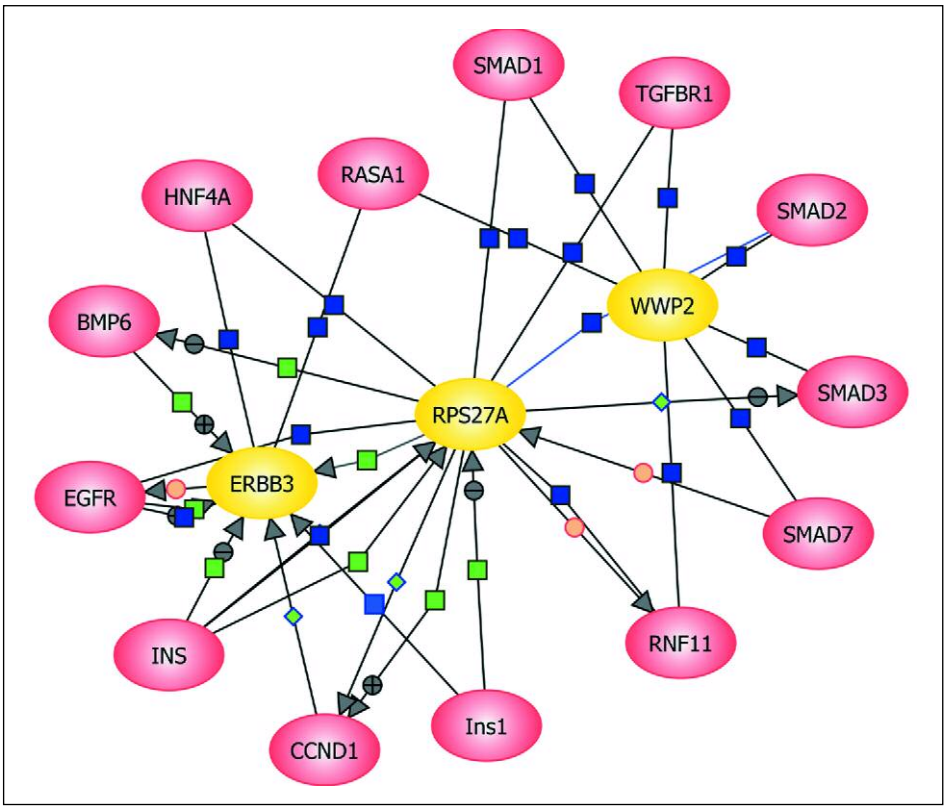

Fig. 7. Scheme of interactions of RPS27A, Wwp2 and c-erbB3 with well known genes involved in the regulation of skeletal muscle growth and metabolism (*PathwayArchitect* software)

In conclusion, the present study is the first showing the transcriptomic index common for muscle growth and metabolism in beef breeds bulls. It consist 48 transcripts involved in protein metabolism and modification (14), signal transduction (5), cell cycle (4), intracellular protein traffic (4), nucleoside, nucleotide and nucleic acid metabolism (4), apoptosis (3), cell structure and motility (3), transport (3) and 2 or single genes engaged in other biological processes. The role of the most genes included to the transcriptomic index in skeletal muscle has not been described yet. Therefore, transcriptomic index may be very useful in revealing new candidate genes to search a new criteria of animal selection in beef production.

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REFERENCES

1. Mullen AM, Stapleton PC, Corcoran D, Hamill RM, White A. Understanding meat quality through the application of genomic and proteomic approaches. *Meat Sci* 2006; 74: 3-16.
2. Gao Y, Zhang R, Hu X, Li N. Application of genomic technologies to the improvement of meat quality of farm animals. *Meat Sci* 2007; 77: 36-45.
3. Switonski M. Postepy genomiki zwierzat domowych. *Nauka* 2008; 1.
4. Hocquette JF, Lehnert S, Barendse W, Cassar-Malek I, Picard B. Recent advances in cattle functional genomics and their application to beef quality. *Animal* 2007; 1: 159-173.
5. Oprzadek J, Dymnicki E, Reklewski Z. Growth rates and carcass composition of young cattle of different breeds (in Polish). *Roczniki Naukowe PTZ* 2007; 3: 25-31.
6. Coffey SG. Prospects for improving the nutritional quality of dairy and meat products. *Forum Nutr* 2007; 60: 183-195.
7. Dekkers JCM. Commercial application of marker-and gene-assisted selection in livestock: Strategies and lessons. *J Anim Sci* 2004; 82: 313-328.
8. Van Eenennaam AL, Li J, Thallman RM *et al*. Validation of commercial DNA tests for quantitative beef quality traits. *J Anim Sci* 2007; 85: 891-900.
9. Lonergan SM, Ernst CW, Bishop MD, Calkins CR, Koohmaraie M. Relationship of restriction fragment length polymorphism (RFLP) at the bovine calpastatin locus to calpastatin activirty and meat lenderness. *J Anim Sci* 1995; 73: 3608-3612.
10. Page BT, Casas E, Quaas RL *et al*. Association of markers in the bovine CAPN1 gene with meat tenderness in large crossbreed populations that sample influential industry sires. *J Anim Sci* 2004; 8: 3474-3481.
11. Juszczak-Kubiak E, Rosochacki S, Wicinska K, Szreder T, Sakowski T. A novel RFLP/AluI polymorphism of the bovine calpastatin (CAST) gene and its association with selected traits of beef. *Anim Sci Pap Rep* 2004; 22: 195-204.
12. Juszczuk–Kubiak E, Sakowski T, Flisikowski K, Wicinska K, Oprzadek J, Rosochacki S. Bovine μ-calpain (CAPN1) gene: New SNP within intron 14. *J Appl Genet* 2004; 45: 457-460.

13. White SN, Casas E, Wheeler TL *et al*. A new single nucleotide polymorphism in CAPN 1 extends I the current tendemess marker test to include cattle of Bos indicus, Bos taurus, and crossbred descent. *J Anim Sci* 2005; 83: 2001-2008.
14. Casas E, White SN, Wheeler TL *et al*. Effects of calpastatin and mu-calpain markers in beef cattle on tendemess traits. *J Anim Sci* 2006; 84: 520-525.
15. Schenkel FS, Miller AP, Jiang Z *et al*. Association of a single nucleotide polymorphism in the calpastatin gene with carcass and meat quality traits of beef cattle. *J Anim Sci* 2006; 84: 291-299.
16. Rosochacki S, Kubiak-Juszczuk E, Barton L *et al*. Preliminary observations upon relation between the G77A polymorphism In CATD gene and lysosomal proteinases activity and sensory traits of meat from bulls of three breeds. *Anim Sci Pap Rep* 2008; 26: 25-35.
17. Schenkel FS, Miller SP, Ye X *et al.* Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. *J Anim Sci* 2005; 83: 2009-2020.
18. Kononoff PJ, Deobald HM, Stewart EL, Laycock AD, Marquess FL. The effect of a leptin single nucleotide polymorphism on quality grade, yield grade, and carcass weight of beef cattle. *J Anim Sci* 2005; 83: 927-932.
19. Nkrumah JD, Li C, Basarab JB, Guercio S *et al.* Association of a single nucleotide polymorphism in the bovine leptin gene with feed intake, feed efficiency, growth, feeding behaviour, carcass quality and body composition. *Can J Anim Sci* 2005; 84: 211-219.
20. Barendse W, Bunch R, Thomas M, Armitage S, Baud S, Donaldson N. The TG5 thyroglobulin gene test for a marbling quantitative trait loci evaluated in feedlot cattle. *Aust J Exp Agr* 2004; 44: 669-674.
21. Thaller G, Kuhn C, Winter A *et al.* DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. *Anim Genet* 2003; 34: 354-357.
22. Grochowska R, Lunden A, Zwierzchowski L, Snochowski M, Oprzadek J. Association between gene polymorphism of growth hormone and carcass traits in dairy bulls. *Anim Sci* 2001; 72: 441-447.
23. Beauchemin VR, Thomas MG, Franke DE, Silver GA. Evaluation of DNA polymorphisms involving growth hormone relative to growth and carcass characteristics in Brahman steers. *Genet Mol Res* 2006; 5: 438-447.
24. Maj A, Zwierzchowski L, Oprzadek J, Oprzadek A, Dymnicki E. Polymorphism in the 5'-noncoding region of the bovine growth hormone receptor gene and its association with meat production traits in cattle. *Anim Res* 2004; 53: 503-514.
25. Maj A, Zwierzchowski L, Oprzadek J, Dymnicki E. Polymorphism in the 5'-noncoding region of the bovine growth hormone receptor gene and its association with meat production traits in Black-and-White cattle. *Meat Sci* 2006; 72: 539-544.
26. Tatsuda K, Oka A, Iwamoto E *et al.* Relationship of the bovine growth hormone gene to carcass traits in Japanese black cattle. *J Anim Breed Genet* 2008; 125: 45-49.
27. Flisikowski K, Oprzadek J, Dymnicki E, Zwierzchowski L. New polymorphism in the bovine STAT5A gene and its association with meat production traits in beef and dairy cattle. *Anim Sci Pap Rep* 2003; 21: 147-157.
28. Wheeler TL, Shackelford SD, Casas E, Cundiff LV, Koohmaraie M. The effects of Piedmontese inheritance and myostatin genotype on the palatability of longissimus thoracis, gluteus medius, semimembranosus, and biceps femoris. *J Anim Sci* 2002; 79: 3069-3074.
29. Bernard C, Cassar-Malek I, Le Cunff M, Dubroeucq H, Renand G, Hocquette JF. New indicators of beef sensory quality revealed by expression of specific genes. *J Agric Food Chem* 2007; 55: 5229-5237.
30. Oprzadek J, Dymnicki E, Oprzadek A, Sloniewski K, Sakowski T, Reklewski Z. A note on the effect of breed on beef cattle the carcass traits. *Anim Sci Pap Rep* 2001; 19: 79-89.
31. Jank M, Zwierzchowski L, Siadkowska E *et al*. Polymorphism in the 5'flanking region of the myostatin gene affects myostatin and TGF-β1 expression in bovine skeletal muscle. *J Anim Feed Sci* 2006; 15: 381-391.
32. Sadkowski T, Jank M, Oprzadek J, Motyl T. Age-dependent changes in bovine skeletal muscle transcriptomic profile. *J Physiol Pharmacol* 2006; 57(Suppl 7): 95-110.
33. Sadkowski T, Jank M, Zwierzchowski L, Siadkowska E, Oprzadek J, Motyl T. Gene expression profiling in skeletal muscle of Holstein Friesian bulls with single nucleotide polymorphism in myostatin gene 5'flanking region. *J Appl Genet* 2008; 49: 237-250.
34. Sturn A, Quackenbush J, Trajanoski Z. Genesis: Cluster analysis of microarray data. *Bioinformatics* 2002; 18: 207-208.
35. Thomas PD, Kejariwal A, Campbell MJ *et al*. PANTHER: a browsable database of gene products organized by biological function, using curated protein family and subfamily classification. *Nuc Acids Res* 2003; 31: 334-341.
36. Yu SL, Chung HJ, Sang BC *et al*. Identification of differentially expressed genes in distinct skeletal muscles in cattle using cDNA microarray. *Anim Biotechnol* 2007; 18: 275-285.
37. Sudre K, Cassar-Malek I, Listrat A *et al*. Biochemical and transcriptomic analyses of two bovine skeletal muscles in Charolais divergently selected for muscle growth. *Meat Sci* 2005; 70: 267-277.
38. Potts JK, Echternkamp SE, Smith TP, Reecy JM. Characterization of gene expression in double-muscled and normal-muscled bovine embryos. *Anim Genet* 2003; 34: 438-444.
39. Cassar-Malek I, Passelaigue F, Bernard C, Leger J, Hocquette JF. Target genes of myostatin loss-of-function in muscles of late bovine fetuses. *BMC Genomics* 2007; 8: 63.
40. Sudre K, Leroux C, Pietu G *et al*. Transcriptome analysis of two bovine muscles during ontogenesis. *J Biochem* 2003; 133: 745-756.
41. Byrne KA, Wang YH, Lehnert SA *et al*. Gene expression profiling of muscle tissue in Brahman steers during nutritional restriction. *J Anim Sci* 2005; 83: 1-12.
42. Lehnert SA, Reverter A, Byrne KA *et al*. Gene expression studies of developing bovine longissimus muscle from two different beef cattle breeds. *BMC Dev Biol* 2007; 7: 95.
43. Wang YH, Byrne KA, Reverter A *et al.* Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. *Mamm Genome* 2005; 16: 201-210.
44. Dymnicki E, Oprzadek K, Reklewski Z, Sloniewski K, Oprzadek A, Krzyzewski J. Growth rate, feed intake and feed conversion in fattening bulls of main beef breeds kept in Poland. *Anim Sci Pap Rep* 2001; 19(3): 57-64.
45. Kirschner LS, Stratakis CA. Structure of the human ubiquitin fusion gene Uba80 (RPS27a) and one of its

pseudogenes. *Biochem Biophys Res Commun* 2000; 270: 1106-1110.
46. Barnard GF, Mori M, Staniunas RJ *et al*. Ubiquitin fusion proteins are overexpressed in colon cancer but not in gastric cancer. *Biochim Biophys Acta* 1995; 1272: 147-153.
47. Ko Y, Hahn T, Lu H *et al*. A novel component of the ubiquitin pathway, ubiquitin carboxyl extension protein 1 is overexpressed in prostate cancer. *Int J Mol Med* 2005; 15: 183-196.
48. Xu HM, Liao B, Zhang QJ *et al*. Wwp2, an E3 ubiquitin ligase that targets transcription factor Oct-4 for ubiquitination. *J Biol Chem* 2004; 279: 23495-23503.
49. Hicke L. Protein regulation by monoubiquitin. *Nat Rev Mol Cell Biol* 2001; 2: 195-201.
50. Lebrasseur NK, Cote GM, Miller TA, Fielding RA, Sawyer DB. Regulation of neuregulin/ErbB signaling by contractile activity in skeletal muscle. *Am J Physiol Cell Physiol* 2003; 284: C1149-C1155.
51. Florini JR, Samuel DS, Ewton DZ, Kirk C, Sklar RM. Stimulation of myogenic differentiation by a neuregulin, glial growth factor 2. Are neuregulins the long-sought muscle trophic factors secreted by nerves? *J Biol Chem* 1996; 271: 12699-12702.
52. Kim D, Chi S, Lee KH *et al*. Neuregulin stimulates myogenic differentiation in an autocrine manner. *J Biol Chem* 1999; 274: 15395-15400.
53. Jo SA, Zhu X, Marchionni MA, Burden SJ. Neuregulins are concentrated at nerve-muscle synapses and activate ACh receptor gene expression. *Nature* 1995; 373: 158-161.
54. Sandrock A, Jr W, Dryer SE *et al.* Maintenance of acetylcholine receptor number by neuregulins at the neuromuscular junction in vivo. *Science* 1997; 276: 599-603.
55. Suarez E, Bach D, Cadefau J, Palacin M, Zorzano A, Guma A. A novel role of neuregulin in skeletal muscle. Neuregulin stimulates glucose uptake, glucose transporter translocation, and transporter expression in muscle cells. *J Biol Chem* 2001; 276: 18257-18264.

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