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Biochimica et Biophysica Acta 1777 (2008) 104–112
Biochimica et Biophysica Acta
BBA
www.elsevier.com/locate/bbabio

Synchronized changes in transcript levels of genes activating cold exposure-induced thermogenesis in brown adipose tissue of experimental animals

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Received 26 May 2007; received in revised form 17 October 2007; accepted 18 October 2007
Available online 6 November 2007

Abstract

To identify genes whose expression in brown adipose tissue (BAT) is up- or down-regulated in cold-exposed rats, we performed microarray analysis of RNA samples prepared from the BAT of cold-exposed rats and of rats kept at room temperature. Previously reported elevations of transcript levels of uncoupling protein (UCP1), type II iodothyronine deiodinase (DIO2), and type III adenylate cyclase (AC3) in the BAT of cold-exposed rats over those in that of rats maintained at room temperature were confirmed. In addition to these changes, remarkable elevations of the transcript levels of several genes that seemed to be associated with the processes of cell-cycle regulation and DNA replication were detected in the BAT of cold-exposed rats, possibly reflecting the significant proliferation of brown adipocytes in response to cold exposure. Up-regulation of the gene encoding sarcomeric mitochondrial type creatine kinase in the BAT of cold-exposed rats was also detected by microarray analysis, but subsequent Northern analysis revealed that the expression of not only the sarcomeric mitochondrial type enzyme, but also that of 2 other subtypes, i.e., cytoplasmic brain type and cytoplasmic muscle type, was elevated in the BAT of cold-exposed rats. Microarray analysis also revealed a significant expression of myoglobin in BAT and its elevation in the BAT of cold-exposed rats, and this result was supported by calibrated Northern analysis. On the contrary, several genes such as regulator of G-protein signaling 2 and IMP dehydrogenase 1 were down-regulated in the BAT of cold-exposed rats. The physiological meaning of these changes accompanying cold exposure was discussed.

Keywords: Brown adipose tissue (BAT); Microarray analysis; Gene expression

1. Introduction

Two kinds of adipose tissues are known to exist in mammals, i.e., white and brown (abbreviated as WAT and BAT, respectively). Both of these tissues store triglycerides; however, their physiological function is completely opposite. Namely, WAT mainly functions to store excess energy in the form of fat, whereas BAT serves to dissipate excess energy in the form of heat. This energy-dissipating function of BAT is known to be mainly achieved by the uncoupling protein (UCP1), which is specifically expressed in brown fat mitochondria. UCP1 dissipates the proton electrochemical potential across the inner mitochondrial membrane, known

Abbreviations: AC3, type III adenylate cyclase; BAT, brown adipose tissue; CK, creatine kinase; CKb, cytoplasmic brain type CK; CKm, cytoplasmic muscle type CK; CKMT1, ubiquitous mitochondrial type CK; CKMT2, sarcomeric mitochondrial type CK; DIO2, type II iodothyronine deiodinase; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; IMPDH, inosine monophosphate dehydrogenase 1; RGS2, regulator of G-protein signaling 2; UCP1, uncoupling protein; VDAC, voltage-dependent anion channel; WAT, white adipose tissue

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0005-2728/$ - see front matter © 2007 Published by Elsevier B.V.
doi:10.1016/j.bbabio.2007.10.014

as the driving force of ATP synthesis, and thus causes the uncoupling of oxidative phosphorylation [1–4].

The thermogenic activity of BAT is known to rise when animals require heat production to maintain their body temperature. To enable efficient heat production in BAT, cold-exposed animals increase the expression of UCP1 in their BAT [5,6]. However, the gene expression of not only UCP1 but also that of other metabolic proteins in BAT would be expected to be regulated to enable effective thermogenesis in BAT under cold conditions. In our previous studies, to examine this possibility we carried out Northern analysis of 39 proteins associated with energy metabolism and its regulation [7,8]. As a result, we found that the expression of several proteins such as heart-type fatty acid-binding protein, heart/muscle type carnitine palmitoyltransferase I, type 4 glucose transporter, and type II hexokinase in BAT was markedly elevated in the BAT of rats exposed to a low temperature [7,8]. Interestingly, these proteins are isoforms that are known to be significantly expressed in heart/skeletal muscle. Therefore, we concluded that energy metabolism occurring in the BAT of cold-exposed rats resembles that occurring in heart/skeletal muscle. A similar study was also conducted by Adams et al., and they concluded that cold exposure of animals stimulated both catabolism and anabolism of fatty acids in BAT [9].

However, information on gene expression profiles in BAT obtained by the Northern approach is limited. As the technology of microarray analysis has become rather commonplace, and the results obtained with it have proven to be significantly reliable, in the present study we carried out this type of analysis to examine changes in gene expression in BAT that accompanied the exposure of rats to a cold temperature.

2. Materials and methods

2.1. Materials

Bacterial expression vectors pET3 and pCold were purchased from Novagen (Madison, MI) and TaKaRa (Otsu, Japan), respectively. RNeasy kit (lipid tissue mini, code 74804) was purchased from QIAGEN (Hilden, Germany). Polyclonal antibody against myoglobin (code FL-154) was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

2.2. Preparation of RNA samples from BAT

For the control experiment, interscapular brown adipose tissue was obtained from 3 male Wistar rats (5 weeks old) kept at room temperature (22 °C). These tissues were combined, and RNA was then promptly prepared from them. To obtain BAT from cold-exposed rats, we kept 3 male Wistar rats (5 weeks old) at 4 °C for 48 h, because this experimental condition is known to cause maximum elevation of mRNA encoding UCP1 in BAT [10]. Their BATs were then removed, combined, and promptly subjected to the procedure for RNA preparation. Total RNA was purified from both groups by using an RNeasy kit, and RNA concentrations were determined by measuring their A_{260} with a Shimadzu spectrophotometer, model UV-1700.

The above procedure was repeated once more with another 3 animals of each group, and individual RNA samples obtained from the 1st and 2nd experimental cycles were referred to as 1st RNA set and 2nd RNA set, respectively.

2.3. Preparation of cDNA probes and Northern hybridization

cDNA fragments of type III adenylate cyclase (AC3), type 2 iodothyronine deiodinase (DIO2), and those with accession numbers of X59736 (sarcomeric mitochondrial creatine kinase, CKMT2), XM_001077474 (spindle pole body component 24 homolog), XM_001065414 (similar to reprimo-like), XM_573992 (similar to cyclin-dependent kinase regulatory subunit 2), XM_001061567 (secretoglobin, family 1C, member 1 [predicted]), NM_053453 (regulator of G-protein signaling 2, RGS2), XM_342650 (IMP dehydrogenase 1, IMPDH1 [predicted]), NM_172091 (glucagon receptor), NM_133295 (carboxylesterase 3), and

Table 1
Primers used for preparation of cDNAs

cDNA [a]	Primer name	Nucleotide sequence	Direction [b]
Type III adenylate cyclase (AC3)	GE161	5′-CTGTTGAAGTGCACATTCCC	D
	GE156	5′-CAACACCTGCAGATTCTCTG	U
Type II iodothyronine deiodinase (DIO2)	GE092	5′-AGTGTCAAGTTGTGGCTGAC	D
	GE112	5′-CTGGTGACTAAAGCTGACTG	U
X59736	GE751	5′-CAACAACGGCTCATCGATGAC	D
	GE660	5′-AATAGCAGTACCTAC	U
XM_001077474	GE1370	5′-TCATGAGCAGATGGTGGAG	D
	GE1371	5′-CAGTGATCCCTTTCTTGGC	U
XM_001065414	GE1372	5′-GAACGTGAGCTTTCTGAACC	D
	GE1373	5′-TAGCCTCGCATTACATCTCC	U
XM_573992	GE1374	5′-ATTGGTCAGTGGTTTCGTCC	D
	GE1375	5′-TTAACCGAGCTCTGTTGCTG	U
XM_001061567	GE1376	5′-AAGCTTTGAAAGGGAGCAGC	D
	GE1377	5′-GATTTAACAGTTCGAGGGTG	U
NM_053453	GE162	5′-CTGACCTTGAATTCAGCCTG	D
	GE163	5′-AACGGACACTGGTTCTACAG	U
XM_342650	GE1378	5′-GCCTACACTCTTACGAGAAG	D
	GE1379	5′-TGGTGAGTCAGGAATGAAGG	U
NM_172091	GE1380	5′-CCATCAGATGCACTATGCTG	D
	GE1381	5′-TACAAGCTGCTGTCTTGCTG	U
NM_133295	GE1386	5′-CACCACATCAGCTGTTATGG	D
	GE1387	5′-CTGAGATTGGTCTCCTCTTC	U
NM_001013185	GE1384	5′-TCCTCATCTGTTCCAAGCTG	D
	GE1385	5′-CAAAGAGCCTATGCTGATCC	U

[a] Except for AC3 and DIO2, names of genes are shown as their accession numbers.
[b] Directions of primers are shown with 1-letter abbreviations: D (downstream) and U (upstream).

NM_001013185 (chaperone, ABC1 activity of bc1 complex like [predicted]) were prepared by RT-PCR using first-strand cDNAs prepared from total RNA of BAT as templates. The amplimers used are summarized in Table 1. cDNA of UCP1 was prepared as described previously [8].

2.4. Preparation of expression vectors

Full-length cDNA or cDNA corresponding to the open reading frame of myoglobin, cytoplasmic brain type creatine kinase (CKb), cytoplasmic muscle type creatine kinase (CKm), ubiquitous mitochondrial type creatine kinase (CKMT1), sarcomeric mitochondrial type creatine kinase (CKMT2), IMP dehydroganase 1 (IMPDH1), regulator of G-protein signaling 2 (RGS2), glyceraldehyde 3-phosphate dehydrogenase (G3PDH), and mitochondrial voltage-dependent anion channel (VDAC1) were prepared by RT-PCR. Primers and templates used for amplification of these cDNAs are summarized in Table 2. These cDNA fragments were subcloned into the following expression vectors: pCold vector (for CKMT2 and RGS2) or pET3 vector (for IMPDH1, G3PDH, VDAC1, myoglobin, CKb, CKm, and CKMT1). The expression vector of acidic ribosomal phosphoprotein P0 (36B4) was prepared as described previously [11].

2.5. In vitro synthesis of mRNA, Northern analysis, and microarray analysis

mRNAs encoding CKb, CKm, CKMT1, CKMT2, myoglobin, and 36B4 were *in vitro* synthesized by using the expression vectors prepared as described above and T7 RNA polymerase, as described previously [12].

Northern blotting was performed as described previously [8]. 10-μg aliquots of total RNA samples obtained from BAT were used for the analyses. Aliquots of *in vitro* synthesized mRNAs were also subjected to Northern analysis to enable quantitative evaluation of individual transcript levels.

Microarray analysis was performed by using an Agilent oligo array system (code G4130A) according to the procedures described previously [12]. Briefly, RNA samples obtained from the rats kept at 4 °C were labeled with Cy5 dye; and those from the rats maintained at room temperature, with Cy3 dye. After mixing of these dye-labeled RNA samples, each mixture was subjected to hybridization on the oligo array. Signals of dyes on the array were detected with an Agilent DNA microarray scanner. With these experiments, expression levels of 21,575 genes in BAT were evaluated.

2.6. Bacterial expression of proteins as positive control of Western blotting

Bacterially expressed proteins were used as positive controls for Western blotting. For this, expression vectors of myoglobin, IMPDH1, RGS2, G3PDH and VDAC were introduced into *E. coli* host cells of strain BL21(DE3)pLysS. Induction of expression of individual genes and preparation of bacterial lysates were carried out as described earlier [13].

2.7. Preparation of antibodies and Western blotting

Polyclonal antibodies against IMPDH1, RGS2, G3PDH, and VDAC1 were raised in rabbits by using oligopeptides with amino acid sequences of RTMSAQIEGGVHGLHSYEKRC (amino acids 614–633 in XM_342650), DKSAGNGPKVEEKREKMKRTC (amino acids 17–36 in NM_053453), EYGYSNRVVDLMAYMASKEC (amino acids 315–333 in NM_017008), and FQLHTNVNDGTEFGGSIYQKVC (amino acids 178–198 in NM_031353), respectively, as immunogens (note that a cysteine residue was artificially introduced at the C-terminal end of each peptide to confer reactivity with maleimide-activated keyhole limpet hemocyanin).

For Western blotting, whole lysates of interscapular BAT (30 μg) were prepared from cold-exposed rats or rats kept at room temperature. Animals used for these experiments were distinct from those used for the preparation of total RNA sample, but animals and tissues were handled in the same manner as those used for the RNA preparation. Furthermore, elevation of the transcript level of UCP1 in the BAT of cold-exposed rats was confirmed. Bacterially expressed target proteins (approximately 100 ng) were also used as positive controls. Immunoreactive protein bands were detected by using an ECL kit and secondary antibody conjugated to horseradish peroxidase.

3. Results

To obtain reliable and reproducible data, we carried out 2 independent microarray experiments by using 2 independently prepared RNA sets from the BATs of rats kept at room temperature or of rats exposed to the cold, as stated in the Methods

Table 2
Primers used for preparation of expression vectors

cDNA [a]	Primer name	Nucleotide sequence [b]	Direction [c]	Region [d]	Template [e]
Myoglobin (NM_021588)	GE1669	5′-CTCTTTA*GAAttCcatATG*GGGCTCAGTGATGG	D	12–44	Muscle
	GE1676	5′-GGATGTT*ggATcc*AAACCAGGTGGGTCAGG	U	906–935	
CKb (NM_012529)	GE1655	5′-CTGCAAGCA*CcAtgg*GTCTGCGTTC	D	31–55	Brain
	GE1656	5′-CAGTGTCTGCA*ggAtCc*TAGCCATCAG	U	1394–1420	
CKm (NM_012530)	GE1653	5′-CACAGCAAAGA*CAtAtg*CTCAGGAGCC	D	14–40	Muscle
	GE1654	5′-CAGCGCCCAAGT*GgAtCC*GTTCCAGC	U	1343–1368	
CKMT1 (NM_001012738)	GE1651	5′-CTGGTAGG*CAtatGC*TGCTACTTCAGC	D	33–60	Brain
	GE1652	5′-GATTGAGAGGC*gGaTCC*TTGGCAAG	U	1525–1549	
CKMT2 (X59736)	GE1649	5′-GATCT*GGtacC*GACAGACTC	D	19–38	Muscle
	GE1650	5′-GCAGTACCTA*gAATTc*TACATG	U	1421–1442	
IMPDH1 (NM_011829)	GE1420	5′-*catATG*GCGGACTACCTGAT	D	106–122	BAT
	GE1421	5′-*catATG*TCAGTACAGCCGCT	U	1637–1650	
RGS2 (NM_053453)	GE1418	5′-*catATG*CAAAGTGCCATG	D	1–15	BAT
	GE1419	5′-*ggatcc*TCTCATGTAGCATG	U	625–638	
G3PDH (X02231)	MB476	5′-CTCATAGAC*cAtATG*GTGAAG	D	60–80	Liver
	MB477	5′-TGGTCCAG*GGaTc*CTTACTC	U	1068–1087	
VDAC (NM_031353)	GE830	5′-GAGA*cCATGG*CTGTGCCTCC	D	61–70	Liver
	GE831	5′-TACA*ggATc*CATTTATGCTTGAAATTCC	U	903–930	

[a] cDNAs are shown with their abbreviated name, and their accession numbers are given in parentheses.
[b] Artificially introduced restriction sites are italicized. Lowercase letters indicate mutated nucleotides used for creation of restriction sites.
[c] Directions of primers are shown as 1-letter abbreviations: D (down stream) and U (upstream).
[d] Locations of individual probes in entire messages.
[e] Source tissues of RNA samples used for preparation of template cDNA libraries.

and methods section. Prior to the microarray analysis, we examined whether the prepared RNA samples of BAT would show essentially the same gene expression profiles as those reported previously. Since the expression of UCP1, type II iodothyronine deiodinase (DIO2), and type III adenylate cyclase (AC3) in BAT is known to be greater in cold-exposed animals than in animals kept at room temperature [14,15], their levels of expression in the 2 RNA sets were examined by Northern analysis. As shown in Fig. 1, for both RNA sets, the expression of each of these 3 genes was markedly elevated in the BAT of cold-exposed rats compared with that in the BAT of rats maintained at room temperature. Thus, having concluded that the prepared RNA samples were suitable for the present study, we performed microarray analysis using these RNA sets.

Both independently prepared RNA sets were subjected to microarray experiments. In these experiments, the RNA samples obtained from BAT of the rats kept at room temperature and those of BAT from the rats exposed to the cold were labeled with the dye Cy3 and Cy5, respectively; and the mixture of these dye-labeled RNA samples was applied to the oligo array. Therefore, by measuring the signal intensities of the fluorescence of these 2 dyes, we could compare the expression profiles of genes in BAT between the rats maintained at room temperature and those exposed to the cold.

First of all, we checked the signal intensities of UCP1, DIO2, and AC3 in the 2 microarray data sets. Signals corresponding to UCP1 were too strong, possibly reflecting its high transcript level even in the BAT of the rats kept at room temperature, and thus were not valuable as a measure of its transcript levels. The spot corresponding to the DIO2 gene was not present on the array used in this study. The signal corresponding to AC3 was about 3-fold higher in the BAT of cold-exposed rat than in that of the rats kept at room temperature, as demonstrated by the Northern analysis (Fig. 1). When we focused on the transcript levels of housekeeping genes, probes of most commonly used as typical housekeeping genes such as β-actin, glyceraldehyde 3-phosphate dehydrogenase or cyclophilin were not present on the array used. The probe for acidic ribosomal phosphoprotein P0 (36B4) was available, but its signal intensities were saturated and not suitable for the evaluation of its transcript level. When we compared the signal intensities of genes reported to be constantly expressed in various tissues [16] such as ribosomal protein L4 (NM_139083), thymosin beta 4 (NM_031136), and proteasome subunit beta type 2 (NM_017284) or its type 3 (NM_017285) between 2 RNA samples of BAT from rats exposed to cold and kept at room temperature, their ratios (Cy5/Cy3) were very small in the ranges between 0.88 and 1.15, indicating that the expression of housekeeping genes was not markedly affected by cold exposure.

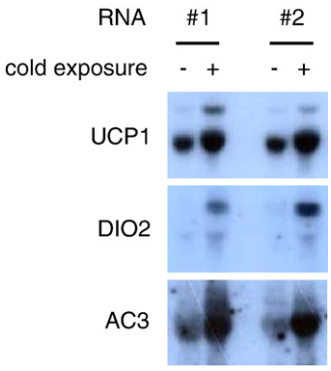

Fig. 1. Transcript levels UCP1, DIO2, and AC3 in the RNA samples prepared from BAT of rats kept at room temperature or exposed to the cold. Two sets of total RNA samples, designated as #1 and #2, prepared from BAT of rats maintained at room temperature (cold exposure −) or exposed to cold (cold exposure +), were subjected to denaturing agarose gel electrophoresis; and transcripts corresponding to type 1 uncoupling protein (UCP1), type II iodothyronine deiodinase (DIO2), and type III adenylate cyclase (AC3) were detected by the Northern technique by using their specific probes.

To understand the physiological implications of the gene expression data, we first tried to identify common features in BAT caused by cold exposure by using the pathway analysis programs GenMAPP and MAPPFinder [17], but neither was effective. Thus, obtained expression profiles of genes in BAT were aligned according to the ratios of signal intensities between Cy5 and Cy3. As a result, 9216 and 8502 genes showed Cy5/Cy3 ratio higher than 1, in RNA sets #1 and #2, respectively. Likewise, 7243 and 7959 genes showed Cy5/Cy3 ratio lower than 1, in RNA sets #1 and #2, respectively. Next, the top 100 genes showing either higher or lower Cy5/Cy3 ratios in the RNA sample of BAT from the cold-exposed rats, compared with those in the RNA samples of BAT from the rats kept at room temperature, were extracted. Thirty-four genes were commonly observed among these top 100 genes to have higher Cy5/Cy3 ratios in the RNA sample of BAT as shown in Table 3, and changes in the transcript levels of the remaining 66 genes were not commonly remarkable in these 2 RNA preparations. Likewise, 23 genes were commonly observed among the top 100 genes showing lower Cy5/Cy3 ratios in the RNA sample of BAT, as shown in Table 4. Since annotations of genes on the oligo array used had not been well updated, nucleotide sequences of individual clones were subjected to BLAST analysis; and the latest annotations of individual genes are given in these tables. Furthermore, to examine the reliability of the obtained results, by Northern analysis we examined the transcript levels of the top 5 genes listed in Tables 3 and 4 in the RNA samples of BAT from the rats kept at room temperature or exposed to the cold. For this, cDNAs encoding individual clones were prepared by RT-PCR; and using these cDNA fragments as probes, we evaluated the expression levels of individual genes in both RNA sets. As shown in Fig. 2, the up- and down-regulation indicated in Tables 3 and 4, respectively, of the gene expressions in the BAT from the cold-exposed rats, in comparison with their expression in the BAT from the rats kept at room temperature, were well confirmed. Thus, we concluded the obtained microarray data to be reliable and valuable for further characterization of changes in the gene expression profiles in BAT in response to the cold environment.

In the BAT of cold-exposed rats, in addition to the 3 genes related to cell-cycle regulation (XM_001065414, XM_573992, and XM_214152), 3 others involved in DNA replication (XM_214477, XM_001064207, and XM_213664), 2 related to chromosome structure (XM_001077474 and XM_342345), and 4 whose products are localized in the nuclei (XM_214527, XM_001058771, XM_227096 and BC099224) were up-regulated, compared with their expression in the BAT of the rats kept at room temperature, possibly reflecting the enlargement of the BAT in the cold-exposed rats.

Table 3
Genes showing higher transcript levels in BAT of cold-exposed rats [a]

Description	Accession number	Signal intensities (RNA #1) [b]			Signal intensities (RNA #2) [b]			Average ratio
		Cy3	Cy5	Cy5/Cy3 ratio	Cy3	Cy5	Cy5/Cy3 ratio	
Sarcomeric mitochondrial creatine kinase	X59736	419.8	2870.0	6.84	209.2	3792.9	18.13	12.48
Spindle pole body component 24 homolog	XM_001077474	3232.4	28,273.6	8.75	2146.2	23,367.3	10.89	9.82
Similar to reprimo-like	XM_001065414	3704.5	33,152.0	8.95	1776.7	16,050.2	9.03	8.99
Similar to cyclin-dependent kinase regulatory subunit 2	XM_573992	1027.7	4099.1	3.99	308.3	3392.7	11.01	7.50
Secretoglobin, family 1C, member 1 (predicted)	XM_001061567	3665.1	13,773.0	3.76	1905.4	19,329.3	10.14	6.95
Similar to Shc SH2-domain binding protein 1	XM_001077162	251.5	1128.7	4.49	135.8	1091.6	8.04	6.26
Hypothetical LOC300207 (predicted)	XM_217031	308.8	1540.8	4.99	356.9	2618.5	7.34	6.16
Centromere protein E (predicted)	XM_342345	952.4	2703.4	2.84	219.8	2057.0	9.36	6.10
Cyclin-dependent kinase inhibitor 3 (predicted)	XM_214152	1196.4	4030.3	3.37	426.0	3508.1	8.24	5.80
Dixin	AY770507	1273.2	7105.4	5.58	1417.1	7721.8	5.45	5.51
Similar to RIKEN cDNA 2810433K01 (predicted)	XM_214527	512.2	1811.4	3.54	223.8	1641.3	7.33	5.43
Hypothetical protein LOC681870	XM_001058771	653.6	2139.1	3.27	262.3	1966.6	7.50	5.39
Geminin (predicted)	XM_214477	863.9	2332.5	2.70	660.9	5019.4	7.60	5.15
Similar to Mki67 protein	XM_227096	2233.9	7631.3	3.42	939.4	6445.4	6.86	5.14
Myoglobin	NM_021588	11,870.3	46,456.3	3.91	6763.9	38348.8	5.67	4.79
Serum/glucocorticoid regulated kinase 2 (predicted)	XM_001070075	913.5	2379.1	2.60	795.9	5412.2	6.80	4.70
Dixin	AY770507	656.8	2628.7	4.00	612.2	3198.0	5.22	4.61
Minichromosome maintenance deficient5, cell division cycle 46 (predicted)	XM_001064207	1203.7	4564.6	3.79	1081.0	5752.1	5.32	4.56
Similar to hypothetical UPF0080 protein KIAA0186	XM_575258	514.9	1691.8	3.29	358.5	2035.2	5.68	4.48
Similar to Acyl–CoA thioesterase 4	XM_001059880	1751.2	4814.5	2.75	880.7	4735.3	5.38	4.06
Similar to CG12279-PA	XM_575783	5474.0	16,723.5	3.06	5531.9	26,920.3	4.87	3.96
Ubiquitin-conjugating enzyme E2C (predicted)	XM_215924	383.8	1446.4	3.77	343.7	1325.8	3.86	3.81
Phosphatidylserine-specific phospholipase A1	NM_138882	449.0	1789.6	3.99	1034.8	3733.5	3.61	3.80
Similar to D15Wsu75e protein (predicted)	XM_235506	861.5	2612.0	3.03	713.8	3205.6	4.49	3.76
Similar to semaF cytoplasmic domain associated protein 2	XM_342359	990.0	2912.4	2.94	1181.5	5264.1	4.46	3.70
Hypoxia induced gene 1	NM_080902	3756.7	11,287.1	3.00	3716.6	16,317.1	4.39	3.70
Similar to IQ motif and WD repeats 1	XM_213926	1731.2	4310.0	2.49	1119.3	5274.7	4.71	3.60
Similar to chromatin assembly factor 1 subunit B	XM_213664	740.3	2136.3	2.89	592.5	2491.6	4.21	3.55
Ubiquitin-like, containing PHD and RING finger domains, 1	BC099224	346.2	1105.4	3.19	456.4	1682.6	3.69	3.44
Unknown	AW143886	361.1	1189.3	3.29	415.3	1441.8	3.47	3.38
Unknown	AW144110	728.0	2117.7	2.91	560.0	2137.1	3.82	3.36
G-protein-coupled receptor 120 (predicted)	XM_215281	2590.0	7801.7	3.01	2812.3	10,338.4	3.68	3.34
Unknown	TC524964	10,500.4	27,580.8	2.63	8322.7	30,392.7	3.65	3.14
Lysophospholipase	NM_144750	1401.4	3580.5	2.55	966.0	3336.1	3.45	3.00

[a] Thirty-four genes identified as constantly and remarkably up-regulated in BAT of cold-exposed rats compared with BAT of rats kept at room temperature are summarized (see text for details). Because distinct two spots were used for detection of dixin and both of them showed up-regulation of this gene, it appeared twice in the table.

[b] RNA samples obtained from the rats exposed to cold were labeled with Cy5 dye; and those from rats maintained at room temperature, with Cy3 dye. After mixing of these dye-labeled RNA samples, each mixture was subjected to hybridization on the oligo array. These experiments were performed for 2 independently prepared RNA sets (RNA #1 and #2). Observed signal intensities of Cy3 and Cy5 dyes for both RNA sets are shown.

It is also noteworthy that the expression of sarcomeric mitochondrial creatine kinase (X59736) was markedly up-regulated in the BAT of cold-exposed rats. Signal intensities of this gene in BAT of rats kept at room temperature were 419.8 and 209.2 in RNA set #1 and #2, respectively, but those in BAT of cold-exposed rats were 2870.0 and 3792.9, respectively. Creatine kinase (EC 2.7.3.2) catalyzes the following reaction: ATP + creatine → ADP + phosphocreatine. There are at least 4 functionally active genes that encode distinct creatine kinases, i.e., those specifying the cytoplasmic brain type (CKb), cytoplasmic muscle type (CKm), ubiquitous mitochondrial type (CKMT1), and sarcomeric mitochondrial type (CKMT2) [18,19]. Interestingly, an increase in the creatine kinase activity in the BAT of cold-exposed animals was reported earlier [20,21]. However, the question as to which of these 4 genes is responsible for the increased creatine kinase activity in BAT of cold-exposed animals has not yet been clearly answered. Of these 4 isoforms, CKb could not be analyzed for its expression level in the present study, because the DNA spot corresponding to this gene was not present on the array. Since signal intensities of DNA spots corresponding to CKm and CKMT1 subtypes were almost negligible in the BAT of rats kept at room temperature or at the cold temperature (data not shown), it was difficult to compare the expression levels of these 4 creatine kinase subtypes. To evaluate the transcript levels of individual creatine kinase subtypes in BAT quantitatively, we carried out a calibrated Northern analysis using *in vitro* synthesized RNA as standards (Fig. 3). From the signal intensities of individual genes observed with RNA samples of BAT compared with those observed with *in vitro* synthesized individual RNA samples, we concluded that the expression level of CKb was most remarkable in BAT, with those of CKMT1 and 2 being almost negligible. It is noteworthy that the expression of these 3 creatine kinase subtypes except for CKMT1 was observed in BAT

Table 4
Genes showing lower transcript levels in the BAT of cold-exposed rats[a]

Description	Accession number	Signal intensities (RNA #1)			Signal intensities (RNA #1)			Average ratio
		Cy3	Cy5	Cy5/Cy3 ratio	Cy3	Cy5	Cy5/Cy3 ratio	
Regulator of G-protein signaling 2	NM_053453	19,178.2	1578.2	0.08	49,520.5	5323.2	0.11	0.09
Regulator of G-protein signaling 2	NM_053453	30,772.9	3528.7	0.11	24,645.0	2681.0	0.11	0.11
IMP dehydrogenase 1 (predicted)	XM_342650	25,339.0	4706.9	0.19	201,339.0	11,548.3	0.06	0.12
Glucagon receptor	NM_172091	21,950.0	3246.5	0.15	20,776.2	2648.8	0.13	0.14
Carboxylesterase 3	NM_133295	70,223.7	13,098.6	0.19	86,408.8	11,666.4	0.14	0.16
Chaperone, ABC1 activity of bc1 complex like (predicted)	NM_001013185	146,492.0	30,058.5	0.21	323,925.0	55,819.5	0.17	0.19
Ankyrin repeat domain 5 (predicted)	XM_215854	4361.9	971.5	0.22	6626.1	1264.7	0.19	0.21
Unknown	TC558298	3535.8	625.2	0.18	3833.5	937.2	0.24	0.21
Unknown	BF564888	5690.2	2108.1	0.37	15,185.2	1843.3	0.12	0.25
Adenylate cyclase 9 (predicted)	XM_220178	11,469.6	3611.4	0.31	31,516.8	5717.0	0.18	0.25
Acyl–CoA synthetase short-chain family member 2 (predicted)	XM_001064684	76,792.8	26,195.7	0.34	102,276.0	16,178.1	0.16	0.25
Similar to solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 23 (predicted)	XM_217310	8453.9	2466.7	0.29	10,992.4	2764.4	0.25	0.27
Adrenergic receptor, beta 3	NM_013108	30,800.5	9353.4	0.30	50,049.5	14,796.0	0.30	0.30
Similar to cell death-inducing DFFA-like effector c	XM_575641	8179.5	3483.6	0.43	21,864.3	3822.6	0.17	0.30
Dihydropyrimidine dehydrogenase	NM_031027	3902.3	1646.1	0.42	11,311.1	2203.3	0.19	0.31
Neuroepithelial cell transforming gene 1	BC100154	1435.5	492.8	0.34	1810.7	535.9	0.30	0.32
Glutathione *S*-transferase A5	NM_031509	35,978.4	13,298.1	0.37	43,754.2	11,940.9	0.27	0.32
Selenium binding protein 2	NM_080892	8515.2	3530.8	0.41	20,825.5	4747.0	0.23	0.32
Influenza virus NS1A binding protein (predicted)	XM_213898	16,500.0	6233.5	0.38	30,073.0	8302.0	0.28	0.33
Unknown	AW142974	2652.0	1126.8	0.42	5771.4	1355.6	0.23	0.33
Farnesyl diphosphate farnesyl transferase 1	NM_019238	11,733.8	4502.4	0.38	15,165.5	4732.2	0.31	0.35
Unknown	AW143067	11,319.8	5046.4	0.45	28,714.7	8080.6	0.28	0.36
RAS, dexamethasone-induced 1	XM_340809	10,342.3	4401.0	0.43	15,665.6	4786.5	0.31	0.37

[a] Twenty-three genes identified as constantly and remarkably down-regulated in BAT of old-exposed rats compared to BAT of rats kept at room temperature are summarized (see text for details).

and that the expression of all 3 of these subtypes was up-regulated in BAT of cold-exposed rats.

When we compared signal intensities of individual genes, the intensity of myoglobin was the most remarkable, and its level was further up-regulated in BAT of cold-exposed rats. Its remarkable expression in BAT and up-regulation in BAT of cold-exposed rat was reconfirmed by Northern analysis (Fig. 3). Since myoglobin is well known to be significantly expressed in muscle tissue and the procedure for isolation of BAT often causes contamination of BAT with muscle tissue, we must pay attention on the observed signal of myoglobin in BAT. To examine the possible contamination of BAT sample with muscle tissue, we evaluated gene expressions in muscle tissue by the same micro-array system. As a result, both myoglobin and CKMT2 were highly expressed in skeletal muscle of the rats fed at room temperature, and their signal intensities were 42,536.5 and 69,457.9, respectively. If the high signal of myoglobin observed in BAT was due to the contamination of BAT with skeletal muscle, the signal of CKMT2 in BAT must be significantly high. However, the signal intensity of CKMT2 in the BAT of rats kept at room temperature was very low, as described above. Thus, we concluded that the significant expression of myoglobin in BAT was not due to the contamination of BAT with skeletal muscle.

When we focused on the genes down-regulated in the BAT of cold-exposed rats compared with their expression in the BAT of the rats kept at room temperature, changes in the transcript levels of NM_053453 (regulator of G-protein signaling 2, RGS2), XM_342650 (IMP dehydrogenase 1, IMPDH1 [predicted]), and NM_013108 (β_3 adrenergic receptor) seemed to reflect the changes in the metabolic status in BAT. In a previous study, we found that the transcript level of the β_3 adrenergic receptor in the BAT of cold-exposed rat was not markedly different from that in the BAT of rats kept at room temperature [8], and so it is difficult to conclude which result reflects precisely the physiological relevance of the transcript level of β_3 adrenergic receptor in BAT. These transcripts would not be stable and would be affected by various factors, as reported previously [22,23].

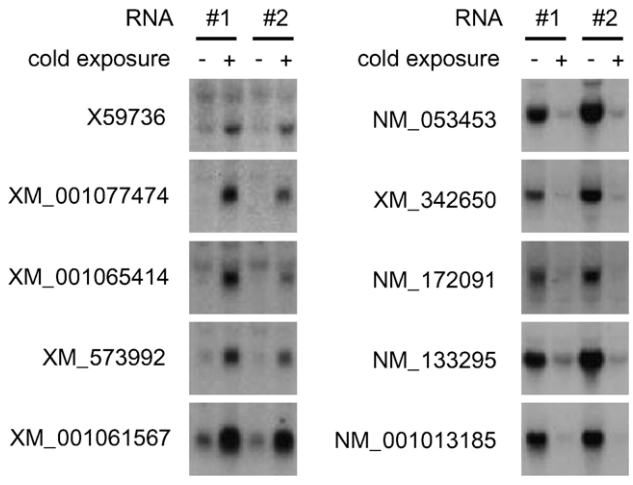

Fig. 2. Confirmation of the changes in the transcript levels of genes by Northern analysis. To confirm the changes in the transcript levels of the top 5 up-regulated and top 5 down-regulated genes, Northern analyses were performed. The RNA samples used were the same as in Fig. 1.

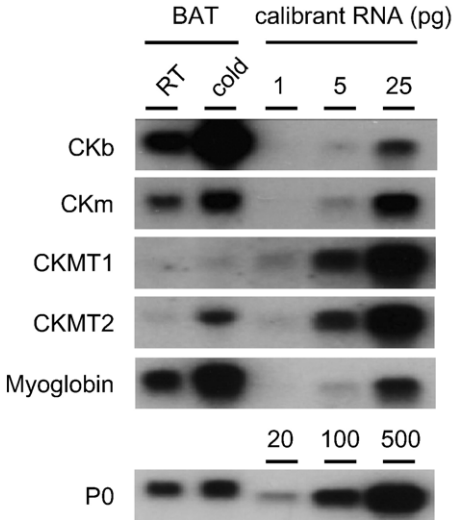

Fig. 3. Calibrated Northern analysis of 4 creatine kinase isozymes and myoglobin. Expression levels of 4 creatine kinase isozymes, i.e., cytoplasmic brain type (CKb), cytoplasmic muscle type (CKm), ubiquitous mitochondrial type (CKMT1), and sarcomeric mitochondrial type (CKMT2), and myoglobin in BAT of rats kept at room temperature (RT) or exposed to cold (cold) were measured. Aliquots (10 μg) of total RNA samples obtained from BAT (RNA set #1, also used for microarray analysis) were subjected to agarose gel electrophoresis. To enable quantitative evaluation of their expression levels, full-length RNAs of individual genes were prepared by *in vitro* transcription, and aliquots of them were subjected to the gel as calibrants. The expression level of acidic ribosomal phosphoprotein P0 (36B4) in RNA samples from BAT was also measured as a control.

Finally, to examine whether these changes in the transcript levels in BAT caused by cold exposure also resulted in changes in the corresponding protein levels, we carried out Western analysis. For this, we prepared specific antibodies against RGS2 and IMPDH1. We also raised antibodies against glyceraldehyde 3-phosphate dehydrogenase (G3PDH) and mitochondrial voltage-dependent anion channel (type 1 isoform, VDAC1), known as constitutively expressed metabolic proteins, as controls. Specific recognition of target proteins by these raised antibodies was confirmed by using bacterially expressed target proteins. The antibody used for detection of myoglobin was a commercial product obtained from Santa Cruz Biotechnology. When whole lysates of BAT obtained from rats kept at room temperature or exposed to the cold were subjected to Western analysis, a strong signal corresponding to G3PDH and a weak one corresponding to VDAC1 were observed with either type of lysate (Fig. 4). Expression of myoglobin in BAT of rats kept at room temperature was not observed, but its faint signal was observed in BAT of cold-exposed rats. Signals corresponding to the RGS2 and IMPDH1 were not observed.

4. Discussion

It is well established that UCP1 plays central roles in the energy-dissipating function of BAT. It works as a proton transporter in the mitochondrial inner membrane, and dissipates the electrochemical potential of proton across the mitochondrial membrane, which gradient is used as a driving force of ATP synthesis. However, to enable efficient energy dissipation in BAT, the gene expression of not only UCP1 but also that of other proteins would be expected to be regulated in BAT. To examine this possibility, in earlier studies we compared gene expression profiles between WAT and BAT. As a result, gene expressions of several proteins involved in fatty acid metabolism were found to be markedly higher in BAT than in WAT [7,8,24–26].

The thermogenic function of BAT is known to be markedly elevated when animals are exposed to the cold. Under these conditions, up-regulation of UCP1 is well known to enable effective thermogenesis in BAT. As mentioned above, our previous studies indicated that certain genes encoding metabolic proteins, especially their subtypes known to be significantly expressed in heart/skeletal muscle, are up-regulated in the BAT of cold-exposed animals [7,8]. Essentially the same conclusion as ours was also obtained by Adams et al. [9]. However, gene expression profiles in the BAT of cold-exposed animals revealed by the Northern or RT-PCR analysis are limited. In the present study, cold-induced changes in gene expression in BAT of experimental animals were systemically investigated by using the technique of microarray analysis.

As a result, in addition to the previously reported genes such as UCP1, DIO2, and AC3, genes related to cell-cycle regulation, DNA replication, and chromosome structure were found to be up-regulated in the BAT of cold-exposed rat compared with their expression in the BAT of the rats kept at room temperature. These results may reflect the enlargement of BAT due to cold exposure, because significant proliferation of brown adipocytes is known to be induced by such exposure [27,28]. In addition to these genes, CKMT2 in the BAT of cold-exposed rat was also remarkably up-regulated. An increase in the creatine kinase activity in the BAT of cold-exposed animals was reported earlier [20,21], but the question as to which of the 4 isoforms is responsible for the increased creatine kinase activity in BAT of cold-exposed animals has not yet been clearly answered. Our present study clearly demonstrated elevated transcript levels of

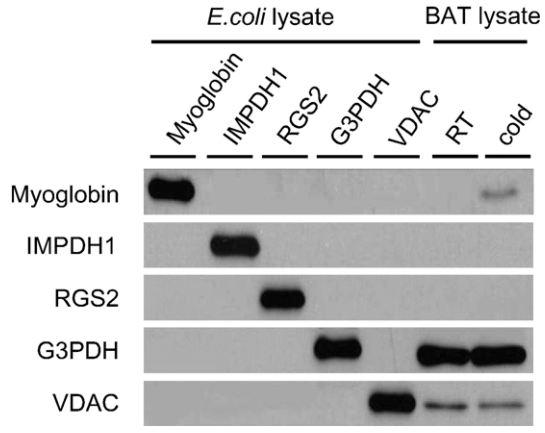

Fig. 4. Western analysis of myoglobin, IMPDH1, and RGS2 in whole lysates of BAT obtained from rats kept at room temperature or exposed to the cold. To examine the expression levels of myoglobin, IMPDH1, and RGS2 proteins in BAT, whole lysates of BAT obtained from rats kept at room temperature or exposed to the cold were prepared. Whole lysates of BAT (30 μg protein) were subjected to SDS-PAGE followed by Western analysis. Myoglobin, IMPDH1, RGS2, G3PDH, and VDAC1 in BAT lysates were detected by their specific antibodies. Bacterially expressed myoglobin, IMPDH1, RGS2, G3PDH, and VDAC1 (approximately 100 ng each) were used as positive controls.

3 creatine kinase subtypes, i.e., CKb, CKm, and CKMT2, in the RNA samples of BAT of cold-exposed rats compared with those in the rats kept at room temperature. Further studies will be necessary for elucidating the roles of individual subtypes of creatine kinase in BAT.

Significant expression of myoglobin in BAT of rats kept at room temperature was also remarkable, and its further elevation in the BAT of cold-exposed rats was observed. Possible contamination of BAT sample with skeletal muscle was clearly excluded by the additional microarray analysis on the gene expression levels in skeletal muscle. Myoglobin is known to deliver oxygen to peripheral tissues. UCP1 selectively expressed in the mitochondria of BAT dissipates electrochemical potential of H^+ across the mitochondrial inner membrane and facilitates oxidation of respiratory substrate. Myoglobin expressed in BAT would be necessary for an effective supply of oxygen to enable facilitated oxidation of respiratory substrates in BAT mitochondria. Elevation of its expression level in BAT of cold-exposed rats is also physiologically reasonable, because oxidation of respiratory substrates would be expected to be accelerated in the BAT of such rats.

In addition to up-regulation of these genes, we observed down-regulation of IMPDH1 and RGS2. IMPDH (EC 1.1.1.205) is a rate-limiting enzyme in the biosynthesis of guanine nucleotides. GDP, known as a negative regulator of UCP1, is synthesized from IMP; and IMPDH is a key enzyme in the synthesis of GDP. When animals are exposed to a cold environment, thermogenesis in BAT should be activated; and hence, synthesis of GDP is not required. Therefore it is not surprising that IMPDH would be down-regulated in the BAT of cold-exposed rats. On the other hand, RGS is known to regulate the GTPase activity of G-protein coupled with adrenergic receptors [29]. Furthermore, its 2nd isoform (RGS2) is known to inhibit AC3 activity [30]. As signals of cold exposure are thought to be mainly mediated by β_3 adrenergic receptors in BAT, RGS2, which negatively regulates the signaling pathway of adrenergic receptors, should be down-regulated in the BAT of cold-exposed rats. Furthermore, up-regulation of their counterparts was also previously reported. Namely, as a counterpart of IMPDH1, guanosine monophosphate reductase (EC 1.7.1.7), which catalyzes the degradation of GMP to IMP, was reported to be up-regulated in the BAT of cold-exposed rats [31]. Its up-regulation in BAT accompanying cold exposure was also observed in the present microarray analysis (data not shown). Because the up-regulation of adenylate cyclase (AC3) in BAT enhances the stimulatory signal of norepinephrine, this could be considered as a counterpart of the down-regulation of RGS2.

In summary, synchronized changes in the transcript levels of various genes involved in the activation of thermogenesis in brown adipose tissue due to cold exposure were revealed by microarray analysis. These results may give us clues for a better understanding of thermogenesis in BAT.

Acknowledgement

The present study was supported by research funding from the “knowledge cluster initiative” of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan.

References

[1] D.G. Nicholls, R.M. Locke, Thermogenic mechanisms in brown fat, Physiol. Rev. 64 (1984) 1–64.
[2] D. Ricquier, F. Bouillaud, The brown adipose tissue mitochondrial uncoupling protein, in: P. Trayhurn, D.G. Nicholls (Eds.), Brown Adipose Tissue, Arnold, 1986, pp. 86–104.
[3] D. Ricquier, F. Bouillaud, The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP, Biochem. J. 345 (2000) 161–179.
[4] B.B. Lowell, B.M. Spiegelman, Toward a molecular understanding of adaptive thermogenesis, Nature 404 (2000) 652–660.
[5] D. Ricquier, G. Mory, F. Bouillaud, J. Thibault, J. Weissenbach, Rapid increase of mitochondrial uncoupling protein and its mRNA in stimulated brown adipose tissue. Use of a cDNA probe, FEBS Lett. 178 (1984) 240–244.
[6] M. Klingenspor, Cold-induced recruitment of brown adipose tissue thermogenesis, Exp. Physiol. 88 (2003) 141–148.
[7] T. Daikoku, Y. Shinohara, A. Shima, N. Yamazaki, H. Terada, Dramatic enhancement of the specific expression of the heart-type fatty acid binding protein in rat brown adipose tissue by cold exposure, FEBS Lett. 410 (1997) 383–386.
[8] T. Daikoku, Y. Shinohara, A. Shima, N. Yamazaki, H. Terada, Specific elevation of transcript levels of particular protein subtypes induced in brown adipose tissue by cold exposure, Biochim. Biophys. Acta 1457 (2000) 263–272.
[9] X.X. Yu, D.A. Lewin, W. Forrest, S.H. Adams, Cold elicits the simultaneous induction of fatty acid synthesis and beta-oxidation in murine brown adipose tissue: prediction from differential gene expression and confirmation in vivo, FASEB J. 16 (2002) 155–168.
[10] D. Ricquier, F. Bouillaud, P. Toumelin, G. Mory, R. Bazin, J. Arch, L. Penicaud, Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue. Evidence for a rapid beta-adrenoreceptor-mediated and transcriptionally regulated step during activation of thermogenesis, J. Biol. Chem. 261 (1986) 13905–13910.
[11] R. Akamine, T. Yamamoto, M. Watanabe, N. Yamazaki, M. Kataoka, M. Ishikawa, T. Ooie, Y. Baba, Y. Shinohara, Usefulness of the 5′ region of the cDNA encoding acidic ribosomal phosphoprotein P0 conserved among rats, mice, and humans as a standard probe for gene expression analysis in different tissues and animal species, J. Biochem. Biophys. Methods 70 (2007) 481–486.
[12] R. Kakuhata, M. Watanabe, T. Yamamoto, R. Akamine, N. Yamazaki, M. Kataoka, S. Fukuoka, M. Ishikawa, T. Ooie, Y. Baba, T. Hori, Y. Shinohara, Possible utilization of in vitro synthesized mRNAs specifically expressed in certain tissues as standards for quantitative evaluation of the results of microarray analysis, J. Biochem. Biophys. Methods 70 (2007) 755–760.
[13] T. Yamamoto, A. Yamada, M. Watanabe, Y. Yoshimura, N. Yamazaki, Y. Yoshimura, T. Yamauchi, M. Kataoka, T. Nagata, H. Terada, Y. Shinohara, VDAC1, having a shorter N-terminus than VDAC2 but showing the same migration in an SDS-polyacrylamide gel, is the predominant form expressed in mitochondria of various tissues, J. Proteome Res. 5 (2006) 3336–3344.
[14] M. Murakami, Y. Kamiya, T. Morimura, O. Araki, K. Imamura, T. Ogiwara, H. Mizuma, M. Mori, Thyrotropin receptors in brown adipose tissue: thyrotropin stimulates type II iodothyronine deiodinase and uncoupling protein-1 in brown adipocytes, Endocrinology 142 (2001) 1195–1201.
[15] J.G. Granneman, Expression of adenylyl cyclase subtypes in brown adipose tissue: neural regulation of type III, Endocrinology 136 (1995) 2007–2012.
[16] L.L. Hsiao, F. Dangond, T. Yoshida, R. Hong, R.V. Jensen, J. Misra, W. Dillon, K.F. Lee, K.E. Clark, P. Haverty, Z. Weng, G.L. Mutter, M.P. Frosch, M.E. Macdonald, E.L. Milford, C.P. Crum, R. Bueno, R.E. Pratt, M. Mahadevappa, J.A. Warrington, G. Stephanopoulos, G. Stephanopoulos, S.R. Gullans, A compendium of gene expression in normal human tissues, Physiol. Genomics 7 (2001) 97–104.
[17] N. Salomonis, K. Hanspers, A.C. Zambon, K. Vranizan, S.C. Lawlor, K.D. Dahlquist, S.W. Doniger, J. Stuart, B.R. Conklin, A.R. Pico, GenMAPP 2: new features and resources for pathway analysis, BMC Bioinformatics 8 (2007) 217.

[18] T. Wallimann, M. Wyss, D. Brdiczka, K. Nicolay, H.M. Eppenberger, Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands — the phosphocreatine circuit for cellular energy homeostasis, Biochem. J. 281 (1992) 21–40.
[19] V.A. Saks, R. Ventura-Clapier, M.K. Aliev, Metabolic control and metabolic capacity: two aspects of creatine kinase functioning in the cells, Biochim. Biophys. Acta 1274 (1996) 81–88.
[20] S.E. Terblanche, T.C. Masondo, W. Nel, Effects of cold acclimation on the activity levels of creatine kinase, lactate dehydrogenase and lactate dehydrogenase isozymes in various tissues of the rat, Cell Biol. Int. 22 (1998) 701–707.
[21] H.H. Berlet, I. Bonsmann, H. Birringer, Occurrence of free creatine, phosphocreatine and creatine phosphokinase in adipose tissue, Biochim. Biophys. Acta 437 (1976) 166–174.
[22] S. Klaus, P. Muzzin, J.P. Revelli, M.A. Cawthorne, J.P. Giacobino, D. Ricquier, Control of beta 3-adrenergic receptor gene expression in brown adipocytes in culture, Mol. Cell. Endocrinol. 109 (1995) 189–195.
[23] P.J. Scarpace, M. Matheny, N. Tumer, Differential down-regulation of beta3-adrenergic receptor mRNA and signal transduction by cold exposure in brown adipose tissue of young and senescent rats, Pflugers Arch. 437 (1999) 479–483.
[24] N. Yamazaki, Y. Shinohara, A. Shima, H. Terada, High expression of a novel carnitine palmitoyltransferase I like protein in rat brown adipose tissue and heart: isolation and characterization of its cDNA clone, FEBS Lett. 363 (1995) 41–45.
[25] Y. Shinohara, T. Daikoku, K. Kajimoto, A. Shima, N. Yamazaki, H. Terada, Expression of NAD^+-dependent isocitrate dehydrogenase in brown adipose tissue, Biochem. Biophys. Res. Commun. 281 (2001) 634–638.
[26] K. Kajimoto, T. Daikoku, F. Kita, N. Yamazaki, M. Kataoka, Y. Baba, H. Terada, Y. Shinohara, PCR-select subtraction for characterization of messages remarkably expressed in brown adipose tissue, Mol. Genet. Metab. 80 (2003) 255–261.
[27] L. Bukowiecki, A.J. Collet, N. Follea, G. Guay, L. Jahjah, Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia, Am. J. Physiol. 242 (1982) E353–E359.
[28] L.J. Bukowiecki, A. Geloen, A.J. Collet, Proliferation and differentiation of brown adipocytes from interstitial cells during cold acclimation, Am. J. Physiol. 250 (1986) C880–C887.
[29] G.B. Willars, Mammalian RGS proteins: multifunctional regulators of cellular signalling, Semin. Cell Dev. Biol. 17 (2006) 363–376.
[30] S. Sinnarajah, C.W. Dessauer, D. Srikumar, J. Chen, J. Yuen, S. Yilma, J.C. Dennis, E.E. Morrison, V. Vodyanoy, J.H. Kehrl, RGS2 regulates signal transduction in olfactory neurons by attenuating activation of adenylyl cyclase III, Nature 409 (2001) 1051–1055.
[31] D. Salvatore, T. Bartha, P.R. Larsen, The guanosine monophosphate reductase gene is conserved in rats and its expression increases rapidly in brown adipose tissue during cold exposure, J. Biol. Chem. 273 (1998) 31092–31096.