Appl Biochem Biotechnol (2011) 165:538–547
DOI 10.1007/s12010-011-9273-4

Differential Effects of Cold Exposure on Gene Expression Profiles in White Versus Brown Adipose Tissue

Masahiro Watanabe · Takenori Yamamoto · Atsushi Yamamoto · Eriko Obana · Kanami Niiyama · Takuya Hada · Toshihiko Ooie · Masatoshi Kataoka · Tomoshige Hori · Hitoshi Houchi · Yasuo Shinohara

Received: 8 October 2010 / Accepted: 25 April 2011 /
Published online: 10 May 2011

Abstract The thermogenic function of brown adipose tissue (BAT) is known to be markedly elevated when animals are exposed to the cold, and intensive studies have been carried out to understand the molecular basis enabling effective thermogenesis in cold-exposed animals. In this study, we used microarray analysis to examine the effects of cold exposure of animals on their gene expression profiles in white adipose tissue (WAT), which seems to function as a counterpart tissue of BAT. The results indicate that the effects of cold exposure on the gene expression profiles of WAT were much more moderate than the effects on those of BAT. Possible reasons for the different responses of BAT and WAT to cold exposure are discussed.

Keywords White adipose tissue · Brown adipose tissue · Cold exposure · Gene expression · Microarray analysis

Abbreviations

BAT	Brown adipose tissue
UCP1	Uncoupling protein 1
WAT	White adipose tissue

Masahiro Watanabe and Takenori Yamamoto contributed equally to the present study.

M. Watanabe · A. Yamamoto · E. Obana · K. Niiyama · T. Hada · Y. Shinohara
Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi-1, Tokushima 770–8505, Japan

M. Watanabe · H. Houchi
Department of Pharmacy, Kagawa University Hospital, Mikicho, Kitagun, Kagawa 761–0793, Japan

T. Yamamoto · A. Yamamoto · E. Obana · K. Niiyama · T. Hada · Y. Shinohara (✉)
Institute for Genome Research, University of Tokushima, Kuramotocho-3, Tokushima 770–8503, Japan
e-mail: yshinoha@genome.tokushima-u.ac.jp

T. Ooie · M. Kataoka
Health Technology Research Center, National Institute for Advanced Industrial Science and Technology (AIST), 2217-14 Hayashicho, Takamatsu 761–0395, Japan

T. Hori
Japan Bioindustry Association, Hatchobori-2, Chuo-ku, Tokyo 104–0032, Japan

Introduction

Two kinds of adipose tissues, white and brown, abbreviated as WAT and BAT, respectively, are present in the mammalian body. These two adipose tissues have completely opposite physiological functions, i.e., WAT functions to store excess energy in the form of triglycerides, and BAT serves as a major site of adaptive thermogenesis, dissipating excess energy in the form of heat [1]. This unique metabolic function of BAT is known to be mainly achieved by the mitochondrial uncoupling protein UCP1, which is specifically expressed in this tissue. It dissipates the electrochemical gradient of H^+ across the inner mitochondrial membrane by acting as a H^+ conductor, and converts the energy of nutrient molecules not into ATP but into heat [2, 3]. In previous studies, we focused on the metabolic processes enabling the efficient thermogenesis in BAT by analyzing the differences in gene expression between WAT and BAT. As a result, the recognition of preferential expression of genes involved in fatty acid metabolism in BAT emerged [4–7]. Furthermore, isoforms of proteins selectively expressed in heart/skeletal tissues were also found to be expressed in BAT [4–7].

For the maintenance of body temperature, the thermogenic function of BAT is stimulated by the cold exposure of animals. Thus, the question as to how the thermogenic function of BAT is stimulated by cold exposure is a major issue in this field, and intensive studies have been carried out to answer this question [3, 8]. We also carried out a microarray analysis to explore the changes in the gene expression in BAT induced by cold exposure, and succeeded in the identification of genes that seemed to be involved in the thermogenic function of BAT under the cold condition [9].

As mentioned above, WAT functions to store excess energy in the form of triglycerides, and hence, it may be considered a counterpart tissue of the BAT. Thus, it is very interesting to know the metabolic processes occurring in the WAT of the cold-exposed animals, but little is known on this issue. In the present study, we used microarray analysis to examine changes in the gene expression in WAT caused by cold exposure.

Materials and Methods

Materials

The oligo array system used (code G4131F) was purchased from Agilent Technologies, Inc. (Santa Clara, CA) and an RNeasy kit (lipid tissue mini, code 74804), from QIAGEN (Hilden, Germany).

Preparation of RNA Samples

Interscapular BAT and epididymal WAT were obtained from three male Wistar rats (5 weeks old) kept at room temperature (22 °C) and from three others exposed to cold (4 °C) for 48 h. Adipose tissues, BAT or WAT, from the three rats of each group were combined; and total RNA was then promptly prepared from them by using an RNeasy kit. RNA concentrations were determined by measuring their A_{260} with a Shimadzu spectrophotometer, model UV-1700. To eliminate the differences between the animals and experiments, we repeated the above procedure once more, and individual RNA samples obtained from the first and second experimental cycles were referred to as RNA #1 and RNA #2, respectively.

Microarray Analysis

A microarray analysis was performed by using an Agilent oligo array system (code G4131F) according to the procedures recommended by the supplier. Eight RNA samples prepared as described above (samples from BAT and WAT of rats kept at room temperature or exposed to cold, prepared in duplicate) were labeled with Cy3 dye and were independently subjected to hybridization on the oligo array. The fluorescence intensity features were detected by the use of an Agilent scanner and were quantified by the Feature Extraction software.

Preparation of cDNA Probes and Northern Blotting

cDNA fragments of *Adam7* and *Rnase10* genes were prepared by RT-PCR using first strand cDNAs prepared from the total RNA of WAT as a template. The primers used were 5′-GGATCCGAAATGTTAAGGAACAG (GE1937) and 5′-GGATCCAAAAGACTTAA CAGCTC (GE1938) for *Adam7* and 5′-CACTGGTGCATCTGTTGTTC (GE1939) and 5′-TCTCCAGTTGTTTCGTGTTG (GE1940) for *Rnase10*. The cDNA of acidic ribosomal phosphoprotein P0 was prepared as described previously [10]. Northern blotting was carried out as described earlier [5].

Results and Discussion

In our previous study, we examined the effects of cold exposure on the expression levels of various genes in BAT by using microarray analysis and succeeded in the identification of genes up/downregulated concomitant with cold exposure of the animals. In the present study, we aimed at exploring the changes in the gene expression profiles of the WAT, which seems to function as a counterpart tissue of BAT. If we could have used the same experimental platform of microarray for the gene expression analysis in WAT as that used in the previous study on BAT, we could have evaluated not only the effects of cold exposure on the gene expression profiles in WAT, but also the differences in the response to cold exposure between BAT and WAT. However, the microarray system used in our previous study (Agilent oligo array system, code G4130A) was changed to its successor version (Agilent oligo array system, code G4131F) prior to the present study, making a quantitative comparison of the results obtained by these two array systems difficult. Thus, to overcome this difficulty, we newly prepared total RNA samples from not only WAT but also BAT of cold-exposed rats and rats kept at room temperature, and four total RNA samples thus prepared were subjected to the current version (code G4131F) oligo array system.

Based on the abovementioned strategies, we carried out a microarray analysis. More precisely, to eliminate the differences between individual experimental animals, we independently prepared two sets of total RNA samples from the WAT and BAT of rats kept at room temperature or rats exposed to the cold (a total of eight RNA samples). Prior to the microarray experiment, to examine whether the prepared RNA samples of BAT would show essentially the same gene expression profiles as those reported previously, we confirmed the elevation of the expression level of uncoupling protein 1 (UCP1) in the BAT of cold-exposed animals compared with the level in the animals kept at room temperature (data not shown).

We also made one improvement of the experimental protocol for microarray analysis. In our previous study, we used the “two color” experimental protocol to seek the changes in

the gene expression caused by cold exposure. Namely, the RNA samples obtained from the animals kept at room temperature and exposed to cold were labeled with Cy3 and Cy5, respectively and were subjected to the microarray analysis. Based on the ratio of signal intensities of Cy5 and Cy3 (Cy5/Cy3), the changes in the transcript level of the individual genes in BAT caused by cold exposure were evaluated. This protocol is conventional and cheap, but quantitative discussions on the changes in the transcript levels of individual genes are difficult because quantum yields would differ between these two dyes. Thus, to overcome this problem, we adopted the "one color" protocol for the present study. The abovementioned RNA samples (eight in total) were subjected to labeling with Cy3, and then subjected to the hybridization with the oligo array. From the obtained raw data set, datum points "flagged" by the Feature Extraction software were omitted, and the remaining data were used for the subsequent analysis.

To visualize the changes in the transcript levels of individual genes in WAT and BAT caused by cold exposure of animals, we prepared scatter plots for RNA samples of WAT and BAT by plotting signal intensities of Cy3 dye observed with animals kept at room temperature and those for the animals exposed to cold on horizontal and vertical axes, respectively. As shown in Fig. 1, changes in the transcript levels of individual genes caused by cold exposure were much remarkable with the RNA samples from BAT than with those from WAT.

Prior to further discussion on the effects cold exposure has on the gene expression in WAT, we first extracted and summarized the genes constantly upregulated in both RNA samples of BAT prepared from cold-exposed rats and those from rats kept at room temperature. In total, 398 genes were shown to be upregulated more than threefold on average in RNA samples of BAT prepared from cold-exposed animals. Of these, four probes, i.e., A_44_P914438, A_42_P484738, A_43_P11472, and A_44_P1042876, used for the detection of the messages encoded by Cdkn1a, Ctgf, Hmox1, and Hspa1a, respectively, appeared multiple times (ten times individually) on the array. By subtracting these overlapping probes, 362 genes were found to be upregulated more than threefold on average in RNA samples of BAT prepared from the cold-exposed rats. We next examined whether the obtained microarray analysis data on the gene expressions in BAT precisely reflected the properties of gene expressions in BAT reported previously. We first focused on the three genes, *UCP1*, type II iodothyronine deiodinase (*Dio2*), and type III adenylate cyclase (*AC3*), used in our previous study as quality controls of the RNA samples prepared from BAT [9], as genes known to be upregulated in the BAT of cold-exposed animals. Unfortunately, data on UCP1 were eliminated by the Feature Extraction due to its saturated signals. The remaining two genes, *Dio2* and *AC3*, were 5.12- and 3.37-fold upregulated (average of two RNA samples), respectively, in the BAT of the cold-exposed rats. Sarcomeric mitochondrial creatine kinase, the expression of which was found in our previous study [9] to be most remarkably upregulated in the BAT of cold-exposed rats in comparison with its level in the BAT of rats kept at room temperature, appeared again as the top gene in the list of upregulated genes (58.8-fold upregulated on average). The expression of type 3 fatty acid-binding protein, found earlier to be remarkably upregulated in the BAT of cold-exposed animals [5], was also upregulated, 3.68-fold on average. Thus, we concluded that the data obtained on the changes in the gene expression profiles in BAT caused by cold exposure of animals precisely reflected the results reported previously. It should be emphasized that the results obtained in the present study on the gene expression profiles in the BAT of rats kept at room temperature or exposed to cold are much more suitable for the quantitative evaluation of the effects of cold exposure on the gene expression profiles in BAT because we used the same dye (Cy3) for the labeling of individual RNA samples.

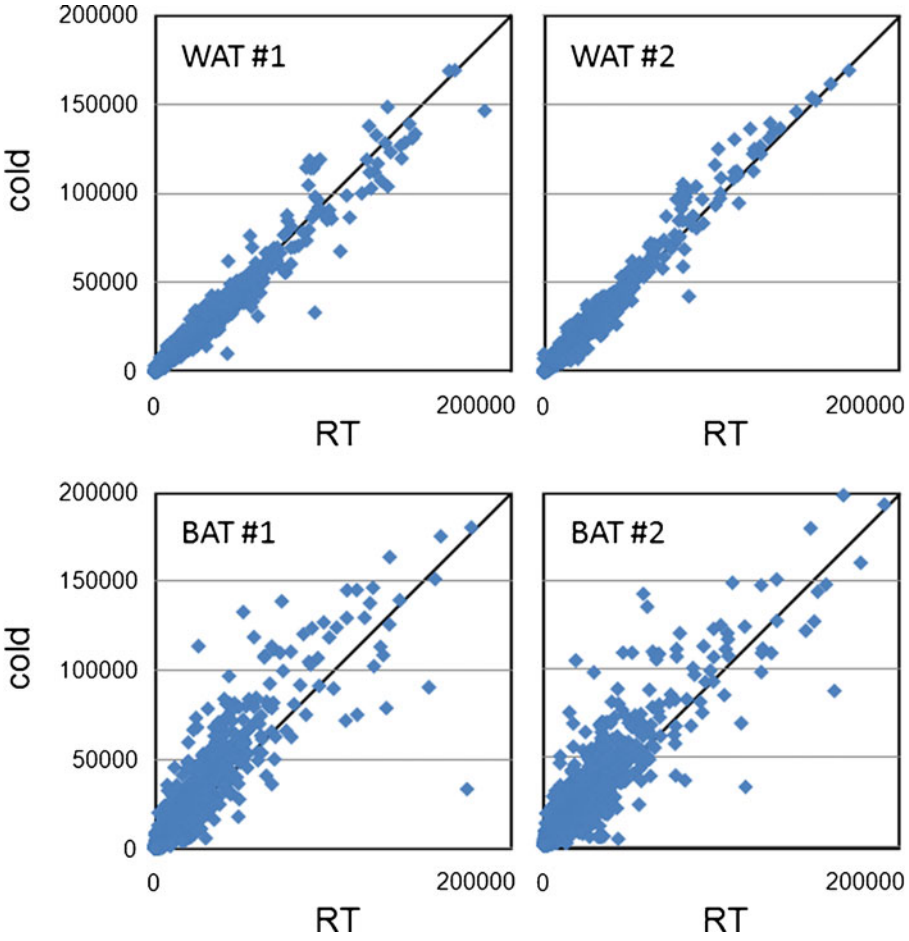

Fig. 1 Scatter plots of the signal intensities of individual genes for animals kept at room temperature and those exposed to cold temperature. For visualization of the effects of cold exposure on the gene expression profiles in WAT or BAT, scatter plots were made for individual RNA sets. In each graph, individual genes were plotted on the *x*- and *y*-axis to show their signal intensities observed for animals kept at room temperature and those exposed to the cold, respectively. To enable the effective evaluation of the cold exposure on the gene expression profiles, we omitted genes showing signal intensities of more than 200,000 (nine and seven genes in WAT #1 and WAT #2, respectively, and 11 and ten genes in BAT #1 and BAT #2, respectively)

Next, to understand the detailed changes in the gene expression profiles in WAT caused by cold exposure of experimental animals, we extracted and summarized those genes constantly up- or downregulated more than threefold in response to the cold, as shown in Tables 1 and 2. When we focused on the genes constantly upregulated in the WAT by cold exposure, we reached the following two conclusions: first, in comparison with the relatively large number of genes upregulated more than threefold on average by cold exposure in BAT (i.e., 362 genes), not so many genes were upregulated in the WAT by cold exposure, i.e., only 14 genes were constantly upregulated more than threefold on average (the probe A_42_P614984, detecting mRNA encoding UCP1, appeared to multiply, but it was counted as a single gene). This conclusion is also evident from the results shown in Fig. 1. Second, the signal intensities

Table 1 Genes showing higher transcript levels in the WAT of cold-exposed rats

Description	Accession no.	Signal intensities (RNA #1)			Signal intensities (RNA #2)			Average of ratios
		RT	Cold	Cold/RT ratio	RT	Cold	Cold/RT ratio	
A disintegrin and metalloprotease domain 7 (Adam7)	NM_020301	26.0	2,262.8	87.03	19.6	7,612.9	387.58	237.30
Ribonuclease, RNase A family, 10 (nonactive) (Rnase10)	NM_001012467	43.0	3,692.7	85.88	33.4	10,421.5	311.93	198.90
Cystatin 8 (cystatin-related epididymal specific) (Cst8)	NM_019258	25.0	585.5	23.42	16.1	1,617.1	100.37	61.89
Lymphocyte antigen 6 complex, locus G5B (Ly6g5b)	NM_001001934	51.0	126.0	2.47	13.9	281.0	20.21	11.34
Lipocalin 6 (Lcn6)	NM_001001519	19.0	79.0	4.16	15.0	196.8	13.08	8.62
Uncoupling protein 1 (mitochondrial, proton carrier) (UCP1)[a]	NM_012682	221.0	779.8	3.53	230.0	1,474.0	6.41	4.97
	NM_012682	235.0	781.8	3.33	231.9	1,480.5	6.38	4.86
	NM_012682	238.0	790.7	3.32	226.7	1,367.4	6.03	4.68
	NM_012682	236.0	787.0	3.33	213.3	1,256.9	5.89	4.61
	NM_012682	229.0	763.7	3.33	234.9	1,376.2	5.86	4.60
	NM_012682	219.0	774.7	3.54	225.5	1,254.5	5.56	4.55
	NM_012682	235.0	774.8	3.30	231.9	1,343.1	5.79	4.54
	NM_012682	248.0	821.2	3.31	230.7	1,325.5	5.75	4.53
	NM_012682	254.0	845.7	3.33	233.7	1,337.0	5.72	4.53
	NM_012682	261.0	802.9	3.08	235.1	1,302.0	5.54	4.31
Lymphocyte antigen 6 complex, locus G5C (Ly6g5c)	NM_198739	59.0	124.9	2.12	45.3	290.1	6.41	4.26
ets variant 4 (Etv4)	NM_001108299	37.0	78.8	2.13	29.2	166.8	5.70	3.92
Tetraspanin 1 (Tspan1)	NM_001004236	294.0	615.7	2.09	254.5	1,419.1	5.58	3.84
Major facilitator superfamily domain containing 2 (Mfsd2)	NM_001106683	36.0	93.0	2.58	51.4	203.8	3.97	3.28
Rat mRNA for pre-optic regulatory factor-1 (PORF-1)	X53231	22.0	60.8	2.76	25.2	88.6	3.51	3.14
Complement component 4 binding protein, alpha (C4bpa)	NM_012516	27.0	99.1	3.67	34.6	87.6	2.53	3.10
Solute carrier family 38, member 5 (Slc38a5)	NM_138854	22.0	51.4	2.34	29.4	108.6	3.69	3.01
Neurotensin receptor 1 (Ntsr1)	NM_001108967	16.0	54.0	3.37	19.7	52.1	2.64	3.01

RT room temperature

[a] Identical probe detecting UCP1 is multiply utilized in the array

Table 2 Genes showing lower transcript levels in the WAT of cold-exposed rats

Description	Accession no.	Signal intensities (RNA #1)			Signal intensities (RNA #2)			Average of ratios
		RT	Cold	Cold/RT ratio	RT	Cold	Cold/RT ratio	
Serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 12 (Serpina12)	NM_138825	685.0	122.5	0.18	458.4	122.3	0.27	0.22
Cerebellin 1 precursor (Cbln1)[a]	NM_001109127	508.0	125.5	0.25	553.6	111.7	0.20	0.22
	NM_001109127	88.0	18.7	0.21	92.4	22.8	0.25	0.23
Hypothetical LOC309150 (predicted) (RGD1308234_predicted)	XR_006515	48.0	10.3	0.21	39.1	12.9	0.33	0.27

RT room temperature

[a] Two distinct probes were used on the array for detection of the message of this gene

of the upregulated genes in WAT were not so high; even after upregulation by cold exposure, the values of the signal intensities of the individual genes were smaller than 2,000, except for those of the top two genes. The expressions of these top two genes, *Adam7* and *Rnase10*, were upregulated about 200-fold by cold exposure, and their probes showed signal intensities of 4,938 and 7,058 (average of the two RNA sets), respectively. The upregulation of these two genes in the WAT of cold-exposed rats was confirmed by Northern blot analysis, as shown in Fig. 2. However, even the signal intensities of these two genes were still not so significant, as compared with those of the 1,260 other genes that constantly showed signal intensities higher than 10,000 in the WAT of cold-exposed rats (data not shown). It should be mentioned that the signal intensities of probes in the currently used array system of the Agilent Company do not simply reflect the expression levels of individual genes because signal intensities of individual probes largely depend upon their locations in the target mRNAs, as reported in our previous study [11]. However, their signal intensities could be essentially regarded to reflect the expression levels of individual genes because most of these probes bind to the 3′ region of the mRNAs of individual genes (data not shown). These two conclusions are essentially the case even for the genes constantly downregulated in the WAT by cold exposure, and hence, the effects of cold exposure on the gene expression profiles for WAT were concluded to be much more moderate than those for BAT under the present experimental conditions. It should be mentioned that there probably are genes that show no remarkable change in their transcriptional level but yet have important roles, as in the case of transcription factors. However, at least, changes in gene expressions reflecting remarkable changes in the metabolic processes observed in the BAT were not induced in the WAT by cold exposure.

Intensive studies on the question as to how the thermogenic function of BAT is stimulated by cold exposure of animals have been carried out, but little is known regarding the changes in the gene expressions in WAT concomitant with cold exposure of animals. The results of our present study clearly show that contrary to the remarkable changes observed in the gene expression in BAT, no remarkable changes were detected in the gene expression in WAT. Then, why were there such remarkable differences observed in the effects of cold exposure on the gene expression between WAT and BAT? We would like to

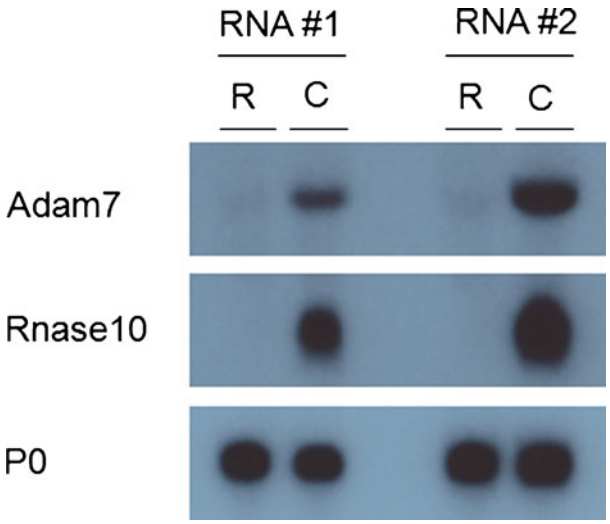

Fig. 2 Upregulated expression of *Adam7* and *Rnase10* genes in the WAT of cold-exposed rats, as confirmed by Northern analysis. To confirm the cold exposure-elicited upregulation of the top two genes listed in Table 1, we carried out Northern blotting. For this procedure, 10-μg aliquots of the total RNA samples obtained from the WAT of rats kept at room temperature (*R*) or exposed to the cold (*C*) were subjected, in duplicate (RNA#1 and #2), to denaturing agarose gel electrophoresis and were transferred onto a nitrocellulose membrane. mRNAs encoded by *Adam7* and *Rnase10* genes were detected by their specific probes prepared as stated in the "Materials and Methods" section. The mRNA of acidic ribosomal phosphoprotein P0 was also measured as a control (P0)

propose two probable answers to this question. The first possibility is that, contrary to the rapid changes in the gene expressions in BAT, those in WAT occur much more slowly. In the present study, we prepared RNA samples from BAT and WAT of rats exposed for 48 h to the cold. This experimental condition is frequently used to examine the effects of cold exposure on the thermogenic function in BAT because upregulation of the uncoupling protein, UCP1, is most remarkable after 48 h. However, it is uncertain whether the effects of cold exposure on the gene expressions in WAT are also most remarkable under this condition. Retarded effects of cold exposure on the gene expressions in WAT would be reasonable since free fatty acids present in the plasma would be firstly utilized as primary metabolites. Triglycerides stored in lipid droplets of brown adipocytes also seem to be used as an acute energy source of thermogenesis because sizes of lipid droplets in the brown adipocytes are known to be reduced by cold exposure of animals [12]. A possible slower response of WAT to cold exposure than that seen for BAT is also supported by the studies of Jakus et al. who reported that the enzymes related to fatty acid synthesis are downregulated in the WAT of rats subjected to prolonged cold exposure for 1 week [13]. However, the expression of these enzymes in the WAT was not changed under the present experimental conditions (data not shown). The second possibility is that changes in gene expression in WAT caused by cold exposure were not remarkable because the amount of WAT present in the body is much larger than the total amount of BAT in the body [14, 15]. To examine whether this interpretation is correct, further careful studies using lean animals would seem to be necessary.

In addition to these discussions, we should also discuss the upregulation of UCP1 in the WAT of cold-exposed rats. As was shown in Table 1, the expression of UCP1 in the WAT

was three- to sixfold upregulated in the cold-exposed rats compared with that in the rats kept at room temperature, although its signal intensity was not remarkable (800–1,500) even after the upregulation by cold exposure. Such ectopic expression of UCP1 in WAT induced by cold exposure of animals or by administration of a β-adrenergic agonist was also observed in past studies [16–19]. The physiological meaning and mechanism(s) responsible for the appearance of UCP1 in WAT are still uncertain, and thus further detailed studies on this point are necessary.

References

1. Nicholls, D. G., & Locke, R. M. (1984). Thermogenic mechanisms in brown fat. *Physiological Reviews, 64*, 1–64.
2. Ricquier, D., & Bouillaud, F. (2000). The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *The Biochemical Journal, 345*, 161–179.
3. Lowell, B. B., & Spiegelman, B. M. (2000). Towards a molecular understanding of adaptive thermogenesis. *Nature, 404*, 652–660.
4. Yamazaki, N., Shinohara, Y., Shima, A., & Terada, H. (1995). 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 Letters, 363*, 41–45.
5. Daikoku, T., Shinohara, Y., Shima, A., Yamazaki, N., & Terada, H. (2000). Specific elevation of transcript levels of particular protein subtypes induced in brown adipose tissue by cold exposure. *Biochimica et Biophysica Acta, 1457*, 263–272.
6. Kajimoto, K., Daikoku, T., Kita, F., Yamazaki, N., Kataoka, M., Baba, Y., et al. (2003). PCR-select subtraction for characterization of messages differentially expressed in brown compared with white adipose tissue. *Molecular Genetics and Metabolism, 80*, 255–261.
7. Unami, A., Shinohara, Y., Kajimoto, K., & Baba, Y. (2004). Comparison of gene expression profiles between white and brown adipose tissues of rat by microarray analysis. *Biochemical Pharmacology, 67*, 555–564.
8. Watanabe, M., Yamamoto, T., Mori, C., Okada, N., Yamazaki, N., Kajimoto, K., et al. (2008). Cold-induced changes in gene expression in brown adipose tissue: implications for the activation of thermogenesis. *Biological & Pharmaceutical Bulletin, 31*, 775–784.
9. Watanabe, M., Yamamoto, T., Kakuhata, R., Okada, N., Kajimoto, K., Yamazaki, N., et al. (2008). Synchronized changes in transcript levels of genes activating cold exposure-induced thermogenesis in brown adipose tissue of experimental animals. *Biochimica et Biophysica Acta, 1777*, 104–112.
10. Akamine, R., Yamamoto, T., Watanabe, M., Yamazaki, N., Kataoka, M., Ishikawa, M., et al. (2007). 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. *Journal of Biochemical and Biophysical Methods, 70*, 481–486.
11. Kakuhata, R., Watanabe, M., Yamamoto, T., Obana, E., Yamazaki, N., Kataoka, M., et al. (2008). Importance of probe location for quantitative comparison of signal intensities among genes in microarray analysis. *Journal of Biochemical and Biophysical Methods, 70*, 926–931.
12. Bukowiecki, L., Collet, A. J., Follea, N., Guay, G., & Jahjah, L. (1982). Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia. *The American Journal of Physiology, 242*, E353–E359.
13. Jakus, P. B., Sandor, A., Janaky, T., & Farkas, V. (2008). Cooperation between BAT and WAT of rats in thermogenesis in response to cold, and the mechanism of glycogen accumulation in BAT during reacclimation. *Journal of Lipid Research, 49*, 332–339.
14. Cinti, S. (2001). The adipose organ: morphological perspectives of adipose tissues. *The Proceedings of the Nutrition Society, 60*, 319–328.
15. Cinti, S. (2005). The adipose organ. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 73*, 9–15.
16. Cousin, B., Cinti, S., Morroni, M., Raimbault, S., Ricquier, D., Pénicaud, L., et al. (1992). Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *Journal of Cell Science, 103*, 931–942.

17. Nagase, I., Yoshida, T., Kumamoto, K., Umekawa, T., Sakane, N., Nikami, H., et al. (1996). Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic beta 3-adrenergic agonist. *Journal of Clinical Investigation, 97*, 2898–2904.
18. Imai, J., Katagiri, H., Yamada, T., Ishigaki, Y., Ogihara, T., Uno, K., et al. (2006). Cold exposure suppresses serum adiponectin levels through sympathetic nerve activation in mice. *Obesity (Silver Spring), 14*, 1132–1141.
19. Xue, Y., Petrovic, N., Cao, R., Larsson, O., Lim, S., Chen, S., et al. (2009). Hypoxia-independent angiogenesis in adipose tissues during cold acclimation. *Cell Metabolism, 9*, 99–109.