FEBS Letters 536 (2003) 232–236 FEBS 26977

A forkhead transcription factor FKHR up-regulates lipoprotein lipase expression in skeletal muscle

Yasutomi Kamei[a,b,*], Junko Mizukami[c], Shinji Miura[b], Miki Suzuki[b], Nobuyuki Takahashi[d,e], Teruo Kawada[d,e], Tomoyasu Taniguchi[c], Osamu Ezaki[b]

[a] *PRESTO, Japan Science and Technology Corporation, National Institute of Health and Nutrition, 1-23-1, Toyama Shinjuku-ku, Tokyo 162-8636, Japan*
[b] *Division of Clinical Nutrition, National Institute of Health and Nutrition, 1-23-1, Toyama Shinjuku-ku, Tokyo 162-8636, Japan*
[c] *Discovery Research Laboratory, Tanabe Seiyaku Co., Ltd., Saitama, Japan*
[d] *Laboratory of Nutrition Chemistry, Division of Food Science and Biotechnology, Graduate School of Agriculture, Kyoto University, Kyoto, Japan*
[e] *Project for Obesity and Lipid Metabolism Regulation, Bio-oriented Research Advancement Institute, Tokyo, Japan*

Received 30 October 2002; revised 12 January 2003; accepted 14 January 2003

First published online 23 January 2003

Edited by Robert Barouki

Abstract Lipoprotein lipase (LPL) plays a role in lipid usage in skeletal muscle by hydrolyzing plasma triglycerides into fatty acids, which are further utilized for β-oxidation. Lipid usage is stimulated during fasting, diabetes mellitus and exercise, concomitant with enhanced LPL expression in skeletal muscle. Here we show that the forkhead type transcription factor FKHR is strongly induced in skeletal muscle in fasting mice, in mice with streptozotocin-induced diabetes and in mice after treadmill running. Ectopic expression of FKHR enhanced LPL gene expression in C2C12 muscle cells in culture. These results implicate FKHR as an important modulator of lipid metabolism in skeletal muscle.

Key words: Lipoprotein lipase; FKHR; PPAR; GLUT4; SREBP; Skeletal muscle

1. Introduction

Lipoprotein lipase (LPL, EC 3.1.1.34) plays a rate-limiting role in lipid usage by hydrolyzing plasma triglycerides into fatty acids, which are further utilized for β-oxidation in skeletal muscle [1,2]. Lipid usage is stimulated during fasting, diabetes mellitus and exercise, concomitant with enhanced LPL expression in skeletal muscle [1,3–5]. LPL is also expressed in tissues such as adipose tissue and liver, and the regulation of the LPL gene has been studied in adipose tissue and liver. Namely, in adipose tissue, LPL is up-regulated by a nuclear receptor PPARγ (peroxisome proliferator-activated receptor γ) [6]. In liver, LPL is up-regulated by both SREBP1c and PPARα [6,7]. The regulation of LPL expression, during fasting, diabetes and exercise, may be mediated by the actions of PPARα or SREBP1c, as both are abundantly expressed in the skeletal muscle [8,9]. However, the precise mechanism of LPL gene regulation in skeletal muscle is unclear.

FKHR (FOXO1), AFX (FOXO4) and FKHRL1 (FOXO3a) are a subfamily of forkhead type transcription factors, also called the FOXO family [10]. In addition, FKHR and AFX have been reported to act as cofactors to various nuclear receptors [11,12], as part of a complex containing CBP/p300 [13,14], which plays critical roles in nuclear receptor signal including PPAR [15–17]. The FOXO family is expressed in skeletal muscle [18]. Indeed, FKHR (forkhead homolog in rhabdomyosarcoma) was originally cloned from a rhabdomyosarcoma because of its aberrant fusion with another transcription factor, PAX3, resulting from a chromosomal translocation t(2; 13) [19]. The resulting fusion protein PAX3–FKHR is thought to play a role in muscle cell transformation and rhabdomyosarcoma [19], but the physiological role of FOXO family proteins in skeletal muscle is unclear.

In this study, we examined the possible involvement of the FOXO family in the regulation of LPL gene expression in skeletal muscle. We found that FKHR is induced in skeletal muscle in which LPL gene expression is increased. Moreover, retroviral overexpression of FKHR in C2C12 muscle cells enhanced LPL gene expression, and addition of PPARα activator enhanced it further. Thus, our data indicated that FKHR activates LPL gene expression in concert with PPARα.

2. Materials and methods

2.1. RNA analysis

Northern blot analysis was performed as described in [9]. The loads of total RNA applied to each gel were shown by ethidium bromide staining of ribosomal RNAs. The cDNA fragments for LPL, AFX, FKHRL1, CD36/FAT (fatty acid transporter), GLUT4, SREBP1 were obtained by polymerase chain reaction from first strand cDNA using mouse skeletal muscle total RNA and HeLa total RNA for AFX. First strand cDNA was prepared using a T-primed first strand kit (Amersham Pharmacia Biotech). The amplified products were subcloned into pGEM-T Easy Vector (Promega), and confirmed by sequencing. The GenBank accession numbers of amplified cDNAs are: LPL, J03302; AFX, X93996; FKHRL1, AF114259; CD36/FAT, L23108; and GLUT4, AF374266. Human FKHR cDNA was obtained by screening a skeletal muscle cDNA library, and confirmed by sequencing. PPARα cDNA was obtained from Dr. K. Umesono.

2.2. Stable cell lines

Phoenix 293 cells [20] were cultured in 90 mm dishes and transfected at 70% confluence by Lipofect Amine Plus (Invitrogen) accord-

*Corresponding author. Fax: (81)-3-3207 3520.
E-mail address: ykamei@nih.go.jp (Y. Kamei).

Abbreviations: LPL, lipoprotein lipase; PPAR, peroxisome proliferator-activated receptor; FAT, fatty acid transporter; STZ, streptozotocin; FKHR, forkhead homolog in rhabdomyosarcoma

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00062-0

ing to the manufacturer's instructions, with 2 μg of pLNCX-derived expression plasmid (Clontech) containing cDNA of FKHR or vector alone. Viral supernatants were harvested 48 h after transfection. The supernatants were added to dishes (90 mm in diameter) of C2C12 cells at 50% confluence in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) and 5 μg/ml polybrene in a final volume of 5 ml. C2C12 cells were purchased from American Type Culture Collection. Cells were split 1:10 24 h after infection and replated in DMEM containing 10% FCS and 1 mg/ml G418 to eliminate uninfected cells. After the drug selection, virally infected stable cell lines were cultured to confluence in DMEM containing 10% FCS. Cells were refed every 2 days. At 1 week post-confluence, cells were incubated for 6 h with or without 10 μM Wy14643 (Calbiochem) and used for the preparation of RNA.

2.3. Animal studies

C57BL/6J mice were obtained from Tokyo Laboratory Animal Science (Tokyo) at 7 weeks of age and allowed free access to laboratory food (on a caloric basis, 11% fat, 60% carbohydrate and 29% protein). The mice were maintained at a constant temperature of 22°C with a fixed artificial light cycle (12 h light and 12 h dark). For treadmill running experiments, mice performed 10% uphill treadmill running exercise at 15 m/min for 45 min periods eight times (totally 6 h), with 5 min rest intervals. After running, mice were allowed free access to the laboratory food. At 2 h and 16 h after the exercise bout, mice were killed and skeletal muscle (quadriceps) was isolated for RNA preparation. For streptozotocin (STZ)-induced diabetes mice experiments, mice were intrapentoneally injected with STZ (Wako Chemical, Japan) at a dose of 100 mg per kg body weight in ice-cold 0.5 mol/l citrate buffer (pH 4.5). Control mice received citrate buffer only. 10 days after injection, mice were killed. Blood was collected from abdominal aorta and glucose was determined using a Tidex kit (Sankyo).

2.4. Statistical analyses

Statistical comparisons were made using Student's *t*-test. Statistical significance is defined as $P < 0.05$.

3. Results

3.1. FOXO family expression is enhanced in skeletal muscle of fasting mice

To study the potential role of the FOXO family in the regulation of LPL gene expression in skeletal muscle by nutritional status, which affects the LPL mRNA level, we examined mice subjected to fasting and refeeding (Fig. 1). Blood glucose levels were decreased by fasting and recovered by refeeding (control, 155 ± 8.9 mg/ml; fasting 24 h, 81 ± 2.2 mg/ml, $P < 0.01$; refed, 137 ± 5.9 mg/ml). As expected, 24 h and 48 h fasting increased the expression of mRNA for LPL. Expression of CD36/FAT, a fatty acid utilization protein [21], was also up-regulated by fasting. Interestingly, fasting induced a parallel seven- to eight-fold increase in FKHR and FKHRL1 mRNAs. AFX mRNA expression was up-regulated two-fold by fasting. PPARα expression was also up-regulated three-fold. All of these inductions were reversed by refeeding. Expression of glucose transporter 4 (GLUT4), an insulin-dependent glucose transporter [22], was not significantly changed by fasting. Refeeding up-regulated the expression of GLUT4, which is considered to contribute to glycogen synthesis [23]. SREBP1, a regulator of lipogenesis genes [24], was decreased by fasting and increased by refeeding. Expression of FOXO family members was not to be correlated with the expression of GLUT4 and SREBP1c. Ethidium bromide staining of 28S RNA was shown (Fig. 1). The content of ribosomal RNA in total RNA was marginally decreased by fasting, which was consistent with previous study [25].

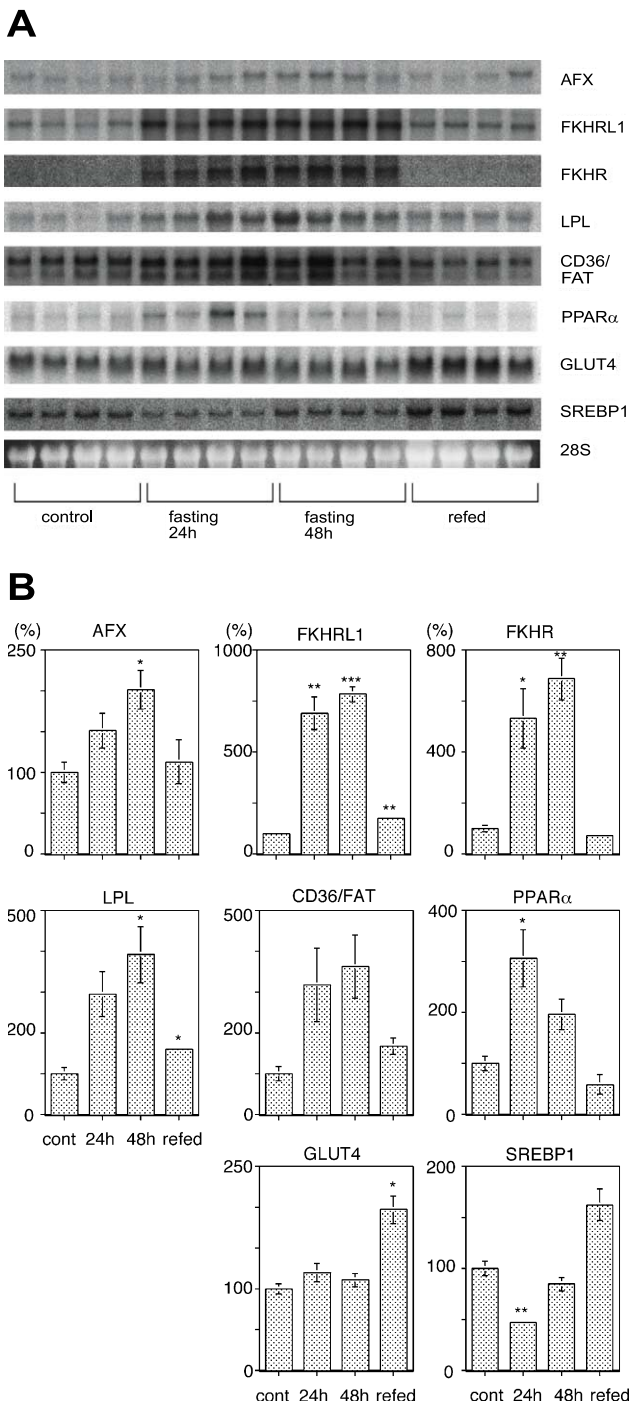

Fig. 1. FOXO family and LPL gene expressions are increased in the skeletal muscle of fasted mice. Mice were divided into four experimental groups. They were either allowed to feed ad libitum (control), or subjected to a 24 h fast or 48 h fast. Others were subjected to a 48 h fast followed by 8 h of feeding (refed). A: Northern analysis was performed on total RNA (20 μg per lane) isolated from skeletal muscle (quadriceps). Each lane represents a sample from an individual mouse. B: Relative densitometric levels of the autoradiogram are shown. Each value represents mean ± S.E. ($n = 4$). ***$P < 0.001$, **$P < 0.01$ and *$P < 0.05$ vs. control.

3.2. Expression of FOXO family members is modulated in skeletal muscle of mice with diabetes

Next, we examined the level of FKHR in skeletal muscle of mice treated with STZ, which is the most commonly used

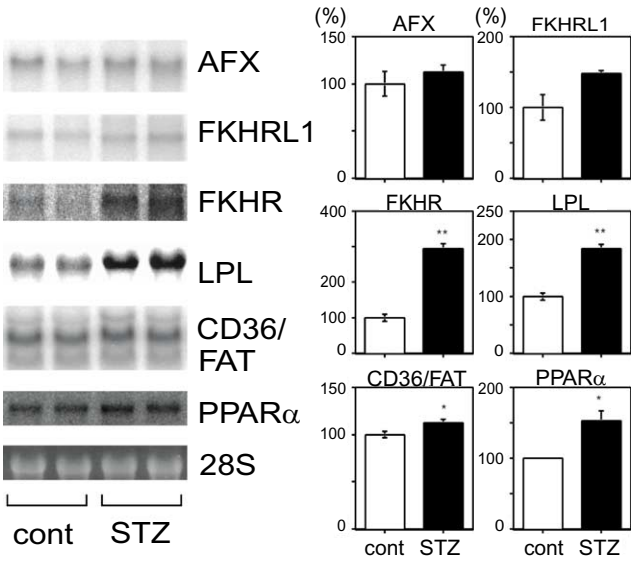

Fig. 2. FOXO family and LPL gene expressions are increased in the skeletal muscle of insulin-deficient mice. Northern analysis was performed on the total RNA (20 μg per lane) isolated from skeletal muscle of the STZ-diabetic mice. Mice of 9 weeks of age received an intrapentoneal injection with sodium citrate solution (control) or STZ (100 mg per kg body weight). After 10 days, the animals were killed. A typical autoradiogram and its relative levels are shown. Each lane represents a sample from an individual mouse. Each value represents mean ± S.E. of four mice. $^{**}P < 0.01$ and $^{*}P < 0.05$ vs. control.

experimental model of diabetes [26]. STZ-treated mice had higher blood glucose levels (543 ± 20 mg/ml) than the controls (181 ± 6.5 mg/ml, $P < 0.001$), indicating that STZ, used in the experiment, worked well to disrupt insulin secretion. As expected, LPL mRNA levels increased as shown in Fig. 2. Expression of CD36/FAT was also significantly increased. Moreover, FKHR mRNA levels were markedly elevated in the skeletal muscle of these mice. AFX and FKHRL1 levels were also elevated. PPARα level was marginally increased.

3.3. Expression of FOXO family members in skeletal muscle of mice after exercise

Mice after a single bout of 6 h running (15 m/min, 10% uphill), showed a three- to five-fold increase of FKHRL1 and FKHR mRNAs in skeletal muscle 2 h after running. At 16 h after exercise, the FKHRL1 and FKHR mRNAs had returned to sedentary levels (Fig. 3). LPL expression was also increased in skeletal muscle 2 h after running, and then returned 16 h after exercise.

3.4. Expression of FKHR in C2C12 myocytes promotes LPL gene expression

We determined the causal relationship between FKHR expression and other genes, which increased in parallel in skeletal muscle, by ectopically overexpressing FKHR in the C2C12 myocyte cell line. We overexpressed FKHR, as FKHR was most up-regulated in fasting, diabetes and exercise, among the FOXO family. C2C12 cells were infected with recombinant particles encoding FKHR or vector alone. Northern blot analysis was performed to confirm the expression of the transduced FKHR gene (Fig. 4). Expression of FKHR led to a marked increase in LPL mRNA compared to the level in cells infected with the vector alone. Thus, LPL appears to be a target of FKHR in skeletal muscle. In contrast to LPL, overexpression of FKHR had only a marginal influence on CD36/FAT levels in C2C12 cells (Fig. 4). Then, we incubated FKHR-C2C12 cells with Wy14643, a synthetic PPARα activator, and measured the expression level of LPL. The basal and Wy14643-induced expression levels of LPL were markedly elevated in the cells overexpressing FKHR (Fig. 4). CD36/FAT expression was also increased by Wy14643, which is consistent with a previous finding that CD36/FAT expression is enhanced by PPAR [27–29]. The Wy14643-induced expression was also marginally increased in FKHR-overexpressing cells (Fig. 4).

4. Discussion

In this study, we showed that the expression of FOXO

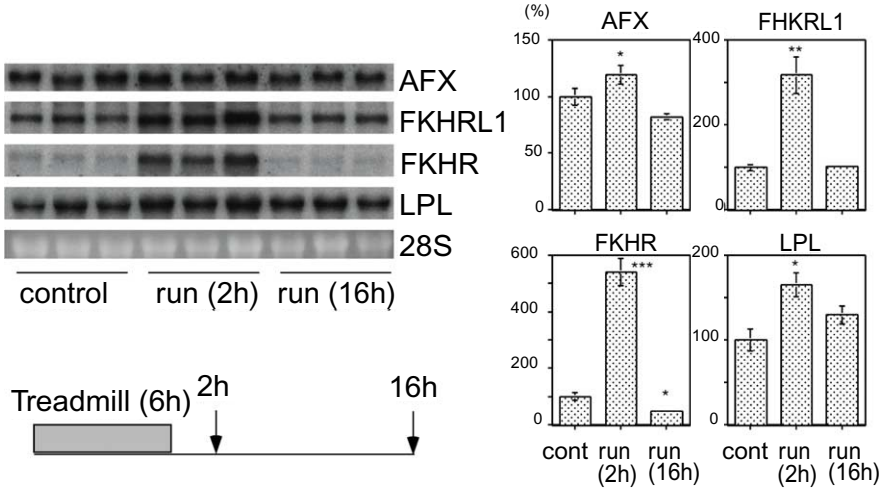

Fig. 3. FOXO family and LPL gene expressions are increased in the skeletal muscle of exercised mice. Mice of 9 weeks of age were either kept sedentary (control) or exercised by a 6 h treadmill run in a day. At 2 h and 16 h after the exercise bout, mice were killed and skeletal muscle was isolated for RNA preparation. Schematic experimental design is shown in the figure. A typical autoradiogram and its relative levels are shown. Each lane represents a sample from an individual mouse. Each value represents mean ± S.E. of six mice. $^{***}P < 0.001$, $^{**}P < 0.01$ and $^{*}P < 0.05$ vs. control.

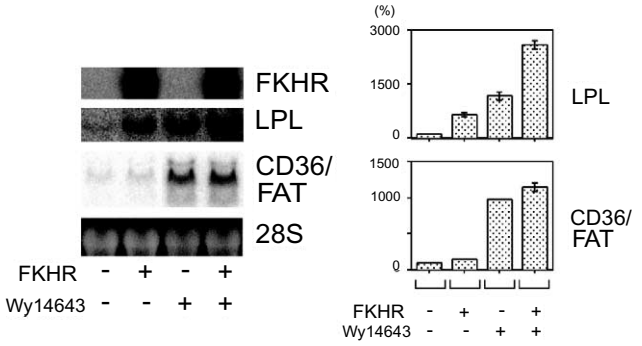

Fig. 4. LPL gene expression is increased in cultured myocytes overexpressing FKHR. FKHR was ectopically expressed in C2C12 cells. The cells were treated with 10 μM Wy14643 (for 6 h). From the cells, total RNA was isolated and analyzed by Northern blotting with ^{32}P-labeled FKHR, LPL and CD36/FAT. A typical autoradiogram and its relative levels are shown. The graph represents densitometric values for Northern blots of LPL and CD36/FAT mRNAs. Each value represents mean ± S.E. of two dishes.

family members, including that of FKHR, concomitant with LPL expression is induced in skeletal muscle of fasting mice and mice with STZ-treated diabetes, and after exercise. Ectopic expression of FKHR in C2C12 muscle cells induced LPL gene expression, in combination with the physiological data described above, indicating that LPL is a target of FKHR in skeletal muscle.

A genetic study of *Caenorhabditis elegans* showed that a forkhead transcription factor DAF16 functions as a suppressor of insulin receptor signaling [30]. The FOXO family is considered the mammalian counterpart of DAF16 [31]. Thus, the FOXO family is suggested to act, in mammals, as a downstream player in insulin signaling. In liver, FKHR is reported to activate the gene expression of glucose-6-phosphatase (G6Pase), a key gluconeogenesis enzyme [32,33]. Both G6Pase and FKHR mRNAs are induced by glucocorticoid [33], whose blood level is high in fasting, diabetes and after exercise [1], and both mRNAs are suppressed by insulin [33]. FKHR may activate gluconeogenesis by increasing G6Pase expression in the liver, and activate fatty acid usage in skeletal muscle by increasing LPL expression and play roles in total glucose metabolism in the human body.

What signal stimulates expression of FOXO family members in fasting, diabetes and exercise in skeletal muscle? As described above, glucocorticoid is a good candidate. On the other hand, conditions like glucose starvation and exercise have been described to increase AMP-activated protein kinase (AMPK) activity [34]. AMPK is a serine/threonine kinase, widely expressed in skeletal muscle, and whose activity reflects the energy status (AMP:ATP ratio) of a cell [34]. We also speculate that AMPK may be involved in the up-regulation of FOXO family in the skeletal muscle. In contrast to our speculation, Barthel et al. reported that treatment of hepatic cells with 5-aminoimidazole-4-carboxamide riboside (AICAR), an activator of AMPK, repressed G6Pase expression and led to disappearance of FKHR protein [35]. Possible involvement of AMPK in the regulation of FOXO family in the skeletal muscle should be studied in the near future.

FKHR can act as a cofactor protein of nuclear hormone receptors [11,12] as part of a nuclear receptor cofactor complex that includes CBP/p300 [13,14]. We observed that FKHR could physically interact with the N-terminal (aa 1–721) and C-terminal (1824–2441), but not other regions of CBP [15] by a GST-pull-down assay (data not shown). LPL gene promoter contains a PPAR-responsive element and some glucocorticoid receptor-responsive elements [6,36]. Ectopic expression of FKHR in C2C12 cells enhanced LPL gene expression, and addition of Wy14643 enhanced it further (Fig. 4). These data suggest that FKHR can activate LPL gene expression in concert with PPARα. The detailed molecular mechanism, including possible functional interaction between FKHR and these nuclear receptors on LPL gene expression, should be clarified in the future.

Transgenic mice specifically overexpressing LPL in skeletal muscle have been reported. High levels of muscle LPL caused insulin resistance in their skeletal muscle [37,38]. Insulin resistance in skeletal muscle is considered to be involved in the pathogenesis of diabetes [37]. In STZ-treated diabetic mice, FKHR expression was induced in skeletal muscle coupled with enhanced LPL expression (Fig. 2). Thus, it is conceivable that enhanced expression of FKHR in skeletal muscle may be involved in the pathogenesis of diabetes. Further study on the molecular mechanism of FKHR action in skeletal muscle is also important from a clinical viewpoint.

Acknowledgements: We thank Y. Kai for technical assistance. This work was supported in part by Special Coordination Funds for Promoting Science and Technology from the Japanese Ministry of Education, Culture, Sports, Science and Technology (Tokyo), by research grants from the Japanese Ministry of Health, Labor and Welfare (Tokyo), and by a Grant from the Promotion of Fundamental Studies in Health Sciences of Organization for Pharmaceutical Safety and Research (OPSR). T.K. was supported by the PROBRAIN grant.

References

[1] McMurray, W.C. (1983) Essentials of Human Metabolism, Harper and Row, New York.
[2] Goldberg, I.J. (1996) J. Lipid Res. 37, 693–707.
[3] Ladu, M.J., Kapsas, H. and Palmer, W.K. (1991) Am. J. Physiol. 260, R953–R959.
[4] Rauramaa, R., Kuusela, P. and Hietanen, E. (1980) Horm. Metab. Res. 12, 591–595.
[5] Greiwe, J.S., Holloszy, J.O. and Semenkovich, C.F. (2000) J. Appl. Physiol. 89, 176–181.
[6] Schoonjans, K., Peinado-Onsurbe, J., Lefebvre, A.M., Heyman, R.A., Briggs, M., Deeb, S., Staels, B. and Auwerx, J. (1996) EMBO J. 15, 5336–5348.
[7] Schoonjans, K., Gelman, L., Haby, C., Briggs, M. and Auwerx, J. (2000) J. Mol. Biol. 304, 323–334.
[8] Amri, E.Z., Bonino, F., Ailhaud, G., Abumrad, N.A. and Grimaldi, P.A. (1995) J. Biol. Chem. 270, 2367–2371.
[9] Ikeda, S., Miyazaki, H., Nakatani, T., Kai, Y., Kamei, Y., Miura, S., Tsuboyama-Kasaoka, N. and Ezaki, O. (2002) Biochem. Biophys. Res. Commun. 296, 395–400.
[10] Kaestner, K.H., Knochel, W. and Martinez, D.E. (2000) Genes Dev. 14, 142–146.
[11] Schuur, E.R., Loktev, A.V., Sharma, M., Sun, Z., Roth, R.A. and Weigel, R.J. (2001) J. Biol. Chem. 276, 33554–33560.
[12] Zhao, H.H., Herrera, R.E., Coronado-Heinsohn, E., Yang, M.C., Ludes-Meyers, J.H., Seybold-Tilson, K.J., Nawaz, Z., Yee, D., Barr, F.G., Diab, S.G., Brown, P.H., Fuqua, S.A.W. and Osborne, C.K. (2001) J. Biol. Chem. 276, 27907–27912.
[13] Nasrin, N., Ogg, S., Cahill, C.M., Biggs, W., Nui, S., Dore, J., Calvo, D., Shi, Y., Ruvkun, G. and Alexander-Bridges, M.C. (2000) Proc. Natl. Acad. Sci. USA 97, 10412–10417.
[14] Mahmud, D.L., G-Amlak, M., Deb, D.K., Platanias, L.C., Uddin, S. and Wickrema, A. (2002) Oncogene 21, 1556–1562.
[15] Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K. and Rosenfeld, M.G. (1996) Cell 85, 403–414.

[16] Mizukami, J. and Taniguchi, T. (1997) Biochem. Biophys. Res. Commun. 240, 61–64.
[17] Glass, C.K. and Rosenfeld, M.G. (2001) J. Biol. Chem. 276, 36865–36868.
[18] Furuyama, T., Nakazawa, T., Nakano, I. and Mori, N. (2000) Biochem. J. 349, 629–634.
[19] Galili, N., Davis, R.J., Fredericks, W.J., Mukhopadhyay, S., Rauscher, F.J., Emanuel 3rd, B.S., Rovera, G. and Barr, F.G. (1993) Nat. Genet. 5, 230–235.
[20] Grignani, F., Kinsella, T., Mencarelli, A., Valtieri, M., Riganelli, D., Grignani, F., Lanfrancone, L., Peschle, C., Nolan, G.P. and Pelicci, P.G. (1998) Cancer Res. 58, 14–19.
[21] Ibrahimi, A., Sfeir, Z., Magharaie, H., Amri, E.Z., Grimaldi, P. and Abumrad, N.A. (1996) Proc. Natl. Acad. Sci. USA 93, 2646–2651.
[22] Ezaki, O. (1997) Biochem. Biophys. Res. Commun. 241, 1–6.
[23] Ryder, J.W., Kawano, Y., Galuska, D., Fahlman, R., Wallberg-Henriksson, H., Charron, M.J. and Zierath, J.R. (1999) FASEB J. 13, 2246–2256.
[24] Shimano, H., Yahagi, N., Amemiya-Kudo, M., Hasty, A.H., Osuga, J., Tamura, Y., Shionoiri, F., Iizuka, Y., Ohashi, K., Harada, K., Gotoda, T., Ishibashi, S. and Yamada, N. (1999) J. Biol. Chem. 274, 35832–35839.
[25] Boss, O., Samec, S., Dulloo, A., Seydoux, J., Muzzin, P. and Giacobino, J.P. (1997) FEBS Lett. 412, 111–114.
[26] Wilson, G.L. and Leiter, E.H. (1990) Curr. Top. Microbiol. Immunol. 156, 27–54.
[27] Tontonoz, P., Nagy, L., Alvarez, J.G.A., Thomazy, V.A. and Evans, R.M. (1998) Cell 93, 241–252.
[28] Teboul, L., Febbraio, M., Gaillard, D., Amri, E.Z., Silverstein, R. and Grimaldi, P.A. (2001) Biochem. J. 360, 305–312.
[29] Sato, O., Kuriki, C., Fukui, Y. and Motojima, K. (2002) J. Biol. Chem. 277, 15703–15711.
[30] Ogg, S., Paradis, S., Gottlieb, S., Patterson, G.I., Lee, L., Tissenbaum, H.A. and Ruvkun, G. (1997) Nature 389, 994–999.
[31] Anderson, M.J., Viars, C.S., Czekay, S., Cavenee, W.K. and Arden, K.C. (1998) Genomics 47, 187–199.
[32] Nakae, J., Kitamura, T., Silver, D.L. and Accili, D. (2001) J. Clin. Invest. 108, 1359–1367.
[33] Barthel, A., Schmoll, D., Kruger, K.D., Bahrenberg, G., Walther, R., Roth, R.A. and Joost, H.G. (2001) Biochem. Biophys. Res. Commun. 285, 897–902.
[34] Hardie, D.G. and Hawley, S.A. (2001) Bioessays 23, 1112–1119.
[35] Barthel, A., Schmoll, D., Kruger, K.D., Roth, R.A. and Joost, H.G. (2002) Endocrinology 143, 3183–3186.
[36] Bey, L., Etienne, J., Tse, C., Brault, D., Noe, L., Raisonnier, A., Arnault, F., Hamilton, M.T. and Galibert, F. (1998) Gene 209, 31–38.
[37] Kim, J.K., Fillmore, J.J., Chen, Y., Yu, C., Moore, I.K., Pypaert, M., Lutz, E.P., Kako, Y., Velez-Carrasco, W., Goldberg, I.J., Breslow, J.L. and Shulman, G.I. (2001) Proc. Natl. Acad. Sci. USA 98, 7522–7527.
[38] Ferreira, L.D.M.C., Pulawa, L.K., Jensen, D.R. and Eckel, R.H. (2001) Diabetes 50, 1064–1068.