Regulation of the Uncoupling Protein-2 Gene in INS-1 β-Cells by Oleic Acid*

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Current evidence suggests that uncoupling protein-2 (UCP2) is a regulator of insulin secretion. It is also known that chronic exposure of pancreatic islets to free fatty acids (FFAs) blunts glucose-stimulated insulin secretion and is accompanied by elevated levels of UCP2. However, the mechanisms regulating expression of UCP2 in β-cells are unknown. Here, we show that UCP2 mRNA and protein levels were increased after a 48-h exposure of INS-1(832/13) β-cells to oleic acid (0.5 mM) by activation of the UCP2 promoter. Furthermore, progressive deletions of the mouse UCP2 promoter (from −7.3 kb to +12 bp) indicated that an enhancer region (−86/−44) was responsible for both basal and FFAs-stimulated UCP2 gene transcription. This enhancer contains tightly clustered Sp1, sterol regulatory element (SRE), and double E-Box elements. While all three sequence motifs were required for basal activity of the UCP2 promoter, the mutations in either the SRE or the E-Box elements eliminated the response to FFAs. The SRE and sterol regulatory element binding protein-1 (SREBP1) appear to be crucial for the response of the UCP2 gene to FFAs, since overexpression of the nuclear forms of the SREBPs increased UCP2 promoter activity by 7–10-fold and restored the ability of E-Box mutants to respond to oleic acid. These data support a model in which SREBP is the major modulator of UCP2 gene transcription by FFA, while E-Box binding factors play a supportive role.

Uncoupling protein-2 (UCP2)1 is expressed in many tissues that are important for regulating carbohydrate and lipid metabolism, most notably pancreatic β-cells, white and brown adipocytes, skeletal muscle, and hypothalamus. Since the discovery of the UCP2 gene and its genetic location in a region linked to hyperinsulinemia (1), several studies have indicated that expression of UCP2 is regulated in tandem with the level of circulating free fatty acids (FFA) (reviewed in Ref. 2). For example, increases in FFAs resulting from fasting or a high fat diet led to a stimulation of UCP2 expression in adipose tissue and muscle (3–5). In isolated rat islets and INS-1 pancreatic β-cells, long term treatment with FFAs can increase UCP2 mRNA (6, 7), but the mechanisms and physiological significance of these changes in UCP2 are still unclear. Recent studies have shown that the amount of UCP2 expressed in pancreatic β-cells is inversely related to the efficacy of glucose-stimulated insulin secretion (8–10). For example, glucose-stimulated insulin secretion is inhibited in rat islets following adenoviral overexpression of UCP2 (8) (10), while in UCP2-deficient (UCP2−/−) mice higher levels of insulin secretion are observed following a glucose challenge (9). Consistent with these observations, it was reported that the increased levels of UCP2 after chronic exposure of INS-1 β-cells to FFA coincided temporally with impaired glucose-stimulated insulin secretion (6, 7). Taken together these results suggest that UCP2 may act as a molecular mediator for the well known chronic effects of FFA (reviewed in Ref. 11) to blunt glucose-stimulated insulin secretion. However, despite this compelling connection between UCP2 and insulin secretion, and the possible role of UCP2 in the development of type 2 diabetes, very little is known about the mechanisms regulating UCP2 expression in β-cells.

In previous work we identified a proximal region (from −86 to −44) of the mouse UCP2 gene as an important regulator of UCP2 promoter activity in adipocytes (12). This region contains several sequence elements that are also found in the transcriptionally important regulatory regions of other genes involved in energy metabolism (reviewed in Refs. 13 and 14). These elements include Sp1, a sterol regulatory element (SRE), and a double E-Box-like motif separated by five nucleotides. In this study we investigated the target elements for FFA-dependent regulation of the UCP2 promoter in the pancreatic β-cell line INS-1(832/13). Our results indicate that in this particular model, SREBP1 participates in the oleic acid-dependent regulation of the UCP2 gene, and the potency of its interaction with the SRE is modulated by adjacent E-Box binding factors.

EXPERIMENTAL PROCEDURES

Plasmids and Constructs—Expression vectors for the nuclear forms of SREBP1a (aa 1–460) and SREBP2 (aa. 1–468) were purchased from M. Sawadogo. Expression vectors for USF1 and USF2 are gifts from M. Sawadogo. UCP2 deletion constructs were designed by cloning UCP2 promoter fragments in pGL3 basic vector (Promega). Point mutation constructs of the −86/−44 enhancer were made by cloning mutated PCR fragments (−86/+12) of the UCP2 promoter in pGL3-basic vector (Promega). The integrity and fidelity of all promoter-reporter constructs were verified by DNA sequencing.

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‡ The abbreviations used are: UCP2, uncoupling protein-2; aa, amino acid; FAS, fatty acid synthase; FCS, fetal calf serum; FFA, free fatty acid; PPAR, peroxisome proliferator-activated receptor; RT, reverse transcriptase; SRE, sterol regulatory element; SREBP1/ADD1, sterol regulatory element binding protein-1/adipocyte differentiation determining factor; USF, upstream stimulatory factor; ANOVA, analysis of variance.
Cell Culture and Transfections—The clone 832/13 of the INS-1 pancreatic β-cell line (15) was a gift from Christopher Newgard (Duke University). Cells were maintained in RPMI 1640 medium at 11.1 mM glucose supplemented with 10 mM HepES, 10% heat-inactivated fetal calf serum (FCS), 2 mM l-glutamine, 1 mM sodium pyruvate, 50 μM β-mercaptoethanol, 100 IU/ml penicillin, and 100 μg/ml streptomycin in a humidified atmosphere (5% CO₂). For transfection experiments, cells were grown to 60–70% confluence in six-well plates and 1.5 μg/well of total plasmid DNA in FuGENETM 6 reagent (Roche Molecular Biochemicals) was added to the cells. For studying the effect of long term oleic acid treatment, the transfection medium was replaced after 48 h with RPMI 1640 medium containing 4% FCS, 1% of fat-free BSA, or 0.5 mg/ml oleic acid (Sigma) bound to BSA (3:1 ratio). Transfected cells were collected after 48 h and assayed for luciferase and β-galactosidase activity using corresponding kits (Promega). Luciferase activity of the UCP2 reporter constructs was normalized to a control pCMV-β-gal reference plasmid.

Immunoblotting—Whole cells or nuclear extracts were lysed, subjected to SDS-PAGE (4–20% gradient gel, Invitrogen), and transferred to nitrocellulose membranes all as described previously (16). Membranes were blocked with 4% (w/v) BSA, fraction 5 (Sigma) in TBST (1× TBS with 0.2% v/v Tween 20), incubated with the respective primary antibodies (the antibody titer was optimized for each antibody) followed by horseradish peroxidase-labeled anti-rabbit IgG (1:10,000 dilution), antibodies to SREBP1 (sc-8984X; Santa Cruz), SREBP2 (sc-8151X; Santa Cruz), USF1 (sc-8983X; Santa Cruz), and USF2 (sc-861X; Santa Cruz) were labeled using a Prime-It RmT random primer labeling kit (Stratagene) according to manufacturer's protocol. RT-PCR reactions were performed using 0.5 μg RNA with a Titan® One Tube RT-PCR System kit (Roche Molecular Biochemicals). Reaction mix contained two forward primers: SREBP1α-specific (5'-CCGCACGCGCATGAGGCACGC) and SREBP1c-specific (5'-GGAGGCAATGATGGACATTG-3') and one reverse primer, common for both SREBP1 isoforms (5'-CAATAAGGCAAGGGAGTACAC-3').

Electrophoretic Mobility Shift Assays—Nuclear extracts from cultured cells were prepared according to a modified method of Dignam et al. (19), and gel shift assays were performed as described previously (18). The DNA binding reaction was performed in the presence of 1 μg of INS-1(832/13) nuclear extracts, 0.5 μg of poly(dI·dC), and 0.1–0.5 ng of end-labeled double-stranded oligonucleotide corresponding to the −76/−44 region of the mouse UCP2 promoter. For supershift experiments, 0.5 μl of antibodies to SREBP1 (sc-89864X; Santa Cruz), SREBP2 (sc-8151X; Santa Cruz), USF1 (sc-8983X; Santa Cruz), and USF2 (sc-861X; Santa Cruz) were included in the binding reactions.

Statistical Analysis—Statistical differences between groups were analyzed by one-way ANOVA and a post-hoc Newman-Keuls test or paired t test as indicated in the figure legends. All results presented are the mean ± S.D.

RESULTS

Fatty Acids Increase Expression of UCP2 in INS-1 Cells through the −86/−44 Enhancer Region—Since UCP2 mRNA levels are increased in pancreatic β-cells after prolonged exposure to FFA (6, 7), we tested whether this response resulted from transcriptional regulation of the UCP2 gene. Fig. 1 shows that prolonged treatment (48 h) of INS-1 cells with 0.5 mM oleic acid resulted in a 2-fold increase of the UCP2 mRNA (Fig. 1A) and protein levels (Fig. 1B) and a similar increase in UCP2 promoter activity (Fig. 1C). Based on these findings we searched for the regulatory regions in the UCP2 promoter that serve as the molecular sensor of FFAs. A series of deletion mutant constructs of the mouse UCP2 promoter was generated spanning the region between −7.3 kb (from the −3' end of the UCP3 gene) to +12 bp from the transcription start site of the UCP2 gene. Both basal and oleate-stimulated activity of the UCP2 promoter in INS-1 cells was completely unaffected by deletions from −7.3 kb to −86 bp (Fig. 2). However, the deletion of the region between positions −86 and −44 nucleotides reduced promoter activity to 3% and abolished stimulation by oleic acid, indicating that this region is responsible for the...
majority of the basal expression as well as the increase of UCP2 gene transcription by oleic acid.

Three Elements in the UCP2 Enhancer, Sp1, SRE, and Tandem E-Boxes, Control Basal Expression, but Only the SRE and E-Boxes Confer Regulation by FFA—In an earlier series of experiments examining UCP2 expression in adipocytes, we described an enhancer region (~86/~44; shown schematically in Table I) in the UCP2 promoter consisting of Sp1, SRE, and double E-Box elements (12). Since the data in Fig. 2 indicate that this region is also important for both basal and fatty acid-stimulated UCP2 gene transcription in INS-1 β-cells, we sought to define the relative contribution of these three sequence motifs to the regulation of UCP2 using a series of mutations (Table I). As shown in the last column of Table I, mutation of the Sp1 element (point mutation “Sp1m”) reduced basal activity of the UCP2 promoter by 71%, whereas a point mutation of the SRE (SREm) reduced basal promoter activity only by 40%. Mutations of the E-Boxes also reduced activity by 55.5 and by 77.4%. Deletion of all the elements up to E-Box2 (to position ~58) reduced UCP2 promoter activity by over 96.6%, to the level of the minimal promoter (~44). Together these data indicate that all three sequence motifs (Sp1, SRE, and double E-Box) contribute to the basal activity of the UCP2 promoter in INS-1(832/13) cells.

We next investigated which of the three motifs in the enhancer are required to mediate the response to 48 h treatment with 0.5 mM oleic acid. In transient transfection experiments in INS-1 cells, mutation of the Sp1 element (Sp1m) did not alter the response of the UCP2 promoter to oleic acid (Fig. 3). However, mutation of either the SRE (SREm) or the distal (EBm1) or proximal (EBm2) parts of the double E-Box element completely eliminated the response of the UCP2 promoter to oleic acid, suggesting that both elements are required for this response. While there was a slight tendency for some residual FFA response in EBm2, it was not significant.

Transcription Factors SREBP1, USF1, and USF2 Bind to the UCP2 Enhancer in INS-1 Nuclear Extracts—To identify transcription factors in INS-1 cells capable of binding the functionally important SRE and double E-Box elements of the UCP2 enhancer, we performed gel shift experiments using nuclear extracts from INS-1 cells and antibodies directed against the candidate transcription factors. Sterol regulatory element-binding protein (SREBP) family transcription factors bind to SREs (20, 21), and at least one member (SREBP1c) has been shown to be expressed in pancreatic β-cells (22). While multiple transcription factors are capable of interacting with the E-Box elements, including SREBP (23), upstream stimulatory factors (USFs) have been reported to be major components of the complex binding to the E-Box element of the fatty acid synthase (FAS) promoter. This interaction has been proposed as important for insulin and glucose responsiveness of the FAS gene (24–26). Likewise, we previously showed that in vitro translated SREBP1c binds efficiently to the SRE, while similarly prepared USF1 and USF2 bind to the E-Boxes in the UCP2 enhancer (12). These findings are consistent with the idea that perhaps SREBP and USF families of transcription factors are involved in the regulation of UCP2. Therefore, using specific antibodies to the transcription factors SREBP1, SREBP2, USF1, and USF2, we tested whether these factors were present in INS-1 nuclear extracts and could specifically bind the elements of the UCP2 enhancer. The results presented in Fig. 4 indicate that SREBP1 (striped arrow) and both USF1 and USF2 (black arrow) were detected among the proteins bound to the UCP2 enhancer in these extracts. Other unidentified factors (gray arrow) were also present that were able to bind to the ~76/~44 region. Therefore, next we examined SREBPs and the USFs in functional assays for their ability to regulate UCP2 gene transcription in INS-1 pancreatic β-cells.

SREBP Isoforms Transactivate the UCP2 Promoter in INS-1 β-Cells—Kakuma reported that SREBP1c could be detected in β-cells (22). However, SREBP1 has two isoforms, SREBP1a and SREBP1c, which differ only in their first exons and promoters (27). The gel shift data in Fig. 4 also indicated the presence of SREBP1 (striped arrow) in INS-1(832/13) cells, but cannot distinguish between the isoforms. We used RT-PCR to specifically identify which of these isoforms were present in INS-1(832/13) cells. The results in Fig. 5A indicate that transcripts for both SREBP1 isoforms (1a and 1c) are expressed in INS-1 cells, while only SREBP1a is found in 3T3-L1 adipocytes (28). Western blotting was used to estimate the relative amounts of the membrane and nuclear forms of SREBP1 (Fig. 5B). In both control and oleic acid-treated INS-1 cells more than half of the SREBP1 protein existed in the active (cleaved) form of 68 kDa, and this form was localized to the nucleus.
Forty-eight hour treatment of INS-1 cells with oleic acid also did not significantly affect the cleavage of SREBP1. Although SREBP1 levels appeared to be slightly elevated in oleate-treated cells, this was not significant in several experiments.

Next, we tested the effects of the nuclear forms of SREBP1a, SREBP1c/ADD1, and SREBP2 on the −7.3-kb UCP2 promoter construct in INS-1 cells. Both isoforms of SREBP1, as well as SREBP2, were able to stimulate UCP2 promoter activity (−7.3 kb/+12 bp) by 7–10-fold (Fig. 6A). In contrast, the strong binding of USFs to the E-Box elements shown in Fig. 4 did not result in equally strong transactivation. In fact, USF1 activated the UCP2 promoter very poorly (−1.8-fold), and USF2 had no effect at all. As shown in Fig. 6B, the strong transcriptional response of the −7.3-kb UCP2 promoter to SREBP1c was still present in the small −86/+12 fragment that contains only the −86/−44 enhancer and basal promoter regions. Perhaps not surprising, the point mutation of the SRE element within the enhancer (SREm) abolished the response to SREBP1c (Fig. 6B). But importantly, since this mutation still leaves the E-Boxes intact, these data strongly suggest that SREBPs activate the UCP2 promoter exclusively through the SRE. This result is also in agreement with our previous observation that, despite the known dual specificity of SREBPs for both elements (23), in vitro translated SREBP1c can bind to the SRE but not the E-Box elements of the UCP2 promoter (12).

**Functional Interactions between the SRE and Overlapping Double E-Box Elements Regulate the Response of the UCP2 Gene to FFA**—The results in Fig. 3 showed that both the SRE and double E-Box sequences were required for oleic acid to increase UCP2 gene expression. These findings are consistent with studies of other genes, in which the SRE often requires other transcriptional elements such as nuclear factor-Y (NF-Y) and Sp1 in close proximity to properly regulate gene expression (reviewed in Ref. 29). Therefore, to investigate whether the Sp1 or E-Box elements in the −86/−44 enhancer are important for SREBP1-dependent transactivation, we examined the ability of various UCP2 enhancer mutants (Table I) to respond to SREBP1c in co-transfection experiments. As shown in Fig. 7, mutation of the Sp1 element (Sp1m) had no effect on the response of the UCP2 promoter to SREBP1c. However, mutations of either the distal (EBm1) or the proximal (EBm2) E-Boxes actually led to a significant increase of UCP2 enhancer activity by SREBP1c. These data suggest that UCP2 expression might be suppressed through the double E-Box element, potentially due to its overlap with the SRE. Recall that the results in Figs. 4 and 6A showed that USF1 and USF2 transcription factors in INS-1 nuclear extract can bind efficiently to
fuel regulation of UCP2 expression in β-cells

Fig. 7. Effect of SREBP1c overexpression on the activity of the −86/−44 enhancer mutants. UCP2 mutant constructs (described in Table I) were co-transfected with vector expressing nuclear form of SREBP1c/ADD1 (aa 1–403) in INS-1(832/13) cells. A, schematic presentation of the mutants used for transfection. B, transfection data. *, significantly different from basal activity; p < 0.001. #, significantly different from −86 stimulated by SREBP1c by one-way analysis of variance and a post-hoc Newman-Keuls test; p < 0.001.

Fig. 8. Competitive interaction between SREBP1c and USF1 in regulation of the UCP2 enhancer. UCP2 reporter constructs were co-transfected with 0.2 µg of SREBP1c expression vector and increasing amount of USF1 (0.2–1.0 µg) as described under "Experimental Procedures." *, significantly different from SREBP1c alone, p < 0.01; **, significantly different from SREBP1c alone, p < 0.001. A, −86 (−86/+12) construct. Values for USF1 (0.2 µg) and USF1 (0.4 µg) are also significantly different from each other, p < 0.05. B, SRE mutant construct (SREm, Table I).

Fig. 9. Overexpression of SREBP1c restores response of the E-Box mutant to oleic acid. INS-1(832/13) cells were co-transfected with −86 or SREm, EBM1 mutant constructs (Table I), and vector expressing active form of SREBP1c (aa 1–403) as described under "Experimental Procedures" and cultured for 48 h in the RPMI medium with 4% FCS and 1% fat-free BSA in the presence or absence of 0.5 mM oleate bound to BSA (3:1). All lanes contain SREBP1c. *, significantly different from −86 +SREBP1c (lane 1), p < 0.001. #, significantly different from EBM1 + SREBP1c (lane 5), p < 0.001.

Since the original isolation of the UCP2 gene and linkage of its chromosomal position with hyperinsulinemia (1), it has been proposed that this protein may play a role in maintaining cell energy homeostasis. Based on this hypothesis a number of studies linked changes in UCP2 expression to obesity and

the double E-Box element, but they activate the UCP2 enhancer very poorly (USF1) or not at all (USF2). These findings together led us to investigate whether USFs act as competitive suppressors of SREBP1-dependent activation of the UCP2 gene. For these experiments the UCP2 enhancer construct (−86/+12) was co-transfected with expression plasmids for SREBP1c (0.2 µg/well) together with increasing amount of USF1. At low concentrations (0.2–0.6 µg/well), USF1 had a significant negative impact on SREBP1c-stimulated UCP2 gene transcription (Fig. 8A). However, at higher concentrations of USF1 (1.0 µg/well) UCP2 promoter activity returned to the level similar to that seen with SREBP1c alone. When the SRE mutant construct (SREm), in which the E-Boxes are still intact, was examined in the same experimental design, UCP2 expression increased linearly as a function of the amount of co-transfected USF1 (Fig. 8B). These data demonstrate that USFs can competitively interfere with the activity of SREBPs, and in the absence of SREBPs they act as weak activators.

Having now demonstrated, first, that increased transcription of the UCP2 gene in INS-1 cells by oleic acid requires both the SRE and E-Box elements of the enhancer and, second, an important role for SREBP1 to regulate the UCP2 gene, we tested whether overexpression of the SREBP1c would affect the response of UCP2 to oleic acid. Fig. 9 shows that 48 h of oleic acid treatment, together with overexpression of SREBP1c, leads to a further augmentation of UCP2 promoter activity beyond the effect of either one alone. Surprisingly, our data indicate that the response of the E-Box mutant to oleic acid could be restored by overexpression of the nuclear form of SREBP1c, while the SRE mutant was inactive (Fig. 9). These data support the existence of functional synergism between the SRE and the E-Box elements. This suggests that the E-Boxes play a supportive role in response of UCP2 to FFA and may be necessary to modulate the activity or binding of SREBPs.

Discussion

Since the original isolation of the UCP2 gene and linkage of its chromosomal position with hyperinsulinemia (1), it has been proposed that this protein may play a role in maintaining cell energy homeostasis. Based on this hypothesis a number of studies linked changes in UCP2 expression to obesity and
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More recently UCP2 has been implicated in the regulation of β-cell insulin secretion (reviewed in Ref. 31). In view of the well-established observation that chronic exposure of β-cells to FFA dampens glucose-stimulated insulin secretion, this effect could depend upon FFA-dependent increases in UCP2 expression. However, the mechanisms regulating UCP2 in β-cells are still not clear. In this study we show that the effect of FFA on UCP2 occurs at the level of transcriptional regulation and that SREBP plays a major role in modulating UCP2 gene transcription by oleic acid. In contrast to the substantial evidence for negative regulation of SREBPs in liver by fatty acids during fasting (reviewed by Horton et al. (32)), Kakuma et al. (29) showed that the SREBP1c isoform was specifically increased in islets of obese, prediabetic (fa/fa) ZDF rats compared with lean (+/−) ZDF rats and was accompanied by increases in transcripts for several lipogenic enzymes that are target genes of SREBP1c. The mechanisms underlying this regulation of SREBP by FFA are unknown. However, a PARP-dependent pathway is one of the likely candidates, since at least certain fatty acids can act as natural ligands for these transcription factors (33), and we have found that PARPγ increases expression of UCP2 indirectly via the −66/−44 enhancer (12). In support of this hypothesis, overexpression of PARPs in INS-1 cells could increase UCP2 mRNA and protein levels (34).

The cooperation between the SRE and E-Boxes for regulation of UCP2 transcription by oleic acid could depend on E-box binding transcription factors functioning as co-regulators of SREBP1. There is a precedent for this idea, since it is known that SREBPs often require co-regulatory factors such as Sp1 and NF-Y to efficiently activate gene expression (20, 35–37). However, at present it is not clear which of the many possible E-box binding factors are involved in regulating the UCP2 gene, and therefore we cannot propose a mechanism. In our studies we used the E-Box binding factors USF1 and USF2 because they are abundant in INS-1 nuclear extracts, and in studies we used the E-Box binding factors USF1 and USF2.

E-Box binding factors are involved in regulating the UCP2 gene. However, at present it is not clear which of the many possible E-box binding factors are involved in regulating the UCP2 gene, and therefore we cannot propose a mechanism. In our studies we used the E-Box binding factors USF1 and USF2 because they are abundant in INS-1 nuclear extracts, and in several models they have been implicated in the control of genes involved in metabolic fuel regulation. For example, the induction of the FAS gene in liver by refeeding was severely delayed in the USF1−/− mice, while expression of SREBP1 was nearly normal (26). However, USFs are not the most likely candidate endogenous factors regulating UCP2, because our data demonstrate that USFs actually function as competitive suppressors of SREBP1c, at least in co-transfection experiments. Another interesting candidate that was the recently described (but not yet cloned) is the “carbohydrate response factor” (ChoRF), which Towle and others (38, 39) have proposed as a glucose-related transcriptional activator of the S14 and FAS genes through their E-Box motifs.

In summary, these results together with our previous observations (12) establish that nutritional regulation of UCP2 gene expression results from the coordinated action of several transcription factors, particularly SREBP- and E-Box-binding proteins. Future work to establish the identity of these E-box factors and the metabolic pathways that link fuel metabolism with UCP2 should allow us to better understand the function of UCP2 not only in β-cells, but perhaps in metabolic active tissues.

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REFERENCES

1. Fleury, C., Neverova, M., Collins, S., Rainbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. D., Surwit, R. S., Riequier, D., and Warden, C. H. (1997) Nature Genetics 15, 269–272
2. Dullo, A. G., and Samec, S. (2001) Br. J. Nutr. 86, 123–139
3. Boss, O., Samec, S., Dullo, A., Seydoux, J., Muzin, P., and Giacobino, J. (1997) FEBS Lett. 412, 111–114
4. Millet, L., Vital, H., Andreelli, F., Loury, R., Rio, J., Riequier, D., Laville, M., and Langin, D. (1997) J. Clin. Invest. 100, 2665–2670
5. Samec, S., Seydoux, J., and Dullo, A. G. (1998) FASEB J. 12, 715–724
6. Lemaellou, N., Muzin, P., Prentki, M., and Assimacopoulou-Jeannet, F. (2001) J. Biol. Chem. 276, 805–810
7. Li, L. X., Skorpen, F., Egeberg, K., Jorgensen, I. H., and Grill, V. (2002) Endocrinology 143, 1371–1377
8. Tontonoz, P., Kim, J. B., Graves, R. A., and Spiegelman, B. M. (1993) Mol. Cell. Biol. 13, 4753–4759
9. Kakuma, T., Lee, Y., Higa, M., Wang, Z., Pan, W., Shimomura, I., and Unger, R. H. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 8306–8311
10. Kim, J. B., Spotts, G. D., Halverson, Y. D., Shih, H. M., Ellenberger, T., Towe, H. C., and Spiegelman, B. M. (1995) Mol. Cell. Biol. 15, 2582–2588
11. Shulman, G. I., Freychet, P. C., and Cherr, J. B. (1999) J. Biol. Chem. 274, 5777–5782
12. Chan, C. B., De Leo, D., Joseph, J. W., McQuaid, T. S., Ha, F. X., Fu, Y., Tsuchima, R. G., Penfather, P. S., Salapatek, A. M., and Wheeler, M. B. (2001) Diabetes 50, 1392–1397
13. Mcgarry, J. D., and Dobbins, R. L. (1999) Diabetologia 42, 128–138
14. Medvedev, A. V., Snedden, S. K., Rainbault, S., Riequier, D., and Collins, S. (2001) J. Biol. Chem. 276, 10817–10823
15. Sullivan, H. S., and Dalla man S. A. (2000) Ann. Rev. Nutr. 18, 331–351
16. Vaulont, S., Vasseur-Cognet, M., and Kahn, A. (2000) J. Biol. Chem. 275, 31355–31359
17. Hohmeyer, H. E., Muller, H., Chen, C., Henkel-Rieger, R., Prentki, M., and Newgard, C. B. (2000) Diabetes 49, 424–430
18. Soeder, K. S., Snedden, S. K., Cao, W., Della Rocca, G. J., Daniel, K. W., Luttrell, L. M., and Collins, S. (1999) J. Biol. Chem. 274, 12017–12022
19. Thomas, P. S. (1990) Proc. Natl. Acad. Sci. U. S. A. 77, 5201–5205
20. Collins, S., Daniel, K. W., Kohli, E. M., Ramakumar, V., Taylor, I. L., and Gettys, T. W. (1994) Mol. Endocrinol. 8, 518–527
21. Dignam, J. D., Lebovitz, R. M., and Roeder, R. G. (1983) Nucleic Acids Res. 11, 1475–1489
22. Briggs, M. R., Yokoyama, C., Wang, X., Brown, M. S., and Goldstein, J. L. (1993) J. Biol. Chem. 268, 14490–14496
23. Tontonoz, P., Kim, J. B., Graves, R. A., and Spiegelman, B. M. (1993) Mol. Cell. Biol. 13, 4753–4759
24. Take, T., Kato, H., and Tsushima, R. G. (1997) J. Lipid Res. 38, 21445–21448
25. Kato, H., and Take, T. (1998) J. Lipid Res. 39, 767–776
26. Kato, H., and Take, T. (2000) J. Biol. Chem. 275, 5200–5207
27. Kato, H., and Take, T. (2001) J. Biol. Chem. 276, 21969–21975
28. Kato, H., and Take, T. (2002) J. Biol. Chem. 276, 8705–8712

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