Genomic organization and promoter function of the mouse uncoupling protein 2 (UCP2) gene

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Abstract We cloned and characterized the mouse uncoupling protein 2 (UCP2) gene and its promoter region. The gene spans approximately 6.3 kb and contains eight exons and seven introns. Two short exons are located in the 5' untranslated region, and each of the remaining exons encodes one of the transmembrane domains. 3'-RACE analysis showed that a polyadenylation signal 257 bp downstream from the stop codon was functional. Primer extension analysis indicated a single transcriptional start site 369 bp upstream from the translational start site. The promoter region lacks both TATA and CAAT boxes but is GCrich. A construct containing 1250 bp of the promoter region showed significant activity in all 6 cell lines examined, and the region between -160 and -678 bp exhibited strong positive regulatory activity. These features of the UCP2 gene are different from those of the UCP1 gene and may contribute to its ubiquitous expression.

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Key words: Uncoupling protein 2; Gene; Promoter

1. Introduction

Mitochondrial uncoupling protein 1 (previously UCP) is exclusively expressed in the brown adipose tissue, which is a thermogenic organ of mammals [1–4]. UCP1 uncouples mitochondrial respiration from ATP production by inducing the exothermic movement of protons from outside to inside the inner mitochondrial membrane and reducing their concentration gradients. Therefore, activation of UCP1 increases consumption of calories and generation of heat [5]. These UCP1-mediated effects on energy expenditure are regulated mostly by changes in the level of sympathetic nervous system activity [6].

Recently, genes encoding novel members of the UCP family (UCP2 and 3) have been cloned [7–10]. Although these proteins show a high degree of homology to UCP1 (59 and 57%), UCP3 mRNA is expressed predominantly in skeletal muscle and UCP2 mRNA was detected in many tissues in humans and rodents. The ubiquitous expression of the UCP2 gene suggested that this molecule may be important for basal metabolic rate and regulation of body weight in mammals.

The partial genomic structure of the mouse and human UCP2 have recently been reported [11,12]. However, the complete structure including the transcriptional start site, number

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of introns and functional polyadenylation signals etc. has not been reported. In the present study, we established the complete structure of the mouse UCP2 gene and characterized its promoter region.

2. Materials and methods

2.1. Isolation of mouse uncoupling protein 2 genomic clones

A C57Bl/6×CBA mouse spleen genomic DNA library (Lambda FIX II; Stratagene, USA) was used in this study. Approximately 1×106 recombinants were screened using a ³²P-labeled mouse UCP2 cDNA (1-UCP2). The 1-UCP2 cDNA was generated by polymerase chain reaction (PCR) using the primers U1, 5'-catcttctgggaggtagcaggaaat-3', and U2, 5'-tttaccacatctgtaggctgggcta-3', with mouse skeletal muscle cDNA as a template. Filter hybridization was performed using the previously described method [13,14]. Two genomic clones were isolated and characterized by restriction endonuclease mapping. The restriction digests were subjected to electrophoresis, transferred onto a nylon membrane, and hybridized with the above probe. All hybridized genomic fragments were subcloned into pGEM4Z or 3Z (Promega, USA) for further restriction analysis and sequenced using a Sequenase 2.0 (Amersham, USA) or SequiTherm Long-Read Cycle sequencing kit (Epicenter Technologies, USA) and a LI-COR model 4000 sequencer (LI-COR, USA).

2.2. Reverse transcription-polymerase chain reaction (RT-PCR) and 5' rapid amplification of cDNA end

PCR was used to confirm the sizes of introns and exon-intron boundaries. Aliquots of 3 µg of total RNA from the skeletal muscle were subjected to reverse transcription for 2 h at 37°C. PCR amplification of cDNA was performed as previously described [14]. Several primers were designed to span each exon/exon boundary (not shown). Amplification products were gel purified, subcloned into the pGEMT-Easy plasmid (Promega, USA) and sequenced.

To obtain the 5' portion of the mouse UCP2 cDNA, 5'-RACE (rapid amplification of cDNA ends) was performed using a mouse skeletal muscle Marathon Ready cDNA (Clontech, USA).

2.3. Primer extension analysis

Primer extension was carried out using the synthetic oligonucleotide PE1, 5'-gagacgaaacacttaaagttcatac-3', corresponding to a sequence located in exon 2. The oligonucleotides were end-labeled with

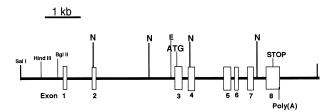


Fig. 1. Schematic representation of the mouse UCP2 gene. *NcoI* (N), *EcoRI* (E), *SalI*, *BglII* and *HindIII* restriction sites are indicated (*SalI*, *BglIII* and *HindIII* are not unique sites, and *SalI* is located in the multiple cloning site of the phage). Boxes denote exons, while thin lines denote introns and flanking regions. ATG represents the translational initiation site; STOP, stop codon; poly(A), polyadenylation signal.

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ACAGGGCTTCTCCCAATGTTGCCCGTAATGCCATTGTGAACTG1GC1GAACTG1GG1GACC1A1GAC G T S P N V A R N A I V N C A E L V T Y D ${\tt CTCATCAAAGATACTCTCCTGAAAGCCAACCTCATGACAGgtgtgttagcgccacttcaagctggt}$ +964 -212 tcaqtctqcctqtcctcacaaagagattqtctgccccgggaatagaaggggaagatctgagactgg AP1 GRE -146 agcctctggggggacctaggggtttcaggcaaccacagatggaaacgcctcctca<u>cccggggggcgt</u> AP2 Sp1 gggccgccctggctccgcctcgtcacgccacgccccgaccacgccctctagacttaagttctgtag CREB Sp1 Sp1 AP2 Sp1 exon 1 gcgcgggttcgcagCTACTGTCAGTTCCGCCCTCGGTGTCGTCGGGTCGCCAGCTTCCTGGGCAGC -14 ${\tt CACCACTCTCCCGCCGTCGGACACAGCCTTCTGCACTCCTGTGTTCTCCTGCGGTCCGgtgaqcqt}$ +53 qccccatcccttcgctggccgcacctgctctctcccactccatctgtcttttctcttttccc $\verb|ttccctggagtcctaatttgccctctctaggtttctttcctcctaaatacgccctttgttatttgc|\\$ ctctgacgt--(1st intron,~740bp)--tgagaaacaaatttggaagtgagccaacgtgtgg exon 2 +122 ATGAACTTTAAGTGTTTCGTCTCCCAGCCATTTTCTATGGAAAATCGAGGGGATCGGGCCATGGTA GCCACCGGCAGCTTTGAAGAACGAGACACCTTTAGAGAAGCTTGACCTTGGAGGCCTCAGCCTGAG ACCTCAAAGCAGCCTCCAGgtaaggagtcccggctaaggaagccttctctcaggcctggggcctag +254 ggctgctcagcaagacaataaaaattcccctcctcccaccctgatgagagcctcttca--(2nd intron, ~2.4kb) -- ctataccaaataagagaggagttcttcactgaatctcagttccttggcag exon 3 tqctqatactqatqqcttctcccctcttgqcctagAACTCCGGCAGAGTTCCTCTGTCTCTTTG CCGATTGAAGGTCCCCGTTTCTCCAATTTCTCTCCATCTTCTGGGAGGTAGCAGGAAATCAGAATC +304 +370M V G F K A T D V P P T A T V K F L G A G T GCTGCCTGCATTGCAGATCTCATCACTTTCCCTCTGGATACCGCCAAGGTCCGGCTGCAGgtgagg +436 A A C I A D L I T F P L D T A K V R L Q atqqaqtqaqqqtcctqatqctqtctaatctgctccctccctgggtcagactccttggcagggatg aggagcctggagctggcgggagaccagcagagccatctctctgtgctcacaaccaccccatcttgg exon 4 I Q G E S Q G L V R T A A S A Q Y R G $\tt TTCTGGGTACCATCCTAACCATGGTGCGCACTGAGGGTCCACGCAGCCTCTACAATGGGCTGGTCG$ V L G T I L T M V R T E G P R S L Y N G L V $\tt CCGGCCTGCAGCGCCAGATGAGCTTTGCCTCCGTCCGCATTGGCCTCTACGACTCTGTCAAACAGT$ +620 AGLQRQMSFASVRIGLYDSVKQ $+686 \quad {\tt TCTACACCAAGGGCTCAGAGCgttgagtattgcagcaaggacagacttgactttagtcttgtctgt}$ F Y T K G S E gcagctcagtggctcctttagacttttcaagaaccccgggaagatcagaggtggaccctgatttct gttatctgagcaccagggctgtctccaagcagggtgtgagcccatg--(4 th intron ~760 qcatqatqcacttgagtgcaagcctgcgggccactaaccccgccccacccctcttgcccacagAT exon 5 GCAGGCATCGGGAGCCGCCTCCTGGCAGGTAGCACCACAGGTGCCCTGGCCGTGGCTGTAGCCCAG A G I G S R L L A G S T T G A L A V A V +775 P T D V V K V R F Q A Q A R A G G G R R Y Q AGCACTGTCGAAGCCTACAAGACCATTGCACGAGAGGAAGGGATCCGGGGCCTCTGGAAAGgtgtg +841 S T V E A Y K T I A R E E G I R G L W K tgctgggtgttggcttttctcccctcctctcctccatgccctgggctcacacaggttctcttttctexon 6

Fig. 2. Nucleotide sequence of the mouse UCP2 gene. Exons are shown in upper case letters. Introns and 5' and 3' untranslated region are indicated in lower case letters. Proposed transcriptional initiation site is shown with an asterisk and is numbered +1. The ATTTA pentamer motif in the 3' untranslated region is underlined. The polyadenylation signal (ATAAA) is shown in bold type and the polyadenylation site is indicated with an asterisk. Putative binding sites for Sp1, AP1, AP2, glucocorticoid response element (GRE) and CREB are underlined and indicated.

 ${\tt agggggggtctagttgtctaaaagactgcatcttgttatcctgcttgcctcgtggtttttaagagttt}$ atatttcttaccattcaaatccaacagggtaaaaaccccactttgcacataaacaggcgagatcaa $\verb|tctgcggcaatggtgggatgcggctgggagagctgacagcctctccccctgctcatggcagATGAC|$ exon 7 +1009 CTCCCTTGCCACTTCACTTCTGCCTTCGGGGCCGGCTTCTGCACCACCGTCATCGCCTCCCTGTT L P C H F T S A F G A G F C T T V I A S P V +1075 GATGTGGTCAAGACGAGATACATGAACTCTGCCTTGGGCCAGTACCACAGCGCAGGTCACTGTGCC D V V K T R Y M N S A L G Q Y H S A G H C A +1141 CTTACCATGCTCCGGAAGGAGGGACCCCGCGCCTTCTACAAGGGgtgagootcagatccccctcac LTMLRKEGPRAFYK agaactccagagaagtgagatgcagccaaaatgtcagacagcccatagttgctgtcacctctgttt tttagaagttaaaatgagcattcagactgggggttggggatgggtcagtggtggagggcttg cttagcatgcttggagaccctgggttctgctccccagcacagcaggagtagggagctgggtactcg $\tt ggattcttgggcatgtggctgggtggatggatgagcccaattgttagaaacaggtggtg$ exon 8 gagggacctccatgacttgtgtttgctctctctagGTTCATGCCTTCCTTTCTCCGCTTGGGATCC G F M P S F L R L G S +1216 TGGAACATAGTGATGTTTGTCACCTATGAGCAGCTCAAAAGAGCCCTAATGGCTGCCTACCAATCT WNVVMFVTYEQLKRALMAAYQS +1282 CGGGAGGCACCTTTCTGAgcctctccatgctgacctggacctgcttcccagccctgccctgtctt REAPF +1348 tttcttcatcctctqcccaqtcccattctcttcccatttcctqcaccccqatttacttcccacctc +1414 acctcctqtqcctctqtactqatqactcacaqtqaqqaqqcctgacaccagaccctgagccctca +1480 gccctttctacagctaagcccacatcttcatcttcatccccagcccagcccagcccagctcagcca

Fig. 2 (continued).

 $|\gamma^{-32}P|$ ATP, hybridized to 5 µg of poly(A)⁺ RNA extracted from the mouse skeletal muscle and extended using AMV reverse transcriptase. Forty micrograms of yeast tRNA were used as a negative control. The primer-extended products were separated on an 8 M urea 6% polyacrylamide gel which was then dried and exposed to Kodak XAR-5 film. The sizes of the resulting labeled primer-extended products were inferred from their positions relative to ϕ x174 RF DNA digested with *Hae*III and a sequencing ladder which was obtained using the same primer (PE1) and a genomic clone containing the region upstream of exon 2 as a template.

2.4. Analysis of 3' untranslated region by 3' rapid amplification of cDNA ends (3'-RACE)

To detect functional polyadenylation signals in the 3' untranslated region of the mouse UCP2 gene, 3'-RACE was performed as described previously [15]. Briefly, the first strand was synthesized with 5 μg of total RNA from the mouse skeletal muscle and an oligo(dT)₁₇+ adapter primer (5'-gactcctgcagacatcgatttttttttttttttt-3'). The first amplification was performed between the adapter (5'-gactcctgcagacatcga-3') (25 pmol of each primer) and a sequence-specific primer (RE1, 5'-tcaaacagttctacaccaagggctc-3'). The second of PCR was performed using 1 μl of the first PCR product with adapter and a downstream internal primer (RE2, 5'-ctgagctggt-gacctatgacctcat-3'). The PCR products were analyzed electrophoretically using 1.5% agarose gels and subjected to Southern blot analysis. Positive fragments were subcloned and sequenced.

2.5. Cell culture and transfection

L6, 3T3-L1, NIH-3T3, CV-1, GH_4C_1 and HeLa cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS). Cells were plated 24 h before transfection into 60-mm tissue culture dishes at subconfluent density. Transient transfection was performed by the calcium phosphate precipitation method with 3 μ g of reporter construct. The cells were then harvested after a further 48 h.

2.6. Plasmid construction

The pGL3 basic vector (Promega, USA) is a promoterless luciferase expression vector. The pGL3 control vector contains an SV40 promoter and enhancer sequence linked to pGL3 basic and was used to monitor transfection efficiency in each cell line and as an internal

standard between different experiments. The mouse UCP2 gene SalI-NcoI fragment containing 1250 bp of the promoter region, 110 bp of exon 1, approximately 760 bp of the first intron and 87 bp of the second exon, was subcloned into the pGL3 basic vector and named p1.2Luc. The p0.7Luc and p0.2Luc constructs containing 678 bp and 160 bp of the promoter region, respectively, were generated by deletion of the SalI-HindIII and Sal-BglII fragments from p1.2Luc.

2.7. Luciferase assay

Assays for luciferase activity were performed using 10-µl aliquots from 400 µl cell lysate and 350 µl of 25 mM glycylglycine buffer (pH 7.8) containing 15 mM MgSO₄, 4 mM EGTA, 16 mM KPO₄, 1 mM dithiothreitol and 2 mM ATP. The reaction was initiated by addition of 200 µl of 0.2 mM p-luciferin and light emission was measured for 10 s using a luminometer. Luc activity was expressed in arbitrary light units (ALU) per microgram of cellular protein.

2.8. Statistical analysis

Statistical analysis was performed by ANOVA and Duncan's multiple range test.

3. Results

3.1. Genomic organization of the mouse UCP2 gene

Comparison of the genomic sequence with the cDNAs obtained by RT-PCR and 5'-RACE established the organization of the mouse UCP2 gene. The gene consists of eight exons and seven introns that are flanked by typical splice donor and acceptor sequences (Fig. 1). Two exons are interrupted by the approximately 760-bp intron 1 in the 5' untranslated region of the mouse UCP2 gene. With respect to the coding region, followed by intron 2 with approximately 2.4 kb, exon 3 started 97 bp upstream of the ATG translation initiation codon. The remaining exons encoded each of the putative transmembrane domains, and exon 8 also encoded the entire 3' untranslated region (Fig. 2).

3.2. Characterization of the 3' untranslated region

Sequence analysis of the 3'-RACE product indicated a single functional polyadenylation signal (ATAAA) 257 bp downstream of the stop codon in the mouse skeletal muscle, and the thymidine residue 19 bp downstream of the signal was the polyadenylation site (Fig. 3A). In addition, an ATTTA pentamer motif was observed 99 bp downstream of the stop codon which may affect mRNA stability (Fig. 2).

3.3. Promoter sequence

Primer extension with 5 µg of poly(A)⁺ RNA from mouse skeletal muscle and the PE1 primer gave a single strong signal at 369 bp from the translational initiation site, while no significant signals were found in yeast transfer RNA (Fig. 3B). Inspection of the sequence of the promoter region indicated that there was no typical TATA box or CAAT box in close proximity to the transcriptional start site. However, the promoter region was GC-rich and several possible regulatory elements were identified including complete sequence matches for Sp1, AP-2, AP-1, CREB, and MyoD binding sites, and the glucocorticoid response element (GRE) (Fig. 2).

3.4. Promoter function of the UCP2 gene in several cell lines

To determine whether the putative promoter region is functional, a SalI-NcoI fragment containing 1250 bp of the promoter region was subcloned into a luciferase reporter plasmid (p1.2Luc) and transfected into 6 different cell lines. As shown in Fig. 4A, significant expression was observed in all 6 cell lines examined. In all cells, the promoterless reporter plasmid (pGL3 basic) expressed luciferase at $\sim 0.3\%$ of the level of that driven by the SV40 promoter. Relative expression of p1.2Luc to that of the SV40 promoter was high in L6 and GH4C1 cells.

In L6 cells, deletion of the region between -1250 and -678 bp from the transcriptional start site led to a slight increase,

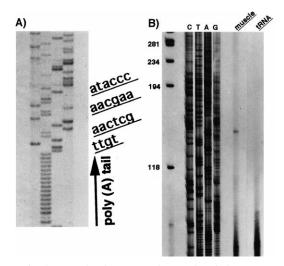
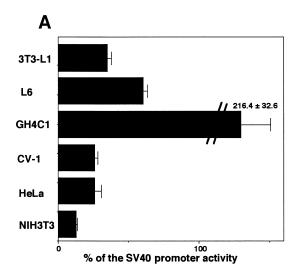


Fig. 3. 3'-RACE and primer extension study of the mouse UCP2 gene. A: Representative sequence data from the 3'-RACE study. B: Primer extension using the oligonucleotide PE1. The end-labeled primer was hybridized to 5 μg of poly(A) RNA from the mouse skeltal muscle (muscle) or yeast tRNA (tRNA) and extended with AMV reverse transcriptase. PE1 gave a positive signal at 369 bp from the translational initiation site. Marker lanes C, T, A, and G indicate sequencing ladder of the mouse UCP2 gene using the primer PE1. φx174 fragments digested with *Hae*III and sizes of fragments are indicated in bp on the left.



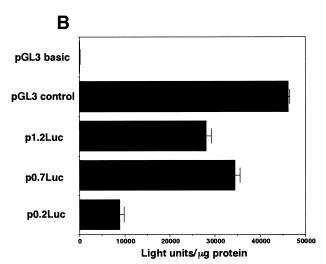


Fig. 4. Analysis of the mouse UCP2 gene promoter. A: 1250 bp of the UCP2 gene promoter was subcloned in front of the luciferase reporter gene (p1.2Luc), and transiently transfected into L6, 3T3-L1, GH4C1, HeLa, NIH3T3 and CV-1 cells. The data are expressed as relative luciferase activity (arbitrary light units/µg protein of the UCP2 promoter/that of the SV40 promoter). Values represent means ± S.E. of triplicate determinations. At least three independent experiments were performed. B: L6 cells were transiently transfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase activity was measfected with the indicated plasmids, and luciferase plasmid plasmids.

but deletion from -678 and -160 bp resulted in a significant decrease in promoter activity (Fig. 4B). These findings indicated a strong positive regulatory activity such as an enhancer in the region between -678 and -160 bp from the transcriptional start site.

4. Discussion

In the present study we established the complete structure of the mouse UCP2 gene which contains eight exons and seven introns and spans a region of 6.3 kb. Although the UCP1 gene contains no introns in the 5' untranslated region, the mouse UCP2 gene contains two exons interrupted by the approximately 740-bp intron 1 [15]. While the UCP1 gene

contains a typical TATA box in the promoter region, the UCP2 gene possesses no typical TATA or CAAT boxes in the appropriate positions. Sequence analysis of the UCP2 promoter region revealed several potential consensus sequences for transcriptional factors. Deletion analysis of the promoter region revealed that several putative SP1, CREB and AP2 binding sites may be important for the basic promoter activity. Furthermore, it is of interest that there were consensus binding sites for MyoD (a transcriptional regulator in myoblasts and skeletal muscle), GRE, AP1 and AP2 in the strong positive regulatory region. Further studies are required to determine whether these binding sites are functional.

We also examined the promoter activity of the UCP2 gene in several cell lines. L6 cells were derived from rat skeletal muscle, while 3T3-L1, CV-1, HeLa, NIH3T3, and GH4C1 cells were derived from rat preadipocytes, embryonic monkey kidney, human chorionic carcinoma, mouse embryonic fibroblasts, and rat pituitary tumor, respectively. Significant promoter activity was observed in all cell lines, and even NIH3T3 cells showing the lowest level of expression exhibited at least 10-fold higher expression (~7000 ALU/10 s/µg protein) than that of the promoterless construct (pGL3 basic). Unexpectedly, very strong promoter activity was observed in the pituitary-derived GH4C1 cells, and the level of expression was higher than that of muscle L6 cells. Therefore, UCP2 may play an important role in the pituitary.

UCP1 gene expression is tightly restricted to the thermogenic brown adipose tissue, and the promoter activity was low in cells derived from other tissues [15–17]. From the results of the present study, therefore, we speculated that the strong and housekeeping gene-like activity of the UCP2 gene promoter may contribute to the ubiquitous expression of the UCP2 gene.

References

- [1] Nicholls, D.G. and Locke, R.M. (1984) Physiol. Rev. 64, 1-64.
- [2] Jacobsson, A., Stadler, U., Glotzer, M.A. and Kozak, L.P. (1985) J. Biol. Chem. 260, 16250–16254.
- [3] Flier, J.S. and Lowell, B.B. (1997) Nat. Genet. 15, 223-224.
- [4] Bouillaud, F., Weissenbach, J. and Ricquier, D. (1986) J. Biol. Chem. 261, 1487–1490.
- [5] Himms-Hagen, J. (1989) Progr. Lipid Res. 28, 67-115.
- [6] Kozak, U.C., Held, W., Kreutter, D. and Kozak, L.P. (1992) Mol. Endocrinol. 6, 763–772.
- [7] Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M.F., Surwit, R.S., Ricquier, D. and Warden, C.H. (1997) Nat. Genet. 15, 269–272.
- [8] Gong, D.W., He, Y., Karas, M. and Reitman, M. (1997) J. Biol. Chem. 272, 24129–24132.
- [9] Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P. and Giacobino, J.P. (1997) FEBS Lett. 408, 39–42.
- [10] Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J.S. and Lowell, B.B. (1997) Biochem. Biophys. Res. Commun. 235, 79–82.
- [11] Argyropoulos, G., Brown, A.M., Peterson, R., Likes, C.E., Watoson, D.K. and Garvey, W.T. (1998) Diabetes 47, 685–687.
- [12] Surwit, R.S., Wang, S., Petro, A.E., Sanchis, D., Raimbault, S., Ricquier, D. and Collins, S. (1998) Proc. Natl. Acad. Sci. USA 95, 4061–4065.
- [13] Yamada, M., Radovick, S., Wondisford, F.E., Nakayama, Y., Weintraub, B.D. and Wilber, J.F. (1990) Mol. Endocrinol. 4, 551–556.
- [14] Yamada, M., Monden, T., Satoh, T., Satoh, N., Murakami, M., Iriuchijima, T., Kakegawa, T. and Mori, M. (1993) Biochem. Biophys. Res. Commun. 195, 737–745.
- [15] Kozak, L.P., Britton, J.H., Kozak, U.C. and Wells, J.M. (1988) J. Biol. Chem. 263, 12274–12277.
- [16] Cassard-Doulcier, A.M., Gelly, C., Fox, N., Schrementi, J., Raimbault, S., Klaus, S., Forest, C., Bouillaud, F. and Ricquier, D. (1993) Mol. Endocrinol. 7, 497–506.
- [17] Cassard-Doulcier, A.M., Larose, M., Matamala, J.C., Champigny, O., Bouillaud, F. and Ricquier, D. (1994) J. Biol. Chem. 269, 24335–24342.