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Transcriptional Regulation of Muscle-Specific Genes during Myoblast Differentiation

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A rapid, highly sensitive method to determine the mRNA level of muscle-specific markers using TaqMan PCR analysis was developed and used to study sequential gene regulation of myoblasts during induced differentiation of C_2C_{12} cells. mRNA levels of muscle regulatory factor (MRF) myogenin, α-actin, thermoregulatory uncoupling protein UCP2 and glucose transporter isotype glut4 increased rapidly during early stage differentiation. In contrast, myf5, β-actin, UCP1 and glut1 mRNA levels gradually decreased during 8 days. However, the mRNA level of other genes such as MyoD, glyceraldehyde-3-phosphate dehydrogenase and hexokinase II changed only slightly in comparison. Muscle-specific uncoupling protein UCP3 mRNA was detected during differentiation and increased rapidly within 8 days. These data clearly show the sequential and the differential regulation of muscle-specific genes in C_2C_{12} cells during multinucleate myotube formation.

Initiation of muscle differentiation is governed by the muscle regulatory factors (MRF), a family of gene products characterized by the basic helix-loop-helix (bHLH) domain: MyoD (1), myogenin (2), myf5 (3) and MRF4 (4). These four proteins are thought to control muscle-specific gene transcription by binding to a specific DNA sequence, called the E-box, after heterodimerization with ubiquitously expressed E2A gene products called "E" proteins (5). These bHLH proteins are also functionally down-regulated through a variety of mechanisms. One of these mechanisms involves heterodimerization with members of the Id family of HLH proteins (Id1 to Id4), which are characterized by the absence of a basic DNA-binding domain (6,7).

It is currently thought that combinatorial interaction between MRF family protein and E proteins leads to terminal myocyte differentiation (8) through the activation of muscle-specific genes including α-actin (9), hexokinase II (HKII) (10), glucose transporter isotype 1 (glut1) (11) and isotype 4 (glut4) (12). One of these proteins, uncoupling protein 3 (UCP3), is preferentially expressed in muscle tissue and has recently been the subject of investigation (13,14). UCP3 belongs to a family of uncoupling proteins such as UCP1 and UCP2. They are inner mitochondrial membrane transporters which dissipate the proton gradient, releasing stored energy as heat (15). However, if these genes were expressed in other tissues, physiologic disruption would surely result. Therefore, interesting questions now arises. How are the expression of the genes for these highly specialized proteins regulated during myocyte differentiation? Additionally, what elements comprise the mechanism of sequential regulatory networks for muscle-specific gene expression by MRF proteins?

In an effort to answer these questions, a rapid and highly sensitive method to determine mRNA levels of muscle-specific differentiation markers using TaqMan PCR analysis was developed. This technique was then used to study the responses of genes in vitro during differentiation of myoblast C2C12 cells.

MATERIALS AND METHODS

Materials. Mouse muscle myoblast cell line C2C12 was purchased from American Type Culture Collection (ATCC CRL 1772, Rockville, MD). Dulbecco's modified Eagle's medium (DMEM) and fetal calf serum (FCS) were obtained from GIBCO BRL (Tokyo, Japan). Horse serum (HS) was from J R Scientific Inc. (Woodland, CA). The TA cloning kit was purchased from Invitrogen (San Diego, CA). Advantage RT for PCR kit was from Clontech Laboratories Inc. (Palo Alto, CA). The TaqMan probe was purchased from Genset K. K.

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Abbreviations: FAM, 6-carboxy-fluorescein; FCS, fetal calf serum; TAMRA, 6-carboxy-tetramethyl-rhodamine; Glut, glucose transporter isotype; G3PDH, glyceraldehyde-3-phosphate dehydrogenase; HKII, hexokinase II; HS, horse serum; MRF, muscle regulatory factors; PCR, polymerase chain reaction; RT, reverse transcription; UCP, uncoupling protein.

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(Tokyo, Japan). The TaqMan EZ RT-PCR Core Reagents was from Perkin-Elmer Applied Biosystems (Tokyo, Japan). Isogen was purchased from Nippon Gene (Toyama, Japan). All other reagents were commercially obtained from standard sources.

Cell culture. C_2C_{12} cells were maintained in DMEM supplemented with 10% FCS in humidified 95% air and 5% CO_2. After plating at a density of 10^5 cells per well in 6-well plates, the cells were incubated for 3 days at 37 in the same medium. As the cells approached confluency (day 0), the medium was replaced with fresh medium supplemented with 2% HS to begin differentiation. The incubation then continued for the specified time (days 1-8). At each time point, the medium was removed by aspiration and the cells were washed with phosphate-buffered saline. Total RNA was isolated using 1 ml of Isogen according to the manufacturer's protocol. Total RNA samples were dissolved in diethylpyrocarbonate-treated water and their concentrations determined by A_{260} and A_{280} (A_{260}/A_{280} = 1.7-1.9) measurements using a Hitachi spectrophotometer U-2000 (Tokyo, Japan).

Determination of mRNA level using the ABI PRISM 7700 System. Oligonucleotide primers and TaqMan probes were designed using Primer Express, version 1.0 (Perkin-Elmer Applied Biosystems Inc.) from the GenBank database as follows: MyoD (Accession# M84918) (16), Myogenin (X15784) (17), Myf5 (X56182) (18), α-actin (X03766) (19), β-actin (X03672) (20), Glut1 (M23384) (21), Glut4 (M23383) (21), HKII (Y11666), UCP1 (U63419) (22) and G3PDH (M32599) (23) (Table 1). The TaqMan probe consists of an oligonucleotide with a 5′-reporter dye and a downstream, 3′-quencher dye. The fluorescent reporter dye, FAM (6-carboxy-fluorescein) is covalently linked to the 5′-end of the oligonucleotide. This reporter dye is quenched by TAMRA (6-carboxy-tetramethyl-rhodamine) typically located at the 3′-end. Fluorescence quenching depends on the spatial proximity of the reporter and quencher dyes. RT-PCR reaction was carried out in 96 sample tubes per assay (25 μl per tube) in a reaction buffer containing 1× TaqMan EZ buffer, 3 mM Mn(OAc)2, 300 μM dA/dC/dG/dUTP, 2.5 unit rTth DNA polymerase, 200 nM primers (forward and reverse), 100 nM TaqMan probe, 0.8-500 ng total RNA. RT reaction conditions were 55°C for 50 min, 60°C for 10 min, 95°C for 2 min for 1 cycle; PCR conditions were 95°C for 15 sec, 58°C for 1.5 min for 40 cycles on an ABI PRISM 7700 Sequence Detector (Perkin-Elmer Applied Biosystems). Each time point was determined as an average from data obtained from n = 8. Linearity of the standard curve for target gene mRNAs (except UCP1 and UCP3) was obtained from a range of 4-500 ng total RNA obtained from C_2C_{12} cells on day 3. In contrast, the linearity of the standard curves for UCP1 and UCP3 mRNAs were obtained from a 20-500 ng total RNA range for UCP1, and a 0.8-500 ng total RNA range for UCP3, using a standard sample prepared from mouse hind limb muscle. Target mRNA content in 96 samples including the linear standard samples was measured simultaneously in one assay run according to the manufacturer's protocol (24). To verify whether the amplification product was a target gene, the amplified RT-PCR products of one sample were sequenced.

A partial cloning of mouse UCP3. An attempt was made to clone the mouse UCP3 cDNA. Total RNA obtained from mouse skeletal muscle was used to prepare a cDNA template using the Advantage RT for PCR kit (Clontech). A male C57/BL mouse at 10 weeks-age was sacrificed, its hind limb skeletal muscles (gastrocnemius) was excised and the total RNA was extracted immediately with Isogen (Nippon gene). Primers (19 mers) for cloning of mouse UCP3 cDNA selected from the consensus sequences between human UCP3 (GenBank Accession# AF001787) (13) and mouse UCP2 (U69135) (25) cDNA sequences were determined. The two oligonulceotides (Forward: 5′-TGCAGCGCCAGATGAGCTT-3′ and Reverse: 5′-TTGGGC-AAAGTTCCTTTCCA-3′) were synthesized using an Oligo 1000M DNA synthesizer (Beckman Instruments Inc., Fullerton, CA). PCR conditions were 95°C for 9 min, 95°C for 20 sec, 58°C for 30 sec for 55 cycles. An amplification product having the corresponding electro-

TABLE 1

Summary of Primer Pairs and TaqMan Probes

Genes	Primer (Forward)	TaqMan Probe	Primer (Reverse)
MyoD	5′-$^{-168}$TTCTTCACCACACCTCTGACA^{-148}-3′	5′-$^{-122}$ACAGCCGGTGTGCATTCCAA^{-101}-3′	5′-$^{-32}$GCCGTGAGAGTCGTCTTAACTT^{-53}-3′
Myogenin	5′-^{866}CCTTAAAGCAGAGAGCATCC^{885}-3′	5′-^{921}CCCTTGTTAATGTCCCTCAGTGGG^{944}-3′	5′-^{993}GGAATTCGAGGCATAATATGA^{973}-3′
Myf5	5′-^{566}ATGTATCAAATGCATGTGCTG^{586}-3′	5′-^{630}TGTGGATCGGATCACGTCTACAGAG^{654}-3′	5′-^{682}CTGTGTCCTGAAGAGCCAG^{664}-3′
α-Actin	5′-^{18}AGGTATGGGTCAGAAGGACTCCTAC^{42}-3′	5′-^{57}CCAGAGCAAGCGAGGTATCCTGACCC^{82}-3′	5′-^{112}TGATGATGCCATGTTCAATGG^{92}-3′
β-Actin	5′-^{739}GTCATCACTATTGGCAACGAG^{759}-3′	5′-^{842}CCATCATGAAGTGTGACGTTGACA^{865}-3′	5′-^{894}CACTGTGTTGGCATAGAGGTC^{874}-3′
Glut1	5′-^{490}ATCGTCGTTGGCATCCT^{506}-3′	5′-^{509}TTGCCCAGGTGTTTGGCTTAGA^{530}-3′	5′-^{555}CAAGTCTGCATTGCCCA^{539}-3′
Glut4	5′-^{149}CACAGAAGGTGATTGAACAGAGC^{171}-3′	5′-^{174}CAATGCCAACGTGGCTGGTAGGC^{196}-3′	5′-^{215}TCCGGTCCCCCAGGA^{201}-3′
HKII	5′-^{1270}GCCAAGCGTCTCCATAAGG^{1288}-3′	5′-^{1290}AGTGAGGAGGCTGGTGCCCGA^{1310}-3′	5′-^{1330}GGAGGAAGCGGACATCACA^{1312}-3′
UCP1	5′-^{202}ACAGAAGGATTGCCGAAAC^{220}-3′	5′-^{225}CAGCGGTCTGCCTGCCGG^{241}-3′	5′-^{262}AGCTGATTTGCCTCTGAATG^{243}-3′
UCP2	5′-$^{-85}$GTTCCTCTGTCTCGTCTTGC^{-66}-3′	5′-$^{-29}$CTTCTGGGAGGTAGCAGGAAATCAG^{-5}-3′	5′-^{18}GGCCTTGAAACCAACCA^{2}-3′
UCP3	5′-^{392}TGACCTGCGCCCAGC^{406}-3′	5′-^{408}CACGGATGTGGTGAAGGTCCGATTT^{432}-3′	5′-^{452}CCCAGGCGTATCATGGCT^{435}-3′
G3PDH	5′-^{72}AAAGTGGAGATTGTTGCCAT^{91}-3′	5′-^{128}CATGTTCCAGTATGACTCCACTCACG^{154}-3′	5′-^{176}TTGACTGTGCCGTTGAATT^{158}-3′

Note. Superscripts indicate the nucleotide number from cDNAs determined in this paper. Nucleotide #1 is the A of the ATG codon that encodes the initiator methionine in each cDNA. TaqMan probe consists of an oligonucleotide with a 5′-reporter dye (FAM) and a downstream, 3′-quencher dye (TAMRA).

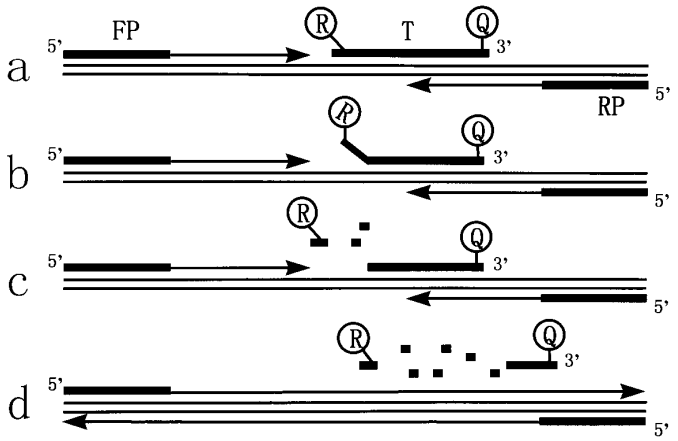

FIG. 1. TaqMan PCR reaction scheme. The design of TaqMan probes, combined with the 5′-3′ nuclease activity of AmpliTaq Gold, allows direct detection of PCR product by the release of a fluorescent reporter (R: FAM and Q: TAMRA) during PCR on the ABI PRISM 7700 Sequence Detector. Two oligonucleotides (FP: forward and RP: reverse primers) and a TaqMan probe (T) indicated in Table 1 were used for quantitative mRNA analysis by TaqMan PCR. a) Polymerization, b) Strand displacement, c) Cleavage, d) Polymerization completed.

phoretic mobilitiy (ca.300 bp) on a 2.5% agarose gel was excised and ligated into TA cloning vector (Invitrogen). The insert was verified by EcoRI digestion, with 9 clones out of 20 having the correct size. The insert was verified by sequencing using a Model 373S DNA sequencer (Perkin-Elmer Applied Biosystems). A partial clone of mouse UCP3 cDNA was obtained from 4 clones with same sequences out of 9 which is similar to human UCP3 (87% identity in nucleotide sequence).

RESULTS AND DISCUSSION

Determination of Relative mRNA Level using RT-TaqMan PCR Analysis

A rapid and highly sensitive method to determine mRNA level of target genes expressed in differentiating myoblast C_2C_{12} cells was developed. In addition, this method allowed for the analysis of multiple samples (96 samples) within 3 h (40 cycles). This technique can generate yes-or-no results much faster than Northern blotting (26), Ribonuclease Protection Assay (27) or several other quantitative RT-PCR techniques (28,29). The design of the TaqMan probes, combined with the 5′-3′ nuclease activity of rTth DNA polymerase, allows for the direct detection of PCR product by the release of a fluorescent reporter during PCR on the ABI PRISM 7700 Sequence Detector (Fig.1) (24,30). When the probe is intact, the proximity of reporter dye to the quencher dye results in suppression of the reporter fluorescence, primarily by Förster-type energy transfer (31). During PCR, if the target of interest is present, the probe specifically anneals between the forward and reverse primer sites (Fig. 1a). The nucleolytic activity of the rTth DNA polymerase enzyme cleaves the probe between the reporter and the quencher only if this region hybridizes to the target (Fig. 1b and 1c). rTth DNA polymerase does not digest free probe. After degradation of the hybridized probe, the shortened probe dissociates from the target and polymerization of the strand continues (Fig. 1d). This process occurs during every cycle and does not interfere with the exponential accumulation of product. The degradation of the oligonucleotide between the reporter and quencher dyes results in increased fluorescence of the reporter proportional to the amount of product accumulated.

At any given cycle within the exponential phase of PCR, the amount of product is proportional to the initial number of template copies. Fig. 2a shows the typical pattern of amplification rate of G3PDH mRNA in a dilution series of the total RNA (0-500 ng per each reaction). Given a range of five known concentrations of total RNA template (0-500 ng), the threshold cycle (Ct) of each concentration was deter-

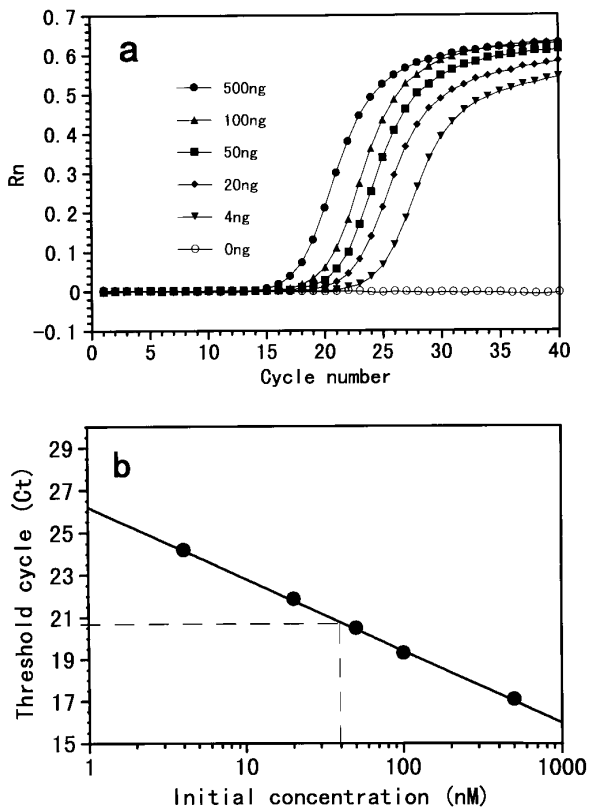

FIG. 2. Determination of mRNA level of target genes using TaqMan PCR. The graphs show: a) An amplification plot (R_n vs. Cycle #) derived from TaqMan PCR, and b) a standard curve to determine initial template concentration. Total RNA sample prepared from C_2C_{12} cells and oligonucleotides for G3PDH mRNA determination were used. Normalized reporter (R_n) represents the fluorescence signal of the reporter dye divided by the fluorescence signal of the passive reference dye. Threshold cycle (*Ct*) represents the PCR cycle at which an increase in reporter fluorescence above a baseline signal can first be detected.

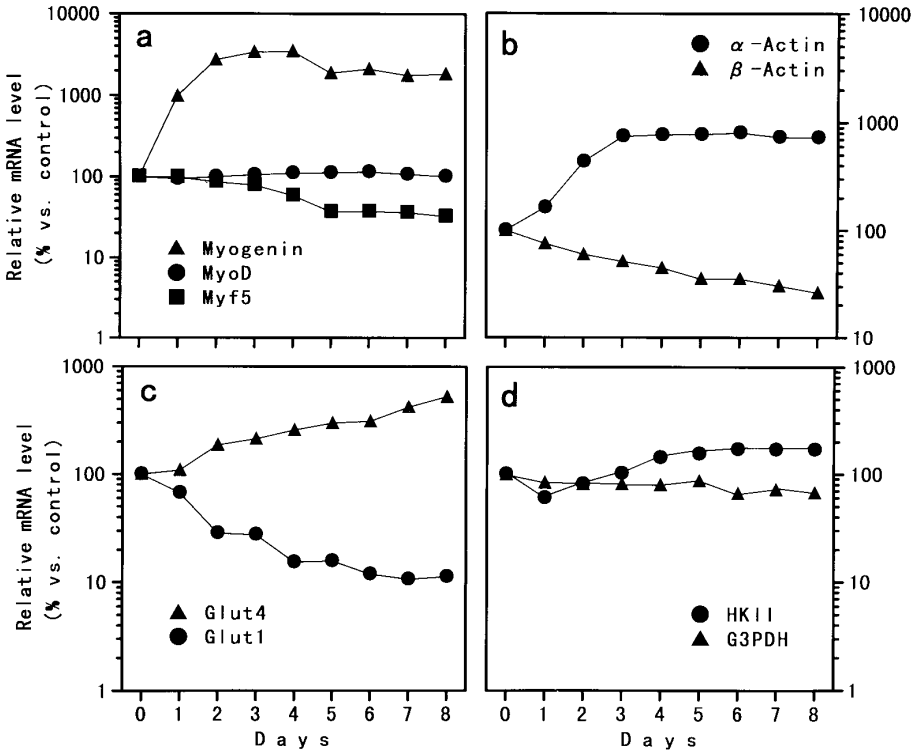

FIG. 3. Time course change of mRNA level of genes during myoblast differentiation. Myoblast C_2C_{12} cells were stimulated to differentiate by incubation in 2% HS-DMEM. Total RNA content and each mRNA level was determined as described in Materials and Methods. Each plot represents the average of three independent experiments with less than 10% of standard deviation. Glut, glucose transporter isotype; HKII, hexokinase II; G3PDH, glyceraldehyde-3-phosphate dehydrogenase.

mined. Then, the Ct vs. (Log N) initial concentration (Fig.2b) was plotted to produce the standard curve. An initial concentration from the standard curve was obtained by interpolation when the initial known concentration bracketed the unknown concentration. Each mRNA concentration in the total RNA sample was determined within the linear range of standard line (4-500 ng) as described above.

Time Course Change of Muscle-Specific Marker Gene mRNA Levels in Mouse Myoblast C_2C_{12} Cells during Differentiation

Using this assay, the time-course of mRNA levels in myoblast cells undergoing differentiation was examined (Fig. 3). Myoblast C_2C_{12} cells were cultured in differentiation medium (DMEM) containing 2% HS as previously described (32). Advanced morphologic changes such as striate, spindled, fused and multinucleate morphologies were markedly observed in C_2C_{12} cells after three days of medium change as shown in previous report (32,33). When stimulated to differentiate, the mRNA level of one muscle regulatory factor (MRF), myogenin, increased very rapidly and reached plateau on day 3 (Fig. 3a). In contrast, the mRNA level of another MRF, myoD, did not change throughout the treatment. The mRNA level of yet another MRF member, myf5, gradually decreased to 67% of initial level on day 8. McKarney et al (34) reported the same results for the rapid induction of myogenin mRNA in androgenic embryonic stem cell cultures during myogenesis. The persistence of MyoD and myf5 expression suggests the proliferation and differentiation are maintained. These results suggest that myogenin is a pivotal factor in initiating myoblast differentiation in C_2C_{12} cells (35).

In the same time-course, changes in muscle-specific gene mRNA levels were determined during myoblast differentiation. After changing to differentiation medium, β-actin mRNA level decreased to about ca. 26% of its initial content by day 8. Conversely, α-actin mRNA level increased by 7.5 times initial value by day 3 and maintained its level until day 8 (Fig. 3b).

Likewise, the glucose metabolism-related genes, glucose transporter isotype 1 (glut1) and isotype 4 (glut4), hexokinase II (HKII) and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were determined in the same samples (Fig. 3c & 3d). The expression of glut1 and glut4 genes in skeletal muscle is developmentally regulated (36,37). Under *in vitro* differentiation conditions using myoblast C_2C_{12} cells (Fig. 3c), the mRNA level of glucose transporter isotype 1, glut1 dramatically de-

creased to 1/10 the initial level by day 8. In contrast, glut4 mRNA level continuously increased to about 5.5 times initial value by day 8. This finding of glut4 induction and glut1 suppression during differentiation was also consistent with the observations reported previously (38).

Hexokinase II (HKII) initiates the first, rate-limiting reaction of glycolysis and catalyzes the formation of glucose-6-phosphate from glucose. It is expressed predominantly in insulin-sensitive tissues such as muscle, just like glut 4 (39). A previous report indicated that the coordinate regulation of HKII and glut4 was seen following contractile stimulation (40) or during development in rat, the first appearance of both transcripts and proteins in muscle coincides with the acquisition of insulin sensitivity around the time of weaning (41). Interestingly, in myoblast C_2C_{12} cells during differentiation, the HKII mRNA level showed a slight decrease at an early stage and then gradually increased without an accompanying change in the G3PDH mRNA level (Fig. 3d). Glut4 transcripts increased steadily with myotube formation of C_2C_{12} cell cultures (Fig. 3c). These findings seem inconsistent with the hypothesis of coordinate control of HKII and glut4 gene expression in muscle (40,41). However, there may be factors missing from C_2C_{12} cells which properly regulate HKII gene expression, or these factors may be affected by 2% HS stimulation. Further experiments are needed, in multiple cell lines, to confirm this observation.

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                248
hUCP3           CCGGCCTGCAGCGCCAGATGAGCTTCGCCTCCATCCGCATCGGCCTCTATGACTCCGTCA
mUCP3           ------TGCAGCGCCAGATGAGCTTTGCCTCCATTCGAATTGGCCTCTACGACTCTGTCA
mUCP2           CCGGCCTGCAGCGCCAGATGAGCTTTGCCTCCGTCCGCATTGGCCTCTACGACTCTGTCA
mUCP1           GGCAA------------ATCAGCTTTGCCTCACTCAGGATTGGCCTCTACGACTCAGTCC

                308
hUCP3           AGCAGGTGTACACCCCCAAAGGCGCGGACAACTCCAGCCTCACTACCCGGATTTTGGCCG
mUCP3           AGCAGTTCTACACCCCCAAGGGAGCGGACCACTCCAGCGTCGCCATCAGGATTCTGGCAG
mUCP2           AACAGTTCTACACCAAGGGCTCAGAGCATGCAGGCATCGGGAGCCGCCTCCT---GGCAG
mUCP1           AAGAGTACTTCTCTTCAGGGAGAGAAACACCTGCCTCTCTCGGAAACAAGATCTCAGCCG

                368
hUCP3           GCTGCACCACAGGAGCCATGGCGGTGACCTGTGCCCAGCCCACAGATGTGGTGAAGGTCC
mUCP3           GCTGCACGACAGGAGCCATGGCAGTGACCTGCGCCCAGCCCACGGATGTGGTGAAGGTCC
mUCP2           GTAGCACCACAGGTGCCCTGGCCGTGGCTGTAGCCCAGCCTACAGATGTGGTAAAGGTCC
mUCP1           GCTTAATGACTGGAGGTGTGGCAGTGTTCATTGGGCAGCCTACAGAGGTCGTGAAGGTCA

                428
hUCP3           GATTTCAGGCCAGCATACACCTCGGGCCATCCAGGAGCGACAGAAAATACAGCGGGACTA
mUCP3           GATTTCAAGCCATGATACGCCT-GGGAACTGGAGGAGAGAG--GAAATACAGAGGGACTA
mUCP2           GCTTCCAGGCTCAGGCCCGGGCTGGTGGTGGTCGGAGATACCAGAGCACTGTCGAAGCCT
mUCP1           GAATGCAAGCCCAGAGCCATCTGCATGGGATCAAACCCCGCTACACGGGGACCTACAATG

                488
hUCP3           TGGACGCCTACAGAACCATCGCCAGGGAGGAAGGAGTCAGGGGCCTGTGGAAAGGAACTT
mUCP3           TGGATGCCTACAGAACCATCGCCAGGGAGGAAGGAGTCAGGGGCCTGTGGAAAGGAACTT
mUCP2           ACAAGACCATTGCACG---------AGAGGAAGGGATCCGGGGCCTCTGGAAAGGGACTT
mUCP1           CTTACAGAGTTATAGCCACCACAGAAAGCTTGTCAACACTTTGGAAAGGGACGACCCCTA

                548
hUCP3           TGCCCAACATCATGAGGAATGCTATCGTCAACTGTGCTGAGGTGGTGACCTACGACATCC
mUCP3           TGCCCAA-----------------------------------------------------
mUCP2           CTCCCAATGTTGCCCGTAATGCCATTGTCAACTGTGCTGAGCTGGTGACCTATGACCTCA
mUCP1           AT-CTAATGAGAAATGTCATCATCAATTGTACA-----GAGCTGGTAACATATGACCTCA
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FIG. 4. Sequence of mouse UCP3 partial cDNA. A partial cDNA of mouse UCP3 gene was cloned as described in Materials and Methods. Nucleotide #1 is the A of the ATG codon that encodes the predicted initiator methionine of human UCP3. Two oligonucleotides used for the cloning are boxed. Single line indicates the forward and reverse primers sites for TaqMan PCR analysis. Double line indicates TaqMan probe site. hUCP, human uncoupling protein; mUCP, mouse uncoupling protein.

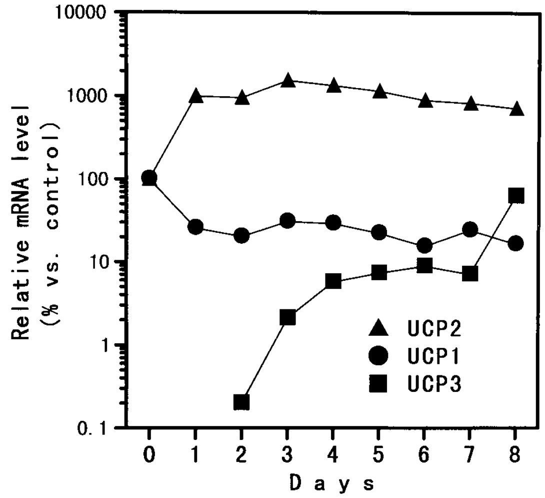

FIG. 5. Time course change of UCP mRNA levels in C_2C_{12} cells during differentiation. Total RNA samples were prepared from C_2C_{12} cells at the indicated times. Relative UCP mRNA levels were determined by RT-TaqMan PCR. Each plot represents the average of three independent experiments with less than 10% of standard deviation. UCP, uncoupling protein.

Changes of mRNA Levels of Mouse Uncoupling Proteins, UCP1, UCP2 and UCP3, in C_2C_{12} Cells during Differentiation

Recent papers reported the cloning of the human thermoregulatory uncoupling protein UCP3, which is preferentially expressed in muscle tissue (13,14). Uncoupling proteins are located in mitochondria and function as agents of thermogenesis (15,22). To determine the time-course change of mRNA levels of UCP isotypes (UCP1 to UCP3) expressed in C_2C_{12} cells undergoing differentiation, a partial cDNA of mouse UCP3, which had not been previously cloned, was recovered as described in Materials and Methods. The cDNA sequences of this partial mouse UCP3 clone is shown in Fig. 4. The mouse UCP3 mRNA level was determined using TaqMan PCR analysis as previously described. Fig. 5 shows UCP3 mRNA level in myoblast C_2C_{12} cells. UCP3 mRNA level prior to differentiation was undetectable. On day 1, even when up to 1 μg of total RNA was tested, no UCP3 mRNA was detected indicating its relative mRNA content is far less than UCP1 and UCP2 (< 1/1000). However, the UCP3 mRNA level increased markedly from day 2 to day 8. In contrast, both UCP1 and UCP2 mRNAs were detectable on day 0 and each mRNA reached a plateau within 1-2 days. These results indicate that different mechanisms govern regulation of these genes during myoblast differentiation in C_2C_{12} cells.

In summary, differential gene transcription during muscle differentiation and maturation was studied by TaqMan PCR analysis. This technique proved useful, efficient, and highly sensitive. This report indicates that different networks of gene expression related to morphologic change, glucose metabolism and thermogenesis exist in differentiating myoblast C_2C_{12} cells. Further experiments are needed to clarify the mechanism of this concerted regulation at transcriptional level.

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