Crucial Role of TCL/TC10βL, a Subfamily of Rho GTPase, in Adipocyte Differentiation*

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The events at the beginning of adipocyte differentiation are not well known. We previously cloned the genes expressed early in the differentiation of mouse 3T3-L1 preadipocyte cells. One of them, similar in sequence to human TC10, was identified as TC10-like/TC10βLong (TCL/TC10βL), a new Rho GTPase by the cloning of full-length cDNA. The expression of TCL/TC10βL increased rapidly right after the addition of inducers for differentiation, whereas the levels of other Rho family genes were unchanged at this stage. The antisense TCL/TC10βL-expressing experiment revealed that the differentiation of 3T3-L1 cells into adipocytes was inhibited. Moreover, the sense TCL/TC10βL-expressing experiment using NIH-3T3 cells, which do not usually differentiate into adipocytes, clearly showed the accumulation of oil droplets as well as the elevated expression of various adipogenic marker genes in the presence of the ligand for peroxisome proliferator-activated receptor γ (PPARγ). These results strongly indicated that TCL/TC10βL has a crucial role in the early stage of adipocyte differentiation, probably linked to the PPARγ pathway. Using a subtraction protocol, the genes specifically regulated by TCL/TC10βL were also isolated. The expression pattern of some of them was similar to TCL/TC10βL expression in adipogenesis, suggesting that the expression of these genes would be regulated by TCL/TC10βL.

The adipose tissue is an important organ for energy storage and lipid homeostasis (1–3). In addition, it secretes many cytokines and other proteins, such as leptin, adiponectin, adipin, tumor necrosis factor-α, and plasminogen activator inhibitor-1. These secreted proteins have crucial roles in regulating food intake and the insulin sensitivity/resistance in diabetes mellitus, as well as in obesity (1, 3, 4). Obesity is a ringleader for many diseases, such as diabetes, hypertension, hyperlipidemia, and also arteriosclerosis (5). Therefore, further insight into the molecular basis of obesity is required.

Three families of transcription factor proteins have been identified and characterized as the master regulators of adipocyte differentiation (6, 7). Peroxisome proliferator-activated receptor γ (PPARγ) is a key transcription factor for the differentiation of preadipocytes into mature adipocytes and activates the many adipocyte-specific genes including the aP2, lipoprotein lipase, and resistin genes (8, 9). The CCAAT/enhancer-binding protein (C/EBP) family also has been identified as master regulators. Notably, C/EBPβ activates the expression of PPARγ, as well as leptin and the insulin receptor (7, 10, 11). The expression of C/EBPβ and C/EBPδ is preceded by that of C/EBPα and induces PPARγ expression (6, 12). Sterol regulatory element-binding protein 1 (SREBP-1) functions as a regulator for lipid homeostasis, and this protein is also known to be an activator for the ligand production of PPARγ (7, 13, 14). Thus, the middle and late stages of adipocyte differentiation are relatively well characterized. However, the events at the early stage of adipogenesis are not fully understood.

In previous reports, we identified 102 genes as inducible at the earliest stage of adipocyte differentiation by a polymerase chain reaction (PCR) subtraction protocol (15, 16). These include genes for transcription factors and signaling proteins. It is of interest that almost half of them are unknown genes that are not found in the data bases. The main reason for this is likely to be that the isolated fragments are too short to identify the genes. Therefore, in a previous study, we performed a preliminary analysis using the rapid amplification of cDNA ends (RACE) technique and, by partial sequencing, identified clone 26 as mouse TC10, similar in sequence to human TC10, one of the Rho family (15). However, recent findings by two groups have revealed the existence of one more Rho family member (17–19). TC10-like (TCL) was identified from human and mouse EST (expressed sequence tag) data bases sharing 95% sequence similarity with human TCL, which has 10 more amino acids in the N-terminal portion (17). TC10β was identified as a mouse ortholog of TC10 (18). Interestingly, TC10β has an isofrom, TC10βL, which has 10 more amino acids at the N-terminus. In the present study, we isolated the full-length cDNA of clone 26 and by sequence analysis revealed that our mouse clone 26 is identical to TC10βL and also to the longer form of TCL (17). Therefore, in the present paper we refer to clone 26 as TCL/TC10βL.

The Rho family of small GTPases makes up a large gene family, which can be divided into two groups; one group includes RhoA, -B, -C, -D, -E, and -L and Rnd1–3, and the other

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‡ The abbreviations used are: PPARγ, proliferator-activated receptor γ; TCL, TC10-like; TC10βL, TC10βLong; C/EBP, CCAAT/enhancer-binding protein; Dex, dexamethasone; DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; Ins, insulin; IPG, isopropyl-1-thio-β-D-galactopyranoside; 5’RACE, 5’-rapid amplification of cDNA ends; RGS, regulator of G protein signaling; RT-PCR, reverse transcriptase-coupled-PCR; SREBP-1, sterol regulatory element-binding protein 1; IBMX, 3-isobutyl-1-methylxanthine.
includes Rac1–3, Rho G, TTF/RhoH, Cdh, Cdc42, TC10, and TCL/TC10βL (20). Rho proteins act as molecular switches to control cellular processes by cycling between the active, GTP-bound and inactive, GDP-bound states. Several lines of evidence in the past few years have revealed that Rho proteins have important roles in many cellular events, such as membrane trafficking, transcriptional regulation, control of cell growth, and development (20–23). Recently, Chiang et al. (24) suggested that the activation of TC10 was essential for insulin-stimulated glucose uptake and GLUT4 translocation in fully differentiated adipocytes. However, the roles of Rho proteins have not been elucidated in terms of adipocyte differentiation.

In our previous report (15), clone 26 (TCL/TC10βL in the present paper) was strongly expressed at the early stage of adipocyte differentiation; this induction is specific to growth-arrested cells, a state that is essential for adipocyte differentiation. These findings prompted us to further characterize the functional roles of TCL/TC10βL. In the present study, we blocked the expression of TCL/TC10βL using the Lac Switch mammalian expression system, which produces antisense TCL/TC10βL. The inhibition of TCL/TC10βL expression in 3T3-L1 cells prevented the cytoplastic accumulation of triglyceride and decreased the expression of adipogenic related genes. We next used a retroviral system to over-express TCL/TC10βL stably in NIH-3T3 cells. The constitutive over-expression of TCL/TC10βL is sufficient to cause the adipocyte differentiation of these cells in the presence of 3-isobutyl-1-methylxanthine (IBMX), dexamethasone (Dex), insulin (Ins), 10% fetal bovine serum (FBS), and the PPARγ ligand BRL49653. Furthermore, using the PCR-cDNA subtraction system, we isolated several clones as candidates for the downstream target genes of TCL/TC10βL. Some of the isolated genes were also induced during the differentiation of 3T3-L1 cells. These results strongly suggest that TCL/TC10βL has crucial roles in the program of adipocyte differentiation.

**EXPERIMENTAL PROCEDURES**

**Cloning of Mouse TCL/TC10βL**—The full-length cDNA for clone 26 was isolated by 5’-RACE analysis. The 5’-RACE reaction was performed using the Marathon cDNA amplification kit (Clontech, Palo Alto, CA) according to the manufacturer’s instructions. The sequences were determined with the automated DNA sequencer DSQ 1000 (Shimadzu Corp., Kyoto, Japan) and ABI PRISM 310 (PerkinElmer Life Sciences). Cell Culture and Differentiation—The mouse 3T3-L1 (ATCC CL173) preadipocytes and mouse NIH-3T3 (clone 611, JCRB 0615, Japanese Cancer Research Resources Bank) fibroblast cells were maintained in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% calf serum. For the differentiation experiment, the medium was changed to DMEM supplemented with 0.5 mM IBMX, 10 μg/ml Ins, 1 μM Dex, and 10% FBS at 2 days postconfluence (15). After 2 days, the cells were transferred to DMEM containing 5 μg/ml Ins and 10% FBS, and then the cells were refed every 2 days. BRL49653, the ligand for PPARγ (a gift from GlaxoSmithKline), was also added at a final concentration of 0.5 μM. The packaging cell line PT67 (Clontech) was maintained in DMEM containing 10% FBS.

**Establishment of Stable Cell Lines Expressing Antisense TCL/TC10βL**—Stable transfectants expressing antisense TCL/TC10βL under the regulation of isopropyl-1-thio-β-D-galactopyranoside (IPTG) were developed using a Lac Switch II inducible mammalian expression system (Stratagene, La Jolla, CA) according to the manufacturer’s instructions. The fragment containing the stretch between −90 and +241 (assigning A in start codon ATG for the first methionine from the 5’-portion as +1) was amplified by PCR using the primers with the restriction sites for SpeI and XhoI, respectively. After enzyme digestion of the amplified fragment, the resultant fragment was subcloned into a site in pOPRSVI/MCS in the antisense orientation. The sequence of the inserted fragment was confirmed as described above. 3T3-L1 cells were grown to 60–70% confluency and co-transfected with pCMVLacL, which expresses the Lac repressor protein, and pOPRSVI/MCS antisense TCL/TC10βL or pOPRSVI without the insert using LipofectAMINE (Invitrogen). The stable transfectant was selected in the presence of both 400 μg/ml neomycin (G418) and 150 μg/ml hygromycin. The drug-resistant clones were removed individually and stored.

**Establishment of TCL/TC10βL-over-expressing Cell Lines**—The TCL/TC10βL expression vector (pDON-AI-TCL/TC10βL) was constructed by ligating the full-length cDNA encoding mouse TCL/TC10βL. The full-length mouse TCL/TC10βL cDNA was amplified from 3T3-L1 cells from total RNA by RT-PCR. The PCR products were ligated into the SalI site of pDON-AI (Takara Biomedicals, Kusatsu, Japan). PT67 cells were cultured in 100-mm dishes and transfected by calcium phosphate coprecipitation with 14 μg of pDON-AI-TCL/TC10βL plasmid. After a 48-h transfection, the viral supernatants were collected and used to infect NIH-3T3 fibroblasts at 50% confluence in 100-mm dishes. Polybrene (4 μg/ml) was added to the viral supernatants before treatment. After 24-h incubation with viral supernatant, cells were split and replaced in DMEM containing 10% calf serum and 400 μg/ml of neomycin (G418) to select infected cells for 2 weeks. The drug-resistant clones were removed individually and stored.

**RNA Isolation, RT-PCR, and Northern Blot Analyses**—The cells were harvested at a specific time. Total RNA was extracted using TRIzol (Invitrogen). The mRNA was isolated from total RNA using Oligotex-dT30 (Daichi Pure Chemicals, Tokyo, Japan) for 5' RACE. For RT-PCR, cDNA was prepared using AMV Reverse Transcriptase XL (Takara Biomedicals) following the manufacturer’s recommended procedures. The PCR was done under the appropriate conditions. The PCR fragments were stained with ethidium bromide, and the intensities were determined with a fluoromager (FluoroImager 505, Amersham Biosciences). For Northern blot analyses, 25 μg of total RNA was electrophoresed on a 1.0% agarose gel containing 2% formaldehyde and then transferred to a Hybond-N+ nylon membrane (Amersham Biosciences). The filter was hybridized with each probe, which was labeled with α-32PdCTP using a random labeling kit (Takara Biomedicals). The radioactivity corresponding to each band was measured with a bioimage analyzer (BAS2000, Fuji Film, Tokyo, Japan).

**PCR Subtraction Method**—The subtraction cloning with PCR was performed using a PCR-select cDNA subtraction kit (Clontech) as described previously (15). For this purpose, two stable cell lines, NIH-TCL/TC10βL (a stable cell line integrated with pDON-AI-TCL/TC10βL) and pCMVLacL (a stable cell line integrated with pDON-AI control vector) were used. These cells were harvested before and at 2 days after induction, and Poly (A)+ RNA was prepared from each cell.

**RESULTS**

**Cloning of Mouse TCL/TC10βL**—We previously isolated genes expressed at the beginning of the differentiation of mouse 3T3-L1 cells (15, 16). Of these, 46 were unknown genes not found in any data bases. Clone 26 was expressed very strongly and specifically during adipocyte differentiation. This induction is not caused by just a change in medium without the differentiation mixture, because the expression of clone 26 was not observed in fresh medium without inducers (15). Moreover, when IBMX was omitted from the differentiation mixture, the induction did not occur, indicating that IBMX is a principal inducer (15). Because clone 26 is obtained as a small DNA fragment, we next attempted to isolate the full-length cDNA of this gene. Using 5’-RACE analysis, PCR products ~800 bp long were obtained, and sequence analysis showed a predicted protein that encodes 214 amino acids (data not shown).

In amino acid sequence, clone 26 showed 79.1% similarity to human TC10, which is one of the Rho family (19). As a result of a data base search, we first regarded this gene as a mouse homolog of human TC10. However, Vignal et al. (17) reported human and mouse TCL (TC10-like), a new GTPase of the Rho family related to TC10. The sequence analysis revealed 95% similarity between human and mouse, with the only major difference being found in the N-terminal portion, where human TCL is 10 amino acids longer than mouse TCL. Interestingly, the amino acid sequence of our clone 26 is the same as that of mouse TCL, except it has 10 more amino acids at the N terminus, which is exactly the same length as the human TCL sequence.

While we were preparing this manuscript, Chiang et al. (18) reported a related TC10 isoform involved in insulin-stimulated glucose transport and referred to TC10 and the new isoform as...
TC10α and TC10β, respectively (18). TC10β has two isoforms, and they named the longer variant TC10βL (TC10βLong). It was found that mouse TC10βL is identical to our clone 26 and of the same length as human TCL and also that TC10β is equal to mouse TCL. Therefore, we refer to our clone 26 as TCL/TC10βL and designated TCL/TC10β as TCL/TC10α. These results indicated functional differences between TC10α and -β. Therefore, we next determined the expression profiles of Rho family genes including TCL/TC10βL and TCL/TC10α.

Northern Blot Analyses of TCL/TC10βL and Other Rho Family Genes during Adipocyte Differentiation—We investigated the expression of TCL/TC10βL and other Rho family members during the differentiation of 3T3-L1 preadipocytes into adipocytes. The expression levels of TCL/TC10βL were quickly elevated after the induction, reaching a peak at 3 h. The expression of TC10α was only slightly detectable before the induction, and it decreased after the induction. However, it increased clearly in the late stage (Fig. 1). The levels of other Rho family members, such as RhoA, Rac1, and Cdc42, were unchanged throughout the incubation, indicating the specific expression of TCL/TC10βL and TCL/TC10α in adipocyte differentiation.

As described above, TC10β has two isoforms that differ in the N-terminal portion. Therefore, we next performed RT-PCR analyses to determine the expression levels of these two isoforms during adipogenesis. Because the first three amino acids were the same between the short and long forms of TC10β, we speculated that the shorter form of TC10β is a splicing isoform and designed PCR primers for the detection of both isoforms. The PCR analyses detected that only TC10βL, suggesting that the long form of TC10β (TCL/TC10βL) was specifically expressed in adipocytes (data not shown); however, we cannot rule out the possibility of sequence differences in the 5’-untranslated region due to the usage of different promoters.

Antisense TCL/TC10βL Prevents the Differentiation of Fibroblasts into Adipocytes—To gain insight into the biological functions of TCL/TC10βL, we first attempted to block the expression of TCL/TC10βL during preadipocyte differentiation using the Lac Switch mammalian expression system. We co-transfected pOPRSV1-TCL/TC10βL antisense and pCMVLac1 into mouse 3T3-L1 cells. As a control, a cell line transfected with pCMVLac1 and pOPRSV1 without the insert was also developed. By selection, using G418 and hygromycin, we isolated stable transformants, antisense TCL/TC10βL-expressing cells and control cells. 5 mM IPTG was added to the medium 12 h before the inducers at a final concentration of 5 mM. The expression levels of TCL/TC10βL and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined by RT-PCR, and the relative intensities of the products are also shown. Adipocyte conversion was inhibited by expressing antisense TCL/TC10βL. The stable transformants expressing antisense TCL/TC10βL and control cells were treated with inducers. IPTG was added to the medium 12 h before the inducers at a final concentration of 5 mM. After 8 days of treatment the cells were stained with Oil Red O, and the oil droplets were stained red. A Northern blot analyses of various adipogenic marker genes. The stable transformants expressing antisense TCL/TC10βL were treated with inducers with or without IPTG. Total RNA was isolated from cells at various time points of incubation and used for Northern blot analyses.

![Figure 1](http://example.com/fig1.png) Figure 1. Northern blot analyses of Rho family mRNAs during adipocyte differentiation. Total RNA was prepared from mouse 3T3-L1 cells at various time points after treatment with inducers. The total RNA (20 μg) was loaded, and the filter was hybridized with each probe. Staining with ethidium bromide (EtBr) for ribosomal RNA is shown as a control.

![Figure 2](http://example.com/fig2.png) Figure 2. Antisense TCL/TC10βL inhibits adipocyte differentiation. A, the expression of TCL/TC10βL mRNA was inhibited by the addition of IPTG in stable transformants expressing antisense TCL/TC10βL. The mouse 3T3-L1 cells, stably expressing antisense TCL/TC10βL under the regulation of IPTG, and integrated empty vector (control) were treated with inducers. IPTG was added to the medium 12 h before the inducers at a final concentration of 5 mM. The expression levels of TCL/TC10βL and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were determined by RT-PCR, and the relative intensities of the products are also shown. B, adipocyte conversion was inhibited by expressing antisense TCL/TC10βL. The stable transformants expressing antisense TCL/TC10βL and control cells were treated with inducers. IPTG was added to the medium 12 h before the inducers at a final concentration of 5 mM. After 8 days of treatment the cells were stained with Oil Red O, and the oil droplets were stained red. C, Northern blot analyses of various adipogenic marker genes. The stable transformants expressing antisense TCL/TC10βL were treated with inducers with or without IPTG. Total RNA was isolated from cells at various time points of incubation and used for Northern blot analyses.
the oil droplets well regardless of the presence of IPTG. On the other hand, the accumulation of oil droplets in the cells expressing antisense TCL/TC10\(\beta\)L was clearly inhibited by the addition of IPTG (Fig. 2B).

To test whether the stage of adipogenesis was really blocked by the expression of antisense TCL/TC10\(\beta\)L, we next determined the expression levels of the fat differentiation-linked genes. The expression of PPAR\(\gamma\), SREBP-1, and C/EBP\(\alpha\), which are all known to be master regulators of adipogenesis, was inhibited to a certain extent (Fig. 2C). Lipoprotein lipase, an adipogenic marker, was also inhibited during the differentiation, whereas the expression of C/EBP\(\beta\) was decreased only slightly, and that of C/EBP\(\alpha\) was unchanged (Fig. 2C).

**Over-expression of TCL/TC10\(\beta\)L Promotes Adipose Differentiation of NIH-3T3 Cells—**We next generated stable transformants constitutively expressing sense TCL/TC10\(\beta\)L using a retrovirus system. The full-length sense TCL/TC10\(\beta\)L was cloned into a retroviral vector, pDONAI-TCL/TC10\(\beta\)L (TCL/ TC10\(\beta\)L) or empty vector alone (control), which were produced in PT67 cells. After selection using G418, the drug-resistant clones were isolated. The Northern blot analysis showed that the endogenous TCL/TC10\(\beta\)L (~3.5 kb) was rarely detected in TCL/TC10\(\beta\)L and control cells, whereas the exogenous TCL/ TC10\(\beta\)L derived from retrovirus was found as a ~4.6-kb long mRNA (Fig. 3A).

Then, using these established cell lines, a differentiation experiment was performed. The TCL/TC10\(\beta\)L and control cells were cultured to confluence and treated with IBMX, Dex, Ins, and 10% FBS. Under the conditions, no morphological change or accumulation of oil droplets was observed after 10 days in either of the cells (data not shown). However, it is of interest that TCL/TC10\(\beta\)L cells started to differentiate into adipocytes when 0.5 mM BRL49653, a PPAR\(\gamma\) ligand, was added to the differentiation medium. As shown in Fig. 3B, the oil droplets were detected in TCL/TC10\(\beta\)L cells, whereas neither morphological change nor the accumulation of oil droplets was observed in control cells (Fig. 3B).

Northern blot analyses were performed to characterize further the phenotype of the TCL/TC10\(\beta\)L cells during the incubation with inducers. Total RNAs were prepared from TCL/ TC10\(\beta\)L and control cells at various times after the treatment with differentiation medium containing 0.5 mM BRL49653. The expression of PPAR\(\gamma\) and SREBP-1, which are master transcription factors for adipogenesis, was elevated in TCL/TC10\(\beta\)L cells during incubation (Fig. 3C). The expression of aP2 and lipoprotein lipase was also increased, although these increases were slight in control cells. However, the expression levels of C/EBP\(\beta\) and C/EBP\(\alpha\) were unchanged in both TCL/TC10\(\beta\)L and control cells. The expression of C/EBP\(\alpha\) was almost undetectable throughout the incubation in both cells. These results strongly suggest that TCL/TC10\(\beta\)L has important functions in the promotion of adipogenesis.

**Isolation of cDNA Clones Up-Regulated in TCL/TC10\(\beta\)L over-expressing Cells—**The sense and antisense experiments indicated that TCL/TC10\(\beta\)L has critical roles in the early stage of adipocyte differentiation. Because the TCL/TC10\(\beta\)L gene has been reported only recently (17, 18), the functions of its product are not well understood yet, and the signaling network through TCL/TC10\(\beta\)L in adipogenesis has not been characterized at all. To solve this issue, we next isolated the genes that seem to be downstream targets of TCL/TC10\(\beta\)L, by subtraction between TCL/TC10\(\beta\)L and control cells.

We designed two different conditions for the characterization of TCL/TC10\(\beta\)L in the differentiation of adipocytes. First, we performed PCR subtraction and isolated the genes in which levels are increased by the expression of ectopic TCL/TC10\(\beta\)L, without the inducers described under “Experimental Procedures” (condition 1). Second, the induced genes were cloned from cells incubated with PPAR\(\gamma\) ligand in addition to the inducers for 2 days (condition 2).

After performing the PCR subtraction protocol, we chose 300 colonies in each condition and sequenced them. As a result, 231 and 234 independent clones were isolated in conditions 1 and 2, respectively. It seems that 56 clones in conditions 1 and 58 clones in condition 2 are unknown genes as determined from a database search. Next, we chose 60 and 43 clones, respectively, from known genes in each condition and performed Northern blot analyses of various adipogenic marker genes. The stable transformant expressing sense TCL/TC10\(\beta\)L and control transformant were treated with the differentiation medium containing IBMX, Dex, Ins, and FBS in the presence of BRL49653. After 10 days of treatment, the cells were stained with Oil Red O. Northern blot analyses of various adipogenic marker genes. The stable transformant expressing sense TCL/TC10\(\beta\)L and control transformant were treated with the differentiation medium containing IBMX, Dex, Ins, and FBS in the presence of BRL49653. Total RNA isolated from cells at various time points of incubation was used for Northern blot analyses.

As expected, the isolated genes include the ones contributing to the signal transduction and cytoskeletal and extracellular...
The expression patterns of several genes were found to be similar, or slightly delayed, compared with the expression of TCL/TC10βL.

**DISCUSSION**

The differentiation of adipocytes is a very complex process. The expression patterns and the functions of several transcription factors have been well characterized. Experiments using knockout mice for PPARγ showed the requirement of this gene for the differentiation into adipocytes (25, 26). The double knockout of C/EBPβ and C/EBPα impaired the synthesis of fat in mice (27). Furthermore, it is reported that the ectopic expression of C/EBPα promotes adipogenesis as well as PPARγ (28). The dominant negative form of SREBP-1 prevented adipocyte differentiation (13). These findings strongly suggested that these three transcription factor families have indispensable roles in adipocyte differentiation. Thus, even though the middle and late stages of adipocyte differentiation have been relatively well characterized, the events in the early stage of adipogenesis have not been fully elucidated.

Previously we isolated many genes induced early in adipocyte differentiation (15, 16). The present paper describes the expression patterns and the functions of several transcription factor families that TCL/TC10βL shares high similarity to other species, the name of the source is given in parentheses.

| Condition 1 | Up-regulated genes in the TCL/TC10βL-expressing stable transformant |
|-------------|-------------------------------------------------------------------|
| TCL/TC10βL  | g) was loaded, and the filter was hybridized with each probe. |
| Ras p21 protein activator 3 | Cnbp, CTHBP, integrin β subunit, Clptm1 |
| caveolin-1 | Calumenin, Cellular nucleic acid binding protein (Cnbp) |
| C/EBPβ, C/EBPα | Tissue plasminogen activator |
| C/EBPβ | Sarco/endoplasmic reticulum Ca2+ ATPase (SERCA2b) |
| C/EBPα | Similar to DnaJ C5 |
| C/EBPγ | Gelsolin |

**Condition 2**

**Transcriptional factor**

- p300/CREB-associated factor (Pcaf)

**Signaling proteins**

- RAB7

**Cellular thyroid hormone binding protein p55 (CTHBP)**

**Cytoskeletal and extracellular structure**

- Fat 1 cadherin
- Integrin β subunit

**Other genes**

- Lactate dehydrogenase 1, A-chain (Ldh1)
- Proteasome β type 1
- 26S proteasome p112 (Rat)
- Aldolase 1A
- Translation initiation factor 3, subunit 9 (human)
- Fatty acid transport protein 4
- Cleft lip and palate associated transmembrane protein 1 (Clptm1)
- Heat shock protein 84

The expression patterns and the functions of several transcription factor families have been well characterized. Experiments using TCL/TC10βL, which shares high similarity to mouse clones, showed the requirement of this gene for the differentiation into adipocytes (25, 26). The double knockout of C/EBPβ and C/EBPα impaired the synthesis of fat in mice (27). Furthermore, it is reported that the ectopic expression of C/EBPα promotes adipogenesis as well as PPARγ (28). The dominant negative form of SREBP-1 prevented adipocyte differentiation (13). These findings strongly suggested that these three transcription factor families have indispensable roles in adipocyte differentiation. Thus, even though the middle and late stages of adipocyte differentiation have been relatively well characterized, the events in the early stage of adipogenesis have not been fully elucidated.

Previously we isolated many genes induced early in adipocyte differentiation (15, 16). The present paper describes the functional role of TCL/TC10βL. The pattern of TCL/TC10βL expression during adipocyte differentiation was specific and different from that of other Rho family members such as RhoA, Rac1, and Cdc42. Interestingly, although TCL/TC10βL shares high similarity to TC10α, the expression pattern of the TC10β gene was also different. TCL/TC10βL was expressed at the beginning of adipogenesis, whereas the expression of TC10α peaked late in the differentiation. It is also reported that TC10α and TCL/TC10βL differ in their impact on insulin-stimulated GLUT4 translocation (18). These results indicated that TCL/TC10βL and TC10α have quite different functions in adipocyte differentiation and adipose tissue. Saltiel’s group (18) also reported that there were two isoforms, TC10β and TC10βL. In our experiments, only the longer form, TC10βL, was detected at the early stage of differentiation, which suggests different roles for the isoforms, although further characterization is definitely needed.

When NIH-3T3 cells expressing TCL/TC10βL differentiated into adipocytes, the PPARγ ligand was required. In our previous paper, we demonstrated that the regulator of G-protein signaling 2 (RGS2) promoted the differentiation in the presence of ligand for PPARγ (29). In this case, the expression patterns of adipogenic genes were quite similar to those of TCL/TC10βL; that is, the expression of PPARγ, but not C/EBPs, was observed. Rosen et al. (30) recently showed that C/EBPα induces adipogenesis through PPARγ and has no ability to promote adipocyte differentiation in the absence of PPARγ, strongly indicating that the adipocytes developed through PPARγ as a unified pathway and that PPARγ seems to be the proximal effector of adipogenesis. It is likely that both TCL/TC10βL and RGS2 are involved in this PPARγ-linked signaling pathway, and this is one reason that neither gene has an effect on the expression of C/EBPs.

Sakaue et al. (31) reported the requirement of fibroblast growth factor 10 (FGF10) in the development of white adipose tissue. By experiments using anti-FGF10, and also FGF10 knockout mice and embryonic fibroblasts, they showed that FGF10 contributes to the expression of C/EBPβ through an autocrine/paracrine mechanism. Therefore, it seems that this...
signaling pathway may be different from the TCL/TC10βL and/or RGS2 pathway, although it is finally linked to a unified pathway through PPARγ.

The functional role of PPARγ ligand in the adipogenesis of TCL/TC10βL-expressing NIH-3T3 cells remains to be investigated. There are at least two possibilities. One is that the endogenous PPARγ, activated by the added ligand, enhances the functions of TCL/TC10βL and RGS2 and also activates the PPARγ pathway. The other possibility is that the ligand BRL49653 binds and activates different receptor proteins.

There is no direct evidence of a relationship between the signaling pathways through TCL/TC10βL and RGS2 at this moment. Therefore, we newly isolated the genes induced to expression by TCL/TC10βL, and found that the expression patterns of several were similar to that of TCL/TC10βL. It is not clear that these genes are the real targets of TCL/TC10βL signaling, because we do not have any experimental evidences to their potential function in relation to TCL/TC10βL signaling pathways through TCL/TC10βL and these isolated genes. However, it is of interest that Ras p21 protein activator 3 was identified as a GTPase-activating protein modulating Ras activity during normal brain development (32). A cellular nucleic acid-binding protein (Cnbp) is a zinc finger DNA-binding protein (33), and a cellular thyroid hormone-binding protein (CTHBP) is known to be involved in insulin action (34). The further characterization of these genes would help us to understand the signaling pathway at the early stage of adipocyte differentiation. The relationship between TCL/TC10βL activity (Rho GTPase activity) and the early stage of adipocyte differentiation is also still unclear. Further studies, including experiments using constitutively active and/or dominant negative mutants of TCL, and also transient inhibition at the early stage of adipocyte differentiation in the various time points using the RNA interference method, are definitely needed to determine whether TCL/TC10βL has a crucial role during the early stage of adipogenesis.

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