# Cloning and characterization of a mammalian fatty acyl-CoA elongase as a lipogenic enzyme regulated by SREBPs

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**Abstract The mammalian enzyme involved in the final elongation of de novo fatty acid biosynthesis following the building of fatty acids to 16 carbons by fatty acid synthase has yet to be identified. In the process of searching for genes activated by sterol regulatory element-binding protein 1 (SREBP-1) by using DNA microarray, we identified and characterized a murine cDNA clone that is highly similar to a fatty acyl-CoA elongase gene family such as Cig30, Sscs, and yeast ELOs. Studies on the cells overexpressing the full-length cDNA indicate that the encoded protein, designated fatty acyl-CoA elongase (FACE), has a FACE activity specific for long-chains; C12-C16 saturated- and monosaturated-fatty acids. Hepatic expression of this identified gene was consistently activated in the livers of transgenic mice overexpressing nuclear SREBP-1a, -1c, or -2. FACE mRNA levels are markedly induced in a refed state after fasting in the liver and adipose tissue. This refeeding response is significantly reduced in SREBP-1 deficient mice. Dietary PUFAs caused a profound suppression of this gene expression, which could be restored by SREBP-1c overexpression. Hepatic FACE expression was also highly up-regulated in leptin-deficient ob/ob mice. Hepatic FACE mRNA was markedly increased by administration of a pharmacological agonist of liver X-activated receptor (LXR), a dominant activator for SREBP-1c expression. These data indicated that this elongase is a new member of mammalian lipogenic enzymes regulated by SREBP-1, playing an important role in de novo synthesis of long-chain saturated and monosaturated fatty acids in conjunction with fatty acid synthase and stearoyl-CoA desaturase.—**Matsuzaka, T., H. Shimano, N. Yahagi, T. Yoshikawa, M. Amemiya-Kudo, A. H. Hasty, H. Okazaki, Y. Tamura, Y. Iizuka, K. Ohashi, J-i. Osuga, A. Takahashi, S. Yato, H. Sone, S. Ishibashi, and N. Yamada. **Cloning and characterization of a mammalian fatty acyl-CoA elongase as an lipogenic enzyme regulated by sterol regulatory element-binding proteins. *J. Lipid Res.* 2002.** 43: **911–920.**

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Biosynthesis of fatty acids is the major function part of lipogenesis in its role as an energy storage system. Fatty acids with lengths of 16–18 carbon atoms, constituting the majority of total fatty acids in the cells, are major products of de novo synthesis in most mammalian tissues. These long chain fatty acids play an important role in cellular biological functions, including: energy metabolism, membrane fluidity, and others. There appear to be several distinct metabolic pathways that produce long chain fatty acids. Cytoplasmic fatty acid synthase (FAS) plays a major role in the de novo synthesis of fatty acids. However, the elongation of fatty acids by this enzyme terminates at palmitic acid (C16:0). The end product of mammalian lipogenesis is usually oleic acid (C18:1n-9) or vaccenic acid (C18:1n-7) (1, 2). Mammals have long been thought to possess a membrane bound enzyme that elongates and/or desaturates saturated fatty acyl-CoAs produced by FAS or derived from dietary resources (3). Stearoyl-CoA desaturase (SCD) has been shown to be committed to the desaturation; however, the gene catalyzing for the C2 elongation of the C16:0 and C16:1 has never been identified.

To date, several enzymes involved in the elongation of long-chain fatty acids in non-mammalian cells have been identified. One such family consists of the yeast ELO genes. The yeast ELO1 gene is involved in the elongation of C14:0 to C16:0 (4). The ELO2 and ELO3 genes were identified based on the homology to the ELO1 gene (5). ELO2 protein is involved in the elongation of saturated and monounsaturated fatty acids up to 24 carbons in length, while ELO3 elongates a broader group of saturated and monounsaturated fatty acids, and is essential for the conversion of C24:0 into C26:0 (5). Another known elongase family, Cig30, which was originally identified as a cold-induced gene in brown fat, is the first mouse fatty acid elongase iden-

Abbreviations: ACC, acetyl-CoA carboxylase; ACBP, acyl-CoA binding protein; ACS, acyl-CoA synthetase; FACE, fatty acyl-CoA elongase; FAS, fatty acid synthase; LXR, liver X-activated receptor; RXR, retinoic acid receptor; SCD, stearoyl-CoA desaturase; SREBP, sterol regulatory element-binding protein; WAT, white adipose tissue.

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tified (6). Ssc1 and Ssc2 were cloned based on the homology to Cig30 (7). Complementation studies in yeast mutants indicated that Cig30 and SSC1 are functionally orthologous to ELO2 and ELO3, respectively. The specific activity of Ssc2 has not been identified. Elovl4, a retinal photoreceptor-specific gene, plays a role in the elongation of very long chain fatty acids and has been reported to be a causative gene for inherited macular degeneration (8). HELO1, as a member of HELO family cloned by homology to ELO2, is involved in the elongation of PUFAs and monounsaturated fatty acids, whereas the specific activity of HELO2 has not been not identified (9). These elongase could consist of a family that share a common structure of at least five membrane-spanning regions and a single histidine-box motif.

Sterol regulatory element-binding proteins (SREBPs) are membrane-bound transcription factors that belong to the basic helix-loop-helix leucine zipper family (10–12). SREBPs have been established as a regulator for biosynthesis of both cholesterol and fatty acids. To exert transcriptional activity on SRE-containing SREBP target genes, SREBPs have to undergo proteolytic cleavage in a complex with a sterol-sensing cofactor, SREBP-cleavage activating protein, that escorts SREBP for a rER-Golgi trafficking, a key step for regulation of cellular cholesterol biosynthesis. There are three isoforms of SREBP that have been characterized, SREBP-1a and -1c (also known as ADD1), and SREBP-2 (13–15). Lipogenic enzymes, which are involved in energy storage through synthesis of fatty acids and triglycerides, are coordinately regulated at the transcriptional level during different metabolic states. Recent in vivo studies demonstrated that SREBP-1c plays a crucial role in the dietary regulation of most hepatic lipogenic genes, whereas SREBP-2 is actively involved in the transcription of cholesterogenic enzymes. These include studies of the effects of the absence or overexpression of SREBP-1 on hepatic lipogenic gene expression (16–18), as well as physiological changes of SREBP-1c protein in normal mice after dietary manipulation, such as placement on high carbohydrate diets, PUFA-enriched diets, and fasting-refeeding regimens (19–22). Previous reports on the regulation of SREBP-1c have all demonstrated the induction to be at the mRNA level. Promoter analysis revealed that the expression of the SREBP-1c gene is regulated by two factors: SREBP itself, forming an autoloop, and liver X-activated receptor/retinoic acid receptor (LXR/RXR) oxysterol receptor (23–25, 35).

In the screening of SREBP-activated genes, we cloned a mammalian fatty acid elongase, designated fatty acyl-CoA elongase (FACE). Our current data suggest that the enzyme activity of this clone explains the missing identity step in the conversion of C16 to C18 fatty acids. In addition, the nutritional regulation of this SREBP-regulated FACE expression is consistent with its roles as a lipogenic enzyme.

## MATERIALS AND METHODS

### Materials and general methods

We purchased fatty acids from Sigma, restriction enzymes from New England Biolabs, redivue [α-$^{32}$P]dCTP (6,000 Ci/mmol) from Amersham Pharmacia, and radioactive [2-$^{14}$C]malonyl-CoA (51 mCi/mmol) from New England Nuclear. Standard molecular biology techniques were used. DNA sequencing was performed with the CEQ™ dye terminator cycle sequencing kit and CEQ2000 DNA Analysis System (Beckman Coulter).

### Preparation of SREBP-1a transgenic liver cDNA library

An expression cDNA library of SREBP-1a transgenic liver (16) was prepared as previously described for construction of a cDNA library of SREBP-1 deficient mouse adipose tissue, except that poly(A)$^+$ RNA was prepared from livers of SREBP-1a transgenic mice (25).

### cDNA cloning and sequencing

From a DNA microarray system using hepatic poly(A)$^+$ RNA of SREBP-1a transgenic and non-transgenic littermate mice (GenomeIncyte), we identified an EST clone (GenBank ID number AA239254) that was activated 19.5-fold in SREBP-1a transgenic liver as compared with wild-type liver. Using this sequence information, a [α-$^{32}$P]dCTP labeled DNA probe was prepared and used in the screening of an SREBP-1a transgenic mouse liver cDNA library by colony hybridization. Positive clones were sequenced; however, the clones were the 3′-fragment of the cDNA. To isolate the 5′ ends of the clones, the 5′-RACE method was used. Poly(A)$^+$ RNA was isolated using oligo-dT Latex (TaKaRa) from the liver of a SREBP-1a transgenic mouse and was used for cDNA synthesis and amplification with the 5′-Full RACE Core Set (TaKaRa). The cDNA sequence was subjected to BLAST search of mouse EST database and UniGene mouse database. The search revealed a cluster of mouse EST sequences (UniGene cluster ID number Mm. 26171) which contained a single open reading frame of 822 bp with similarity to Cig30, another known mouse fatty acid elongase. The putative elongase gene was tentatively designated FACE. Primers TMBSP1 (5′-TGG ATG CGG ACG CTG GGA GG–3′) and TMBAP1 (5′-AGT TGC ACT CAG CGA GTC CT-3′) were designed based on the putative FACE sequence and used to amplify the full length FACE cDNA from SREBP-1a transgenic mouse liver cDNA. The 1.1 kb PCR amplified product was subcloned into pGEM-T easy vector (Promega) and sequenced. The FACE cording sequence was isolated from this plasmid by *Not*I digestion and blunt ended using DNA Blunting Kit (TaKaRa), and inserted into *Sma*I site of expression CMV7 vector.

### Preparation of microsomes from transfected HEK-293 cells

HEK-293 cells were grown at 37°C in an atmosphere of 5% $CO_2$ in DMEM containing 25 mM glucose, 100 U/ml penicillin, and 100 μg/ml streptomycin sulfate supplemented with 10% FBS on 100 mm culture plates. At 80% confluency, the mouse FACE expression plasmid or the empty plasmid CMV7 (10 μg) was transfected into cells using SuperFect Transfection Reagent (Qiagen) according to the manufacturer's protocol. After transfection, cells were incubated with DMEM plus 10% FBS for 24 h. The microsomal fractions from the cells were prepared as previously described, with some modification (26). Twenty four hours after the transfection, cells were washed with PBS and scraped in 5 ml of ice-cold 0.25 M sucrose, 0.02 M HEPES, pH7.5. The cells were washed and resuspended in 3 ml of ice-cold sucrose/HEPES and dounce-homogenized. The homogenate was centrifuged 1,000 *g* for 7 min at 4°C. The pellet was resuspended in 1 ml of sucrose/HEPES, dounce-homogenized, and the suspension was centrifuged at 1,000 *g* for 7 min at 4°C. The supernatants were combined and re-centrifuged at 2,000 *g* for 30 min at 4°C. Supernatant from this centrifugation was centrifuged at 105,000 *g* for 60 min at 4°C. The resultant pellets were resuspended in 100 μl of 0.1 M Tris-HCl, pH 7.4 and used for fatty acid elongation assay.

### Fatty acid elongation assay

Microsomal fatty acid elongation activity was assayed by the measurement of [2-$^{14}$C]malonyl-CoA incorporation into exogenous acyl-CoAs as described previously (27), with some modification. The assay mixtures (0.25 ml total, including protein addition) contained 100 μM Tris-HCl, pH 7.4, 60 μM palmitoyl-CoA, 500 μM NADPH, and 30 μg of freshly obtained microsomal protein. After 2 min of preincubation at 37°C, the reaction was initiated by the addition of 60 μM malonyl-CoA (containing 0.037 μCi of [2-$^{14}$C]malonyl-CoA) and carried out for 5 min at 37°C. The incubation was terminated by addition of 0.5 ml of 15% KOH in methanol and saponified at 65°C for 45 min. Then the samples were cooled and acidified with 0.5 ml of ice-cold 5 N HCl. Free fatty acids were extracted from the mixture three times with 1 ml of hexane (total vol 3 ml). The pooled hexane fractions were dried under vacuum, and after addition of 3 ml of scintillation mixture, the radioactivity incorporated was counted (BECKMAN LS6500). Blanks were carried out in parallel reactions incubated without microsomal fractions.

### Animal experiments

All mice were housed in a controlled environment with a 12-h light/dark cycle and free access to water and diet. For fatty acid or drug experiments, 7-week-old male C57BL/6J mice (21–23 g) were purchased from CLEA (Tokyo, Japan) and adapted to the environment for 1 week. Prior to sacrifice, each group of animals was fed a diet containing the indicated fatty acids and drugs for 7 days. SREBP-1a, and -1c, and -2 transgenic mice (16, 18, 28) and wild-type controls (non-transgenic littermates of SREBP-1a transgenic mice) were put on a high protein/low carbohydrate diet for 2 weeks to induce the transgene expression, and were fasted for 12 h prior sacrifice. For fasting and refeeding treatment, SREBP-1 deficient (29) and wild-type mice were fasted for 24 h and fed a high sucrose/fat free diet for 12 h. Ob/+ mice on a C57Bl/6 background were purchased from Jackson Laboratories. Ob/+ mice were crossed to obtain leptin deficient ob/ob mice and wild-type mice. At 12 weeks old, ob/ob and wild-type mice were sacrificed in the early light phase following a 2 h fast.

### Total RNA preparation and Northern blotting

Total RNA was extracted from mouse livers, white adipose tissue (WAT), and various tissues using TRIZOL Reagent (Life Technologies, Inc.). RNA samples were run on a 1% agarose gel containing formaldehyde and transferred to a nylon membrane (Hybond-N, Amersham Pharmacia Biotech). The probes used were labeled with [α-$^{32}$P]dCTP using the Megaprime DNA Labeling System kit (Amersham Pharmacia Biotech). The cDNA probe for mouse FACE was prepared by digesting the cloned cDNA with *Not*I. The cDNA probes for mouse SREBP-1 and ribosomal phosphoprotein PO (36B4) were prepared as described previously (17). The membranes were hybridized with the radiolabeled probe in Rapid-hyb Buffer (Amersham Pharmacia Biotech) at 65°C and washed in 0.1 × SSC, 0.1% SDS at 65°C. The resulting bands were quantified by exposure of the filters to BAS2000 with BAS station software (Fuji Photo Film Co., Ltd).

## RESULTS

### Cloning of FACE as an SREBP-activated gene

DNA microarray analysis identified an EST clone whose expression was increased 19.5-fold in the livers of SREBP-1a transgenic mice as compared with wild-type mice. Using this EST clone (GenBank ID number AA239254) as a probe, we obtained the full-length 6 kb cDNA by screening an SREBP-1a transgenic liver cDNA library, followed by 5′ RACE. As shown in **Fig. 1A**, nucleotide sequence of this clone revealed that it encodes a putative protein of 267 amino acid residues with a theoretical molecular mass of 31.6 kDa and very basic pI of 9.38. We found a human homolog (GenBank ID number AK027031) of mouse FACE after searching BLAST database. The predicted amino acid sequence revealed that 96% identical and 97% similarity in mouse and human homologs (Fig. 1B). Hydropathy analyses by the Kyte-Doolittle algorithm (30) suggest that this predicted protein contains five transmembrane regions, typical for members of the elongase family (Fig. 1C). An HXXHH motif, often present in desaturase/hydroxylase enzymes containing di-iron-oxo cluster (Fe-O-Fe) proteins (31), was found between predicted transmembrane regions II and III, at amino acid positions 141–145, and could function to receive electrons from either cytochrome b5 or a cytochrome b5-like domain in an NAD(P)H-dependent way (Fig. 1B, C). The COOH terminus of the FACE polypeptide contains a lysine residue in position −3 (KKXX-like motif), suggesting that the predicted protein is located in the endoplasmic reticulum (ER) membrane (Fig. 1B) (32). The predicted amino acid sequence of mouse FACE showed a considerable similarity to Cig30 (44% identical), Ssc1 (29%), Ssc2 (26%), and Elovl4 (26%), all of which are known to be involved in the elongation of fatty acids by two carbon atoms, and they all contain 100% conserved HXXHH motif characteristic of this protein family (**Fig. 2**).

### Expression of FACE in HEK-293 cells and substrate specificity of FACE activity

This cDNA clone is highly likely to encode a mammalian fatty acid elongase, and is tentatively designated FACE. When the FACE cDNA was over-expressed in HEK293 cells supplemented with various fatty acids, gas chromatography analysis of the cellular fatty acids showed a trend that overexpression of FACE caused a slight increase in the relative amounts of stearic acid (C18:0) and oleic acid (C18:1n-9), accompanying a decrease in palmitic acid (C16:0) and myristic acid (C14:0) in the cells as compared with mock transfected cells (CMV7) (data not shown). These data suggested that the cells transfected with FACE were capable of synthesizing C18:0 from C16:0, indicating that FACE is involved in the elongation of C16:0 and that FACE may have an elongase acitivity for C14:0.

To verify that FACE plays a role in the conversion of C16:0 to C18:0, we performed in vitro microsomal fatty acid elongation assays. Microsomal fatty acid elongation activity was assayed by the measurement of [2-$^{14}$C]malonyl-CoA incorporation into exogenously added acyl-CoA esters of saturated and mono-unsaturated fatty acids with chain length 12 to 18, namely lauroyl-CoA (C12:0), myristoyl-CoA (C14:0), palmitoyl-CoA (C16:0), palmitoleoyl-CoA (C16:1n-7), stearoyl-CoA (C18:0), and oleoyl-CoA (C18:1n-9). Compared with control cells that had been transfected with empty plasmid CMV7, the transfection of the FACE cDNA resulted in two ~5-fold increases in elongase activity when

**A**


1 TGGATGCGGACGCTGGGAGGAGAGCCCCTGAGCTAGGAGCTGGGAGCAGAGGCGCAGAGAACACGTAGCGACTCCGAAGATCAGCCCCA 89
90 ATG AAC ATG TCA GTG TTG ACT TTA CAA GAA TAT GAA TTC GAA AAG CAG TTC AAC GAG AAC GAA GCC ATC CAA TGG 164
1 M N M S V L T L Q E Y E F E K Q F N E N E A I Q W 25
165 ATG CAG GAA AAC TGG AAG AAG TCT TTC CTG TTT TCT GCG CTG TAC GCT GCC TTT ATC TTT GGT GGT CGG CAT CTG 239
26 M Q E N W K K S F L F S A L Y A A F I F G G R H L 50
240 ATG AAC AAG CGA GCC AAG TTT GAA CTT CGG AAG CCG CTC GTG CTC TGG TCG CTG ACT CTT GCC GTC TTC AGT ATA 314
51 M N K R A K F E L R K P L V L W S L T L A V F S I 75
315 TTC GGT GCT CTT CGA ACT GGT GCT TAC ATG CTG TAC ATT CTG ATG ACC AAA GGC CTG AAG CAG TCA GTT TGT GAC 389
76 F G A L R T G A Y M L Y I L M T K G L K Q S V C D 100
390 CAG AGT TTT TAC AAT GGA CCT GTC AGC AAA TTC TGG GCT TAT GCA TTT GTG CTC AGC AAA GCA CCC GAA CTA GGT 464
101 Q S F Y N G P V S K F W A Y A F V L S K A P E L G 125
465 GAC ACG ATA TTC ATC ATT CTG AGG AAA CAG AAA CTG ATC TTC CTG CAC TGG TAC CAC CAC ATC ACT GTG CTC CTG 539
126 D T I F I I L R K Q K L I F L H W Y H H I T V L L 150
540 TAC TCC TGG TAC TCC TAC AAA GAC ATG GTC GCT GGG GGT GGT TGG TTC ATG ACT ATG AAC TAT GGC GTG CAT GCC 614
151 Y S W Y S Y K D M V A G G G W F M T M N Y G V H A 175
615 GTC ATG TAC TCT TAC TAC GCC TTG CGG GCT GCG GGT TTC CGA GTC TCC CGG AAG TTT GCC ATG TTC ATC ACC TTG 689
176 V M Y S Y Y A L R A A G F R V S R K F A M F I T L 200
690 TCC CAG ATC ACT CAG ATG CTG ATG GGC TGT GTC ATT AAC TAC CTG GTC TTC AAC TGG ATG CAG CAT GAC AAC GAC 764
201 S Q I T Q M L M G C V I N Y L V F N W M Q H D N D 225
765 CAG TGC TAC TCC CAC TTT CAG AAC ATC TTC TGG TCC TCG CTC ATG TAC CTC AGC TAC CTT GTG CTC TTC TGC CAT 839
226 Q C Y S H F Q N I F W S S L M Y L S Y L V L F C H 250
840 TTC TTC TTT GAG GCC TAC ATC GGC AAA GTG AAG AAA GCC ACG AAG GCT GAG TAG TGTCAGGGCTGAGGAGGAAGTCATAGCT 921
251 F F F E A Y I G K V K K A T K A E * 267
922 CAGGGTCATCACGAAAAATAATCGACAAAAGAAAAATGGCACAAGGAATCCCATATGGTGCAGCTAAAACAAAACAAAACATCCGTATGAGCAGGCACGA 1021
1022 GGCCCAAGGCAGCTTGGGACTGAAGATTAGGTTGTAAGTTTATGATCCTTTCTGGGTGAGGACTCGCTGAGTGCAACTCTTATCTCAAAGCACGGCTGCT 1121
1122 GAGGGGACCCCTTCCCTCTGGCCTGTCAACTCTAGAACACACTAGATGCAAAGGCAGCCACGGGCAAAGAGATTGGGCAGAGATTAGTGGACGGCCAGCA 1221
1222 AAACACTGCAGGAAGCAGGTGGGGGGAGGAATCTACTCAGCCTTTTTGTTTTGTTTTGTTTTGTTTTGTTTTCTCTAAGGATAAAGGAGTTTCC 1321
1322 CCTTTTCAAACGATGTGAGCACACACACACACACACACACACACACACACACACACACACACACACGCAATCTTTTCAACACGAAACCAGAGCTAAAAGAAA 1421
1422 AGATAAACATGGGAGAGACAGGGTTTCTATCTGGGACAGCAATGCTTTTGCAAAAGGCTAGGCCTTTTAAAGAAAGGTGAGCTTGTAACTCCTTGATAAA 1521
1522 AGATGTCTTAATTATTTTTACTGCAACTGAAAGTAAAGAGGTAGAGCCTTTCCCCTTCTGCACAGCCTCAGGGCTTGTATGTTCGCTACAACCAAACACA 1621
1622 GGACAGTACTTCCCCCATGATACTTTATTACTGGGAGAAAGAAACCCCTGTAGTTGAAACACCACACTGACAACTGTTATTTCTGCTCTCCGACGAGAAT 1721
1722 TCAAGCATCCGTTGTTCAGTTGCCCCAAACTTTAGTGACGGAGGAGTAAATGCAGAACTGAAAGGGAAGAAGCTCAGCTGGCTGGCTTGAAAATGGAGTC 1821
1822 TTGTACCATGTGTAACAAATGCCAGCCCATCGTCCCTGGAGCTGAACAGGGAGGAAGGGCTATGGGCAGAGACTAGAGCCGGATTCATCCAATGTGCAGA 1921
1922 CAGCGTGTTCGCCTCCCTCCCTGTTCGACCTCACACATAATCCTGGCTTTCTAAATGAGGCCCTGTGACACACTCTGTGCTTTCTATATTTTTGTGACTT 2021
2022 TCAAACACAGATCTGCAGGGCTCTGCCTGATTTGGGGTAAACACTGTGTTTCTGCAGCCTCTGCATTTGCTCCCTTCAGCAGTGCAGAGGCTTGAGAAGT 2121
2122 GCCCTCTGCTGGCTTAGTGAGAAGCTTCAACAAACACTTCACAGTAGTGTTGAAATAACTGACCACTAAGGGCCTGCGGAGATTAAACCCTAAGTTCTAA 2221
2222 GTGCTGTCAAACACCTGACATATATTTGACCAAATCAGAAATTTTTTAGGTGACTTTCACTTGAGAACTCAGAAAGTCAATGTATTAAGAGCCATATTCT 2321
2322 GAAAGAAAGAAAGAGAAAGAGAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAAGAAGAAAGAAAGAAAGAAAGAAAGCAAGCT 2421
2422 AGACAATGTTAACTGTAATATTTCAGTCCTCTACAAGCCAAATAAATGTGTCGATGTTCCTAATATTTCAAAGACGCAATTACATAAAGTTGTTCAATGT 2521
2522 GATATAATAGTATTCTAATTGGTTAAGTAGTTTAATGATTAGGCAAACTAGCAGAAACAATTAGAGGCTGCTACACCACGTAGATTATACACACATAGCC 2621
2622 ACGTACGTGAGGTACACGGAGCTATAAAGCTCAAATCAATAGTAACACGATTTTTGGCTAGCAGAAACTATCACCTATCTTCCTTTACTTGAGAGTGTAC 2721
2722 AGTAAAAGGGATTTTTTTCAAATTATTTTTATATTATTTTAGCTTTAATTGTGCTGTCATTCATGGAACAGCTGCTCAGCAGCCTTCCTGTGAGAGATGA 2821
2822 CAGGGGTGTTTTCGTGTGGCTTGTTTACGTGTGGAATTGGAAAAGAATAAAATCTGATTCCCTTCTGTGGGAATGGGATCAAGGGTAGACAAAGGACCCA 2921
2922 TGTAGATCAAGTCATAACTGAACGACCAGGGAAGGGAGCCAGGCGGGGGCGGGAGTCAGCTGTGGCTTTCATGTTACCATTGTGTGGTGGCTGATGGACG 3021
3022 GGACGTGGTTGGAGGGATGTTTTCTTACTTGGGGTAGAAGCTAACCGGAGATGAAAGTCTAGAAGCCACTCTGTCCAGTGGAATCTTAGGGTGTACTTGT 3121
3122 TCCTTTGAGCTTTGTAAATGCACAATAGTGTACAATAATAAGCCTTTCCCTTGCCTTATGGAAGAAAGTGAGCAAGATAATGAAAACCAAGAAGCCACCT 3221
3222 ATCAGTTGAATTGAGTCTAATCAAAAGGCCAGCCTGAGGCTCCTCTGGTCGGTTCAGTTTAATTTAGATATTTACCATAGAATACAGGTACAATATGGAA 3321
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3722 CCCCACTATGCTGCAATAGATTATCTTTCTATTGAAGATTTCTCGCTGCATTCCAATCCCAGTGTGGTGAAAACTTAATTCCTGGATTGAATGAACACAA 3821
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3922 CACAGTAGGAGATTTTTATCTGGACCTTCTAGTGTCAACTGTGAGGAGAAACAACTATCATTATTATTTATTTATTGTATTTATATCCAATGCCAGGCCA 4021
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4122 CATATTCATTGCTGCAGGGGAAAGCAGGTTTATTAATCCTCCCGCTGTCTTTATGAGACCGATTGACCAATGTAGCTCAGGCAGAAGTTTCATGTGGGTG 4221
4222 GGATATCTACAGGAGCCTAGGAAAACACTTCCAGAGAAGATAACCACAGACTGTTGTTTTTGTTCATTTGTTTTATATCTTCATGGTAAGATAAGCCTGT 4321
4322 CACGGAGTTACAAGGCACCATGACACTAAGGTAGAATGTTCCAGAAGTCTGGCTACCTTCCCAGGCTGCTCAGTTACCTGGGAGTGTCTAGTTACTATCT 4421
4422 TGTTCTGACGAGAGGAGCTTTTGCTCAAGAACTGCCAGATACAGACACCAAGTCAGCCCTGGCACACTCTACAACCTCCGGGCATAGGTAATGGGTCTTT 4521
4522 GACTATTGGATTGCCTCAGTGTCAAGTGAGTTCCTAGAAGAAGAGACCGAGTAGGCTCTACCCCCCAGACTCCACCACACTCTGAGTTGCATTGACAGGA 4621
4622 TCGGTGTCTAGACACAGTTCTTTGTGAAGTGTCAATGCTAGAGACAGTTGTGAGGAGATCATGATGACAGCCCAGAACTTTCTAGCTTTCAAATGCATCC 4721
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4822 CATTTCCTGGTGCCTGAGATCCCAGCCATCAGAAAGTGATTTGGGTGAGAATTCACAACATATATGTCACCTCTGCATATTGAAGTGACATCTAATAAAA 4921
4922 CAAGGACGTCCTATTTTGTCTGAACCCGCTGAATGAAGCTCTGTTATCCTAGTTAGTCATTGGGCCGCCATCCTCTGTACCCGATAGTGACACAAAACAG 5021
5022 ATGTCGGTGCCTGTACAAGAATTCTCAGTGCCTGTTGTGACAGACTGTGCTTAGAAGAAACATTCGTGAGCCATAAAGCAGGAACCACAGATGAAAGGGC 5121
5122 CAGTTAAAAGTCCACCTGCTCCAAGTATCATAGAAAACCCAAAAGCCTGTTGTATAATCTGGTATTGTCCCCATCCCCAGATGCTTTGAAAACTAGGATT 5221
5222 CTCAGAGCATGGATACCCACGCTTCCATCTTCCCACAAACATTTCCTAGAGTTGTACTGGTGGGTGCAGCCCTAGGTGGTTGGTTGGGGGAAGTCTTGGA 5321
5322 AGCTGTACTTTGATTGCAGGTCAAGCAAAGCCAAATCCAGATATTTCTGTGTCACTCACCAGTTGTCCATGTCCACCCACAAAACAATTGTATTATAGTC 5421
5422 AAGTTGTCCTAGCTGATTGGTCCTCAAATAAGGATGCAACTATGTTTGCAACCCAGTTAGGACACATTTGAAAGAACCTGACTCACTAGCATCTAAACAA 5521
5522 TATCATTTCCCCAATGCTTGGTGGCACTTCAGACTTTTGTTCTCCTGGTTGATCAAGGTGTTGCCTGGTGGTGCCGCCTCCTAGTGTGAATATTTCAGTT 5621
5622 AAGTGTGGGTCTGAGCATGACCGGGCTGGGCTTAGCTCACTGCTACTTGGAAAATGACTGGCATTCTGCTTCCTAGGCCCTAAACCCATATTCAGAGGGA 5721
5722 AAATTCACTATCAAGCCTCACAGCGAAATCACAGCAGTGTTGGAATTCTTATTTTCAAGTGCTTATCTCACAACATTGAAAAATATTTTTGGTGTATTAA 5821
5822 GATTTAAAATAAAGTCATCATAAACTTTTGAATTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 5893

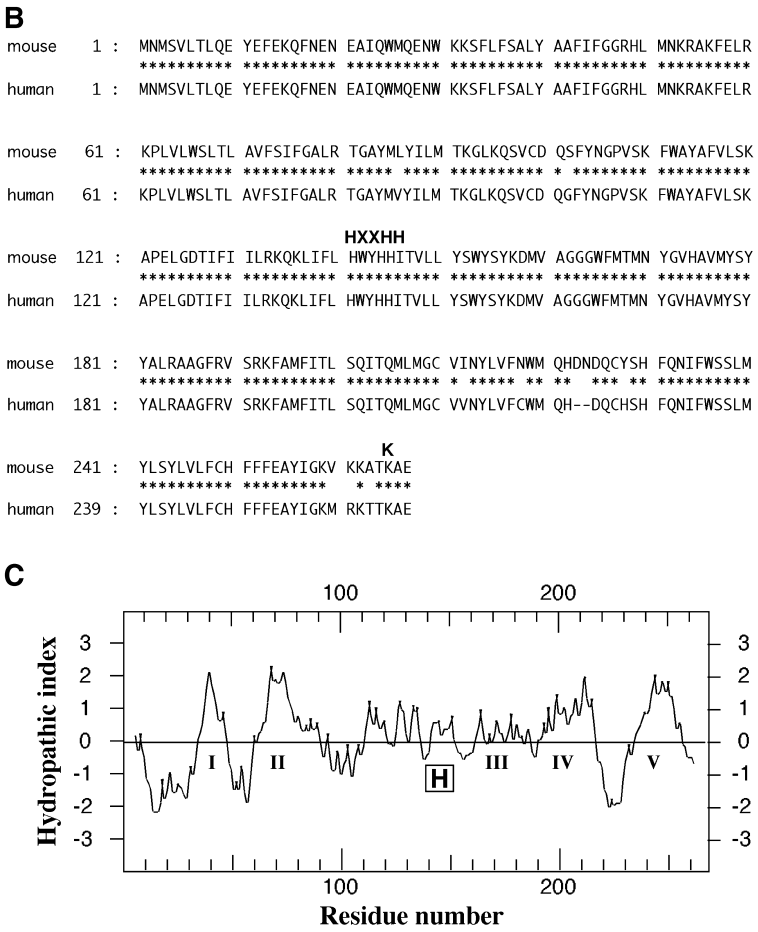


**Fig. 1.** Sequence and homology analysis of fatty acyl-CoA elongase (FACE) cDNA and protein. A: Nucleotide sequence of mouse FACE cDNA. Predicted amino acid sequence is shown below. Open reading frame and putative transmembrane domains are underlined and numbered with Roman numerals. Polyadenylation signal consensus sequences are double-underlined. Sequence data have been deposited with the GenBank accession number AB072039. B: Amino acid sequence comparison of mouse and human FACE. The dioxy iron-binding HXXHH motif and COOH-terminal lysine signal responsible for retention in endoplasmic reticulum are indicated above protein sequence. C: Hydropathy profile of mouse FACE protein. Roman numerals indicate five regions above the horizontal bars predicted to span the membrane according to the algorithm of Kyte and Doolittle. The region containing the histidine motif is indicated H.

added lauroyl-CoA, myristoyl-CoA, palmitoyl-CoA and palmitoleoyl-CoA (**Fig. 3**). When stearoyl-CoA and oleoyl-CoA were used as a substrate, there were no significant changes in the elongation activity. These data demonstrated that FACE possesses elongase activity specific to C12–C16 saturated and monounsaturated fatty acids.

### Tissue distribution of mouse FACE mRNA

Expression of FACE was examined in various tissues as estimated by Northern blot analysis. Whereas the dominant transcript of mouse FACE is approximately 6.0 kb in size, mouse tissues also contain a minor transcript that is approximately 2.5 kb in size, (**Fig. 4**), presumably due to the presence of two (poly)A signal sequences in the 3′-UTR (Fig. 1A). The expression was high in adrenal gland, liver, WAT, brain, testis, and skin where lipogenesis and steroidogenesis are active. Lower expression was found in kidney, heart, skeletal muscle, lung, and spleen, whereas the dominant transcript in testis is 2.5 kb.

### Activation of FACE expression in livers from SREBP transgenic mice

To confirm SREBP-1a activation of FACE expression and to estimate the effect of other nuclear SREBP isoforms, Northern blot analysis was performed on liver RNAs from nuclear SREBP-1a, -1c, and -2 transgenic and wild-type mice. As shown in **Fig. 5**, hepatic mRNA levels of FACE were robustly increased by over-expression of nuclear SREBP-1a, -2, and -1c in this order of magnitude. The shorter FACE transcript was barely detectable from livers of fasted wild-type mice, but became prominent in SREBP transgenic livers. This minor short transcript was also nutritionally regulated in the same manner as the major transcript, consistent with the notion that the dual transcripts are due to different (poly)A signals. The data indicate that every isoform of SREBP can activate the expression of FACE gene, with the SREBP-1a isoform being the most effective.

```
              10        20        30        40        50
FACE         MNMS----VLTLQEYEFEKQFN-------ENEAIQWMQENWKKSF
CIG30     MDTSMNFSRGLKMDLMQPYDFETFQD-------LR---PFLEEYWVSSF
SSC1            MEAVVNLYHELMKHAD-------PRIQSYPLMGSPLLIT
SSC2         MEQLKAFDNEVNAFLDNMFGPR------DSRVRGWFLLDSYLPTF
ELOVL4    MGLLDSEPGSVLNAMSTAFNDTVEFYRWTWTIADKRVADWPLMQSPWPTI
                              .                   .

              60        70        80        90        100
FACE      LFSALYAAFIF-GGRHLMNKRAKFELRKPLVLWSLTLAVFSIFGALRTGA
CIG30     LIVVVYLLLIV-VGQTYMRTRKSFSLQRPLILWSFFLAIFSILGTLRMWK
SSC1      SILLTYVYFILSLGPRIMANRKPFQLRGFMIVYNFSLVILSLY-IVYEFL
SSC2      ILTITYLLSIW-LGNKYMKNRPALSLRGILTLYNLAITLLSAY-MLVELI
ELOVL4    SISTLYLLFVW-LGPKWMKDREPFQMRLVLIIYNFGMVLLNLF-IFRELF
               Y   .   G   M  R    ..  . .    . .

             110       120       130       140       150
FACE      YMLYILMTKGLKQSVCDQSFYNGPVSKFWAYAFVLSKAPELGDTIFIILR
CIG30     FMATVMFTVGLKQTVCFAIYTDDAVVRFWSFLFLLSKVVELGDTAFIILR
SSC1      MSGWLSTYTWRCDPIDFSNSPEALRMVRVAWLFMLSKVIELMDTVIFILR
SSC2      LSSWEGGYNLQCQNLDSAGEG-DVRVAKVLWWYYFSKLVEFLDTIFFVLR
ELOVL4    MGSYNAGYSYICQSVDYSNDVNEVRIAAALWWYFVSKGVEYLDTVFFILR
                     .                   .  SK  E  DT   .LR

             160       170       180       190       200
FACE      KQ--KLIFLHWYHHITVLLYSWYSYKDMVAGGGWFM--TMNYGVHAVMYS
CIG30     KR--PLIFVHWYHHSTVLLFTSFGYKNKVPSGGWFM--TMNFGVHSVMYT
SSC1      KKDGQVTFLHVFHHSVLPWSWWWGIKIAPGGMGSFH-AMINSSVHVVMYL
SSC2      KKTNQITFLHVYHHASMFNIWWCVLNWIPCGQSFF-GPTLNSFIHILMYS
ELOVL4    KKNNQVSFLHVYHHCTMFTLWWIGIKWVAGGQAFF-GAQMNSFIHVIMYS
          K.   . F.H .HH  .                 F    .N  .H .MY

             210       220       230       240       250
FACE      YYALRAA--GFRVSRKFAMFITLSQITQMLMGCVINYLVFNWMQHDNDQC
CIG30     YYTMKAA--KLKHPNLLPMVITSLQILQMVLGTIFGILNYIWRQEKG--C
SSC1      YYGLSALGPVAQPYLWWKKHMTAIQLIQFVL-VSLHISQYYFMPS----C
SSC2      YYGLSVF-PSMHKYLWWKKYLTQAQLVQFVL-TITHTLSAVVKP-----C
ELOVL4    YYGLTAFGPWIQKYLWWKRYLTMLQLVQFHV-TIGHTALSLYTD-----C
          YY .       .        .T  Q. Q  .                    C

             260       270       280       290       300
FACE      YSHFQNIFWSSLMYLS-YLVLFCHFFFEAYIG------------------
CIG30     HTTTEHFFWSFMLYGT-YFILFAHFFHRAYLRP------------------
SSC1      NYQYPIIIHLIWMYGTIFFILFSNFWYHSYTK-------GKRLPRAVQQN
SSC2      GFPFGCLIFQSSYMMT-LVILFLNFYIQTYR---KKP-VKKELQEKEVKN
ELOVL4    PFPKWMHWALIAYAIS-FIFLFLNFYTRTYNE-PKQSKTGKTATNGISSN
                     .    LF .F   .Y

             310       320
FACE      ----------KVKKATKAE
CIG30     ----------KGKVASKSQ
SSC1      G--------APATTKVKAN
SSC2      GFPKAHLIVANGMTDKKAQ
```

**Fig. 2.** Amino acid sequence alignment of mouse FACE homologs. Amino acid positions conserved in the homologs are indicated under the protein sequence. The HXXHH motif, characteristic of desaturase/hydroxylase enzymes containing a diiron-oxo cluster (Fe-O-Fe) is underlined.

### Nutritional regulation of FACE expression in SREBP-1 deficient mice

Nutritional regulation of hepatic FACE mRNA levels was estimated in wild-type and SREBP-1 deficient mice. These mice were fasted and then refed a high sucrose/fat free diet. As shown in **Fig. 6A**, the mRNA levels of FACE were suppressed in livers of fasted wild-type mice and markedly activated by refeeding in the wild-type mice, showing nutritional regulation of FACE as a lipogenic enzyme. The mRNA level of FACE in the fasted SREBP-1$^{-/-}$ mice was low and essentially similar to those of fasted normal mice. However, the SREBP-1$^{-/-}$ mice had a lower induction of FACE upon refeeding compared with wild-type mice. These data demonstrate that the hepatic expression of FACE is at least partly under the control of the SREBP-1.

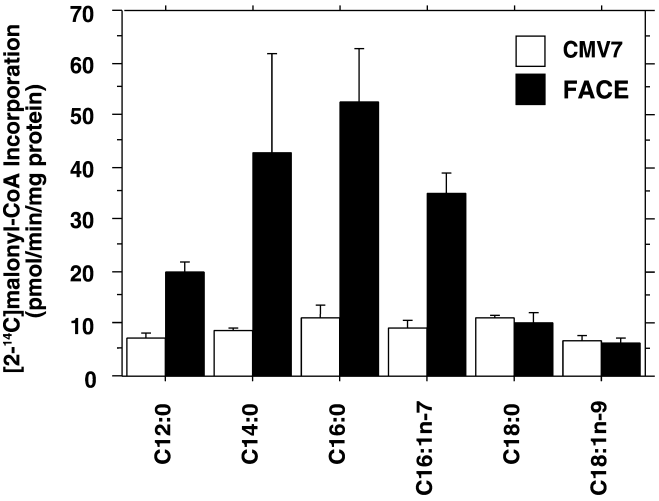


**Fig. 3.** Microsomal fatty acyl-CoA chain elongation activity from HEK-293 cells transfected with FACE or CMV7. Total fatty acid elongation activities in the presence of NADPH were determined in microsomal fractions prepared from HEK-293 cells transfected with FACE or empty vector CMV7. Specific activity was expressed as pmol of radioactive malonyl-CoA incorporated into hydrophobic long chain fatty acid fractions by 1 mg of microsomal protein in 1 min. Values represent the mean ± SEM (n = 3).

In addition to liver, adipose tissue is another organ in which lipogenic enzymes are thought to respond to fasting-refeeding at the transcription level. As shown in Fig. 6B, fasting-refeeding changes in mRNA levels of FACE from adipose tissues of wild-type mice were observed similar to those in the liver. SREBP-1$^{-/-}$ adipose tissues exhibited modestly impaired induction of FACE in both fasted and refed states. These data demonstrate that FACE mRNA level in lipogenic organs are nutritionally regulated in a lipogenic fashion, and are regulated by SREBP-1,

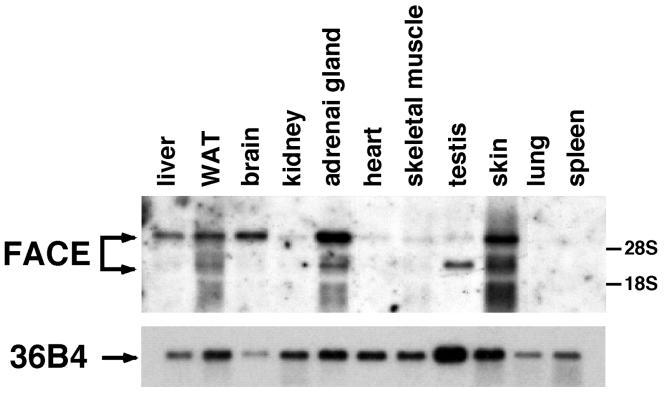


**Fig. 4.** FACE mRNA levels in mouse tissues. Northern blotting analysis was performed with 10 μg of total RNA isolated from variety of mouse tissues with the indicated cDNA probes. WAT, white adipose tissue.

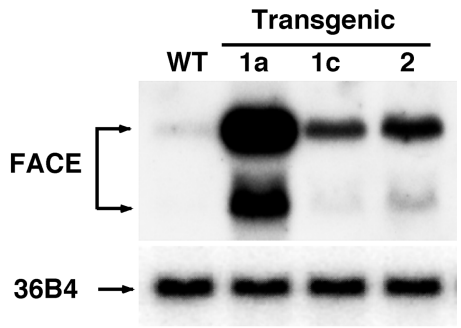


**Fig. 5.** Northern blots analysis of FACE mRNA in livers from transgenic (Tg) mice that overexpress each nuclear form of SREBP-1a, -1c, and -2. Total RNA (15 μg) pooled equally from three mice was subjected to Northern blotting, followed by hybridization with the FACE cDNA probe. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

although SREBP-1-independent expression is also involved in FACE expression.

### Effects of PUFAs on FACE expression

Numerous dietary studies indicate that hepatic activity and expression of lipogenic enzymes are suppressed by diets rich in PUFAs such as vegetable or marine oils (20–22). We used Northern blot analysis to compare mouse hepatic FACE expression levels in mice fed fat-free high carbohydrate diet with the addition of various fatty acids

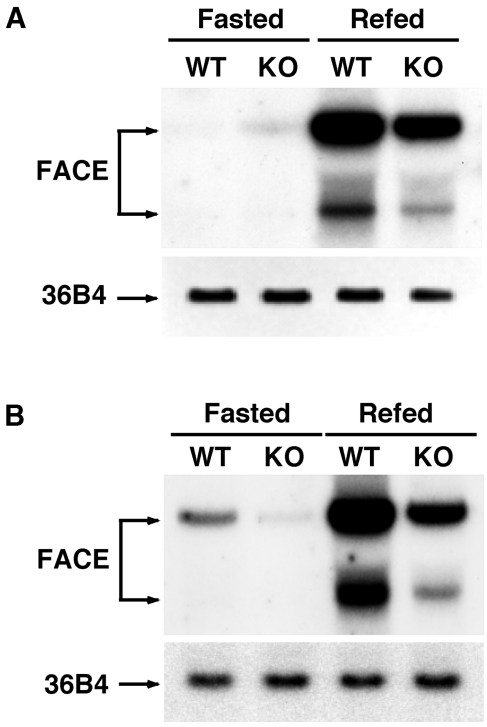


**Fig. 6.** Northern blots analysis of FACE mRNA in livers (A) and WAT (B) from fasted or refed wild-type (WT) and SREBP-1 knockout (KO) mice. Total RNA was extracted from the livers of the mice in each treatment group. Equal aliquots of 15 μg were pooled and subjected to electrophoresis and blot hybridization with the FACE $^{32}$P-labeled cDNA probe. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

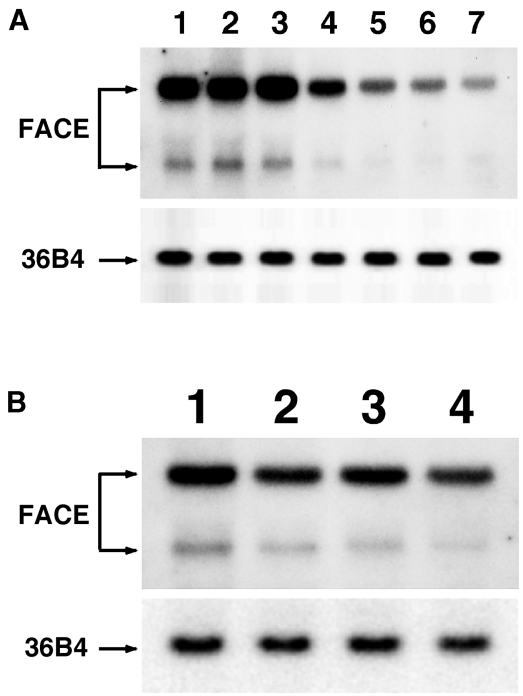


**Fig. 7.** Northern blot analysis of FACE mRNA from livers of wild-type (A) or TgSREBP-1c (B) mice fed a diet containing various fatty acids. A: mice (three male C57BL/6J, 8 weeks old) were fed the indicated diet for 7 days and sacrificed in a non-fasted state. Diets were as follows: lane 1, a high carbohydrate fat-free diet; lane 2, a high carbohydrate diet with 20% tristearin (18:0); lane 3, 20% triolein; lane 4, 5% linoleate ethyl ester (18:2); lane 5, 5% EPA ethyl ester (20:5); lane 6, 20% sardine fish oil; lane 7, 20% tuna fish oil. B: Wild-type mice were fed a high carbohydrate fat-free diet (lane 1), and homozygous TgSREBP-1c mice were fed a high protein diet with 20% triolein (lane 2), 5% EPA ethyl ester plus 20% triolein (lane 3), or 20% sardine fish oil (lane 4) for 7 days and sacrificed in a nonfasted state. Total RNA (10 μg) pooled equally from livers of each group was subjected to Northern blotting, followed by hybridization with the FACE cDNA probe. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

for 7 days. **Figure 7A** shows that stearate (C18:0, lane 2) and oleate (C18:1n-9, lane 3) did not affect the expression of FACE. However, when the high carbohydrate diet was supplemented with linoleates (lane 4) or eicosapentaenoic acid (EPA) (lane 5) the expression of FACE was considerably suppressed, and the reduction was more marked with fish oils rich in EPA and docosahexaenoic acid (DHA) (lanes 6, 7). This specificity to PUFA corresponded to the pattern of suppression observed for other lipogenic enzymes that are regulated by SREBP-1 (20).

To examine whether the PUFA suppression of FACE was ascribed to the decrease in mature SREBP-1, we also tested PUFA suppression of FACE expression in SREBP-1c transgenic mice (Fig. 7B). This line of transgenic mice express similar amounts of nuclear SREBP-1c protein, derived from the transgene, to the physiological level of nuclear SREBP-1c in the livers from normal refed mice (20). The sustained level of nuclear SREBP-1c completely abolished PUFA suppression of FACE mRNA levels. These data suggest that endogenous SREBP-1c is involved in maintaining FACE expression in a fed state.

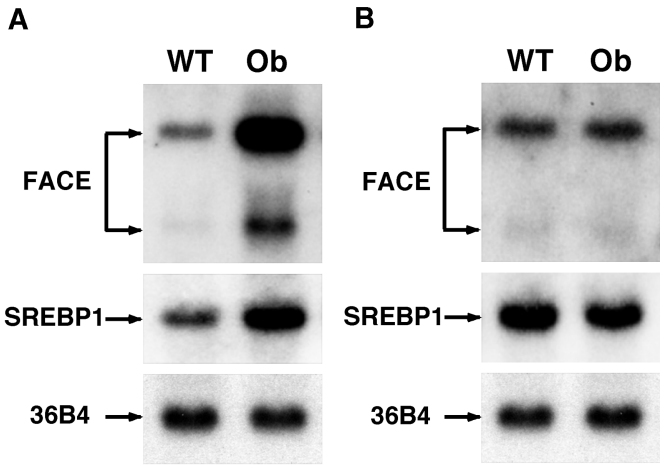


**Fig. 8.** Northern blot analysis of FACE mRNA from livers (A) and WAT (B) of wild-type or ob/ob mice. Mice were fed chow diet and sacrificed in a non-fasted state of early light phase following a 2 h fast. Total RNA (10 μg) pooled equally from three mice was subjected to Northern blotting, followed by hybridization with the FACE or SREBP-1 cDNA probe. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

### Activation of FACE expression in livers from ob/ob mice

Leptin deficient mice (ob/ob) are an excellent murine model for obesity, insulin resistance, and diabetes. Previous studies indicate that the mRNA level of SREBP-1 and SREBP target genes involved in fatty acid biosynthesis were significantly elevated in livers from ob/ob mice compared with wild-type mice, but decreased in WAT (33, 34). We examined FACE mRNA levels in livers and WAT by Northern blot analysis. As shown in **Fig. 8A**, hepatic mRNA levels of FACE and SREBP-1 from ob/ob mice fed ad libitum were elevated compared with their respective wild-type controls. In contrast, the mRNA levels of FACE and SREBP-1 did not show a marked change in adipose tissues from ob/ob and wild-type mice (Fig. 8B). These data are consistent with the way lipogenic enzymes are regulated in ob/ob mice further supporting FACE regulation by SREBP-1c.

### Effects of LXR agonist and PPAR ligands on FACE expression

The oxysterol receptors, LXRα and LXRβ, have been shown to activate SREBP-1c promoter and expression of its downstream lipogenic enzyme genes (24, 25, 35). As shown in **Fig. 9**, administration of T0901317, a pharmacological LXR agonist (35), significantly increased hepatic FACE mRNA levels, whereas the PPARα agonist Wy-14,643 and the PPARγ agonist pioglitazone had no effect. These data again suggest that endogenous SREBP-1c is involved in the expression of FACE. The FACE expression level was not highly induced by the LXR ligand in adipose tissue; the mechanism is currently unknown.

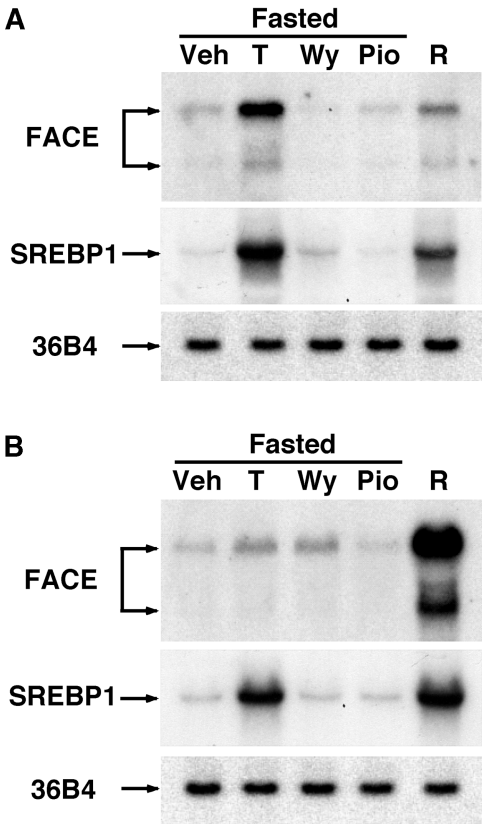


**Fig. 9.** Northern blot analysis of FACE mRNA from livers (A) and WAT (B) of mice administered T0901317, Wy-14,643, or pioglitazone. T0901317, a pharmacological agonist for liver X-activated receptor (LXR) (T, 50 mg/kg), Wy-14,643 for PPARα (Wy, 50 mg/kg), pioglitazone for PPARγ (Pio, 50 mg/kg), or vehicle (Veh, 0.5% carboxymethyl-cellulose) was administered orally to the mice that were fasted and sacrificed 18 h after the treatment. Some mice were fasted for 24 h and refed (R) a chow diet for 12 h. Total RNA (10 μg) pooled equally from three mice was subjected to Northern blotting followed by hybridization with the FACE cDNA probe. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

## DISCUSSION

In the current study, we have described the cloning and characterization of a cDNA that corresponds to a mammalian fatty acid elongase, which we have termed FACE. Mouse FACE cDNA encodes 267 amino acid residues and contains five transmembrane regions, a histidine-rich motif (HXXHH), and ER retention signal (KKXX-like), all typical for members of fatty acid elongase family (6–8). Difficulty in identification of the enzyme responsible for this elongation could be due to its hydrophobicity. Expression studies suggest that FACE has specific activity for the elongation of C16 fatty acids C16:0 and C16:1n-7, a step that FAS cannot commit. Now it can be speculated in the cellular fatty acid synthesis that C16:0 produced by fatty acid synthase in the cytosol is transferred to ER membranes, where ACS, ACBP, FACE, and SCD are sequentially involved to produce the main final product, oleyl-CoA, as schematized in **Fig. 10**. It well fits the fact that oleate is a good endogenous substrate for triglyceride and cholesterol ester formation. In the process of preparing this manuscript, cloning of the same gene from the similar strategy and detailed characterization of the enzyme activity was reported (36). It was shown to have elongase

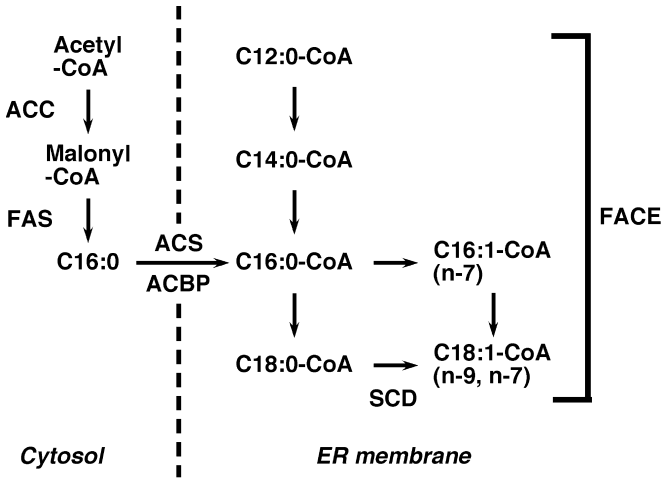


**Fig. 10.** Enzymes involved in mammalian fatty acid elongation. ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; SCD, stearoyl-CoA desaturase; ACS, acyl-CoA synthetase; ACBP, Acyl-CoA binding protein; FACE, fatty acyl-CoA elongase.

activity for C12–C16 fatty acids. Our study demonstrates that FACE also has an elongase activity for conversion of C16:1n-7 to C18:1n-7. It was reported consistently that C18:1n-7 as well as C18:1n-9 is increased in the fatty compositions of liver of rats in a refed state (1).

The FACE cDNA contains an unusually long 3′-untranslated region (UTR) of about 5 kb, which is similar to SCD (37, 38). The similarity of SCD and FACE in both protein and gene structures and the coordinating functions of both proteins suggests that these genes could be derived from a common ancestral gene. The role of such a long 3′-UTR is currently unknown; however, FACE responds to many stimuli such as nutrient intake and hormones, a dynamic response that may require additional post-transcriptional control in addition to transcriptional control by SREBP-1. Indeed, several RNA structural motifs (e.g., AUUUA) characteristic of mRNA destabilization sequences are seen in the 3′-end of the non-coding region. These AU-rich elements (ARE) may play active roles in the selective degradation of several mRNAs in response to various factors (39).

The SREBP-activation as a clue to the cloning of this gene and subsequent studies on in vivo regulation of FACE in mice clearly demonstrate that expression of FACE is controlled by SREBP-1 as lipogenic gene. C18:1 is the major end-product of de novo synthesis of fatty acids in mammalian livers. Consistently, the major fatty acid constituent of livers of SREBP-1a transgenic mice was oleate, suggesting that SREBP activates the whole reactions of this pathway (40). Both FAS and SCD are well-established SREBP targets. Therefore, it is reasonable that FACE is also regulated by SREBPs. Transgenic mouse studies suggest that any isoform of SREBPs can activate FACE expression. Therefore, FACE could also be under sterol-regulation by SREBP-2 in addition to lipogenic regulation by SREBP-1. To establish FACE as an SREBP target gene, detailed promoter analysis on the FACE gene is needed, although the presence of SRE-like and NF-Y sites in the 5′ flanking sequence convincingly suggests that FACE is a direct target of SREBP (36). Remaining FACE mRNA level, although decreased, in lipogenic organs of SREBP-1 null mice could be due to the compensatory activation of SREBP-2 (29), or some unknown SREBP-independent mechanism. Since FACE is expressed in essentially all tissues tested here, it could play a role in a basal cellular function in addition to lipogenesis as an energy storage system.

Our current study established that FACE is a new member of the lipogenic enzyme family, based upon its fatty acyl elongation activity, SREBP activation, and lipogenic regulation in numerous diet studies, such as overshooting induction at refeeding and PUFA suppression. Theoretically, the role of FACE in lipogenesis seems crucial because no other known enzymes have been reported to show the same activity as observed in this enzyme. However, the importance of this gene in lipogenesis as an energy storage system or in more basal cellular functions awaits analysis of effects of the FACE gene disruption, such as characterization of gene knockout mice.

BLAST analysis of the genome resources by NCBI revealed that human and FACE is mapped to chromosome 4 (4q25) and mouse FACE is mapped to chromosome 3. It is interesting that the location of FACE gene is very close to a locus that has been reported to be linked to human exceptional longevity by a genome-wide scan (41). It was reported that elongase activity estimated by C18:0-C16:0 ratio in the muscle is significantly related to adiposity in humans (42), suggesting that FACE could be related to human obesity. Further studies might unveil clinical relevance of FACE to human diseases or pathophysiological states.

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