Dual regulation of mouse Δ^5- and Δ^6-desaturase gene expression by SREBP-1 and PPARα

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Abstract **In the process of seeking sterol regulatory element-binding protein 1a (SREBP-1a) target genes, we identified and cloned a cDNA clone encoding mouse Δ^5-desaturase (D5D). The hepatic expression of D5D as well as Δ^6-desaturase (D6D) was highly activated in transgenic mice overexpressing nuclear SREBP-1a, -1c, and -2. Disruption of the SREBP-1 gene significantly reduced the expression of both desaturases in the livers of SREBP-1-deficient mice refed after fasting. The hepatic expression of both desaturases was downregulated by dietary PUFA, which were reported to suppress SREBP-1c gene expression. Sustained expression of hepatic nuclear SREBP-1c protein in the transgenic mice abolished the PUFA suppression of both desaturases. Although these data suggested that SREBP-1c regulates D5D and D6D expression, there was no difference in either the D5D or D6D mRNA level between fasted and refed normal mouse livers, indicating a mechanism for fasting induction of both desaturases. Administration of fibrate, a pharmacological ligand for peroxisome proliferator activating receptor α (PPARα), caused a significant increase in expression of both desaturases. The data suggested that D5D and D6D expression is dually regulated by SREBP-1c and PPARα, two reciprocal transcription factors for fatty acid metabolism, and could be involved in lipogenic gene regulation by producing PUFA.**—Matsuzaka, T., H. Shimano, N. Yahagi, M. Amemiya-Kudo, T. Yoshikawa, A. H. Hasty, Y. Tamura, J-i. Osuga, H. Okazaki, Y. Iizuka, A. Takahashi, H. Sone, T. Gotoda, S. Ishibashi, and N. Yamada. **Dual regulation of mouse Δ^5- and Δ^6-desaturase gene expression by SREBP-1 and PPARα.** ***J. Lipid Res.*** **2002.** 43: **107–114.**

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PUFA such as arachidonic acid (AA, 20:4n-6), EPA (20:5n-3), and DHA (22:6n-3) play pivotal roles in a number of biological functions including brain development, cognition, reproduction, inflammatory responses, and hemostasis. In addition to being crucial components of membrane phospholipids and functioning in key steps of cell signaling, PUFA have a unique ability to suppress the transcription of hepatic genes encoding lipogenic enzymes (1–4). The availability of 20- and 22-carbon (n-3 and n-6) PUFA is determined by the synthesis of γ-linolenic acid (18:3n-6) and stearidonic acid (18:4n-3) and by the subsequent elongation and desaturation of these fatty acids to 20:4n-6 and 22:6n-3 (5). One of two rate-limiting steps in the production of these polyunsaturated fatty acids is the desaturation of eicosatrienoic acid (20:3n-6) and eicosatetraenoic acid (20:4n-3) to 20:4n-6 and 20:5n-3, determined by the activity of Δ^5-desaturase (D5D). The other step is the desaturation of linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) to 18:3n-6 and 18:4n-3 catalyzed by Δ^6-desaturase (D6D). Studies based on enzymatic activity suggest that the liver is the primary site for 20-carbon polyenoic fatty acid synthesis because the liver is an organ with a great amount of D6D and D5D activity (6). Both enzymes are coordinately regulated by various dietary conditions and hormonal levels in the liver. For instance, D6D activity is induced by peroxisome proliferators and by the supplementation of insulin to diabetic rats (7, 8). It is also highly suppressed by dietary PUFA, indicating that these enzymes are involved in feedback regulation in the production of AA, EPA, and DHA. Remarkable progress in understanding the molecular basis of the nutritional regulation of these desaturases has been made by cloning mammalian D5D and D6D cDNAs (9, 10). The predicted amino acid sequences were suggested to contain a cytochrome b_5-like domain that might dispense with the presence of a separate cytochrome b_5. Suppression of D5D and

Abbreviations: AA, arachidonic acid; D5D, Δ^5-desaturase; D6D, Δ^6-desaturase; PPAR, peroxisome proliferator activating receptor; SRE, sterol regulatory element; SREBP, sterol regulatory element-binding protein.

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D6D activities by dietary unsaturated fatty acids was shown to be associated with decreased D5D and D6D mRNA levels (9, 10).

Sterol regulatory element-binding proteins (SREBP) are membrane-bound transcription factors that belong to the basic helix-loop-helix leucine zipper family (11–13). A sterol-regulated cleavage is required for SREBP to enter the nucleus and activate the transcription of genes involved in cholesterol and fatty acid synthesis by binding to a sterol regulatory element (SRE) or its related sequences, including E-boxes, within their promoter regions (14, 15). There are three forms of SREBP that have been characterized: SREBP-1a and -1c (also known as ADD1) and SREBP-2 (16–18). Most organs, including the liver and adipose tissue, express predominantly SREBP-2 and the c isoform of SREBP-1 (19). Lipogenic enzymes, which are involved in energy storage through the synthesis of fatty acids and triglycerides, are coordinately regulated at the transcriptional level during different metabolic states. 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 (20–22), as well as physiological changes of SREBP-1c protein in normal mice after dietary manipulation, such as placement on high carbohydrate diets, polyunsaturated fatty acid-enriched diets, and fasting-refeeding regimens (23–27). 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 the oxysterol liver X receptor-retinoid X receptor (28, 29). The mechanism for PUFA suppression of the SREBP-1c promoter is under investigation.

Both D5D and D6D play crucial roles in the production of PUFA, which in turn suppresses SREBP-1. In addition, D5D, D6D, and SREBP-1c are nutritionally regulated. These lines of evidence suggest that D5D and D6D expression might be related to SREBP-1 activity. In the screening of SREBP-activated genes, we cloned and identified a mouse D5D gene. Our current studies suggest that nutritional expression of D5D and D6D is regulated by both SREBP-1 and peroxisome proliferator activating receptor α (PPARα).

EXPERIMENTAL PROCEDURES

Materials and general methods

We purchased 20:3n-6 from Sigma (St. Louis, MO), Redivue [α-^{32}P]dCTP (6,000 Ci/mmol) from Amersham Pharmacia Biotech (Piscataway, NJ), and restriction enzymes from New England BioLabs (Beverly, MA).

Standard molecular biology techniques were used. DNA sequencing was performed with a CEQTM dye terminator cycle sequencing kit and CEQ2000 DNA analysis system (Beckman Coulter, Fullerton, CA).

Preparation of SREBP-1a transgenic liver cDNA library

An expression cDNA library of SREBP-1a transgenic liver (21) 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 (29).

Cloning of mouse D5D cDNA

From a DNA microarray system using hepatic poly(A)$^+$ RNA of SREBP-1a transgenic and nontransgenic littermate mice, we identified an expressed sequence tag (EST) clone (GenBank accession number AA068575) that was activated 8-fold in SREBP-1a transgenic liver as compared with wild-type liver, and resembled the mouse D6D. Using this sequence information, an [α-^{32}P]dCTP-labeled DNA probe was prepared and used in the screening from an SREBP-1a transgenic mouse liver cDNA library by colony hybridization. Positive clones were sequenced and the clones containing an open reading frame were used for expression experiments.

Expression of mouse D5D

HEK-293 cells were grown at 37°C in an atmosphere of 5% CO_2 in DMEM containing 25 mM glucose, penicillin (100 U/ml), and streptomycin sulfate (100 μg/ml) supplemented with 10% FCS on 100-mm culture plates. At 80% confluence, the mouse D5D expression plasmid or the basic plasmid CMV7 alone (10 μg) was transfected into cells, using SuperFect transfection reagent (Qiagen, Valencia, CA) according to the manufacturer protocol. After transfection, cells were treated with either 100 μM 20:3n-6 diluted in ethanol or ethanol alone for 24 h and then used for fatty acid analysis.

Fatty acid analysis

Total fatty acid extracted from HEK-293 cells transfected with the mouse D5D expression plasmid or CMV7, in the presence of 100 μM 20:3n-6, was analyzed by gas chromatography of methyl ester derivatives. Lipids were extracted and transmethylated with methanolic HCl, and the fatty acid methyl esters were analyzed as described previously (10).

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. Before sacrifice, each group of animals was fed a diet containing the indicated fatty acids and drugs for 7 days. Fasting and refeeding regimens for wild-type and SREBP-1 knockout mice were previously described (22). Transgenic mice overexpressing the nuclear form of SREBP-1a, -1c, and -2 in the liver under the control of the phospho*enol*pyruvate carboxykinase promoter were previously described (21, 30, 31). SREBP-1c and -2 transgenic mice were homozygous for the transgene (32). These three transgenic lines and wild-type controls (nontransgenic littermates of SREBP-1a transgenic mice) were put on a high protein/low carbohydrate diet to induce transgenic mice for 2 weeks, and were fasted for 12 h before sacrifice. For fasting and refeeding treatment, SREBP-1-deficient and wild-type mice were fasted for 24 h and fed a high sucrose/fat-free diet for 12 h.

Total RNA preparation and Northern blotting

Total RNA was extracted from mouse livers and various tissues with TRIzol reagent (Life Technologies, Rockville, MD). RNA samples were run on a 1% agarose gel containing formaldehyde and transferred 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 D5D was prepared by digesting the cloned cDNA with *Not*I and *Eco*RI. The cDNA probes for mouse D6D and ribosomal phosphoprotein P0 (36B4) were prepared as described previously (10, 22). 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 BASstation software (Fuji Photo Film, Tokyo, Japan).

RESULTS

Cloning mouse D5D as an SREBP-activated gene

DNA microarray analysis identified an EST clone whose expression was increased 7-fold in the livers of SREBP-1a transgenic as compared with wild-type mice. This clone (GenBank accession number AA068575) was originally named an EST clone slightly similar to mouse D6D in the database. Mouse D6D was cloned and reported (10). The sequences suggested that this clone should encode something similar, but not identical, to D6D. Using this EST clone as a probe, we cloned the cDNA clone that contains an open reading frame (ORF) from an SREBP-1a transgenic liver cDNA library. The DNA sequence analysis revealed that the predicted amino acid sequence was highly similar to human and rat D5D (9). Alignment of the predicted amino acid sequences for mouse and human D5D is shown in **Fig. 1**. Eighty-eight percent of the amino acid sequence for the mouse and human homologs was identical. The high homology includes the presence of a cytochrome b_5 domain, transmembrane domains, and histidine-rich regions, all of which were reported for human D5D and mouse D6D.

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mD5D   1 :  MAPDPVPTPG PASAQLRQTR YFTWEEVAQR SGREKERWLV IDRKVYNISD FSRRHPGGSR
            ****  *       **    * **** ***** ** * ***** *********  * ********
hD5D   1 :  MAPD--PLAA ETAAQGLTPR YFTWDEVAQR SGCE-ERWLV IDRKVYNISE FTRRHPGGSR

mD5D  61 :  VISHYAGQDA TDPFVAFHIN KGLVRKYMNS LLIGELAPEQ PSFEPTKNKA LTDEFRELRA
            ********** ********** **** ***** ****** *** *********  **********
hD5D  58 :  VISHYAGQDA TDPFVAFHIN KGLVKKYMNS LLIGELSPEQ PSFEPTKNKE LTDEFRELRA

mD5D 121 :  TVERMGLMKA NHLFFLVYLL HILLLDVAAW LTLWIFGTSL VPFILCAVLL STVQAQAGWL
            ********** ** *** *** ****** *** **** ****   ** ****** * ********
hD5D 118 :  TVERMGLMKA NHVFFLLYLL HILLLDGAAW LTLWVFGTSF LPFLLCAVLL SAVQAQAGWL

mD5D 181 :  QHDFGHLSVF GTSTWNHLLH HFVIGHLKGA PASWWNHMHF QHHAKPNCFR KDPDINMHPL
            **********  ** ****** ********** ********** *********** *********
hD5D 178 :  QHDFGHLSVF STSKWNHLLH HFVIGHLKGA PASWWNHMHF QHHAKPNCFR KDPDINMHPF

mD5D 241 :  FFALGKVLPV ELGREKKKHM PYNHQHKYFF LIGPPALLPL YFQWYIFYFV VQRKKWVDLA
            ****** * * ***  **  * ********** ********** **********  *********
hD5D 238 :  FFALGKILSV ELGKQKKNYM PYNHQHKYFF LIGPPALLPL YFQWYIFYFV IQRKKWVDLA

mD5D 301 :  WMLSFYARIF FTYMPLLGLK GFLGLFFIVR FLESNWFVWV TQMNHIPMHI DHDRNVDWVS
            **  ** * *  ** ******  ********* ********** ********** ***** ****
hD5D 298 :  WMITFYVRFF LTYVPLLGLK AFLGLFFIVR FLESNWFVWV TQMNHIPMHI DHDRNMDWVS

mD5D 361 :  TQLQATCNVH QSAFNNWFSG HLNFQIEHHL FPTMPRHNYH KVAPLVQSLC AKYGIKYESK
            **********  **** **** ********** ********** ********** ** ** * **
hD5D 358 :  TQLQATCNVH KSAFNDWFSG HLNFQIEHHL FPTMPRHNYH KVAPLVQSLC AKHGIEYQSK

mD5D 421 :  PLLTAFADIV YSLKESGQLW LDAYLHQ
            *** *****   ********* *******
hD5D 418 :  PLLSAFADII HSLKESGQLW LDAYLHQ
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Fig. 1. Predicted amino acid sequence and homology analysis of mouse and human D5D. The cytochrome b_5 domain is underlined. Transmembrane domains are shown as dashed lines, and three histidine-rich regions are in boxes.

TABLE 1. Fatty acid profiles of HEK-293 cells transfected with mouse Δ^5-desaturase

	+100 μM 20:3n-6	
Fatty Acid	CMV7	D5D
	mol% of fatty acids	
C14:0	2.80 ± 0.16	2.80 ± 0.14
C16:0	25.63 ± 0.28	26.58 ± 0.78
C16:1n-7	3.24 ± 0.03	3.26 ± 0.08
C18:0	11.23 ± 0.36	11.58 ± 0.51
C18:1n-9	15.10 ± 0.24	15.39 ± 0.08
C18:2n-6	2.69 ± 0.12	2.58 ± 0.08
C20:3n-6	26.55 ± 0.82	20.65 ± 0.88[a]
C20:4n-6	5.69 ± 0.24	8.98 ± 0.10[b]
C22:4n-6	2.20 ± 0.13	4.43 ± 0.35[c]
C22:5n-3	1.79 ± 0.08	1.88 ± 0.25
C22:6n-3	1.84 ± 0.11	1.78 ± 0.08

Fatty acid composition of HEK-293 cells transfected with the mouse Δ^5-desaturase and CMV7, grown in the presence of 100 μM 20:3n-6. Results are expressed as the mol percentage of total fatty acids and as means ± SEM for three independent cultures. Statistical significance was assessed by unpaired *t*-test.

[a] $P < 0.01$ versus CMV7.
[b] $P < 0.0005$ versus CMV7.
[c] $P < 0.005$ versus CMV7.

Expression of mouse D5D

The high similarity of the predicted amino acid sequence from the ORF of the cloned mouse cDNA to the human D5D amino acid sequence suggested that the cDNA encodes the mouse D5D. To confirm this, the cDNA was expressed in HEK-293 cells supplemented with C20:3n-6. As shown in **Table 1**, gas chromatography analysis of the cellular fatty acids revealed that overexpression of this putative D5D clone increased the relative amounts of C20:4n-6 and C22:4n-6, accompanying a decrease in C20:3n-6 in the cells as compared with mock-transfected cells. The data suggested that the cells transfected with the cDNA were capable of synthesizing 20:3n-6 from eicosadienoic acid (20:2n-6), indicating that the cDNA product has a D5D activity.

Tissue distribution of mouse D5D mRNA

Expression of D5D and D6D was examined in various tissues as estimated by Northern blot analysis. Mouse D5D is a single transcript that is approximately 4.5 kb in size (**Fig. 2**). The expression was high in the adrenal gland, liver, brain, and testis, tissues where lipogenesis and steroidogenesis are active. The tissue distribution pattern of mouse D6D expression was similar, suggesting that D5D and D6D cooperatively produce polyenoic fatty acids.

Activation of D5D and D6D expression in liver of SREBP transgenic mice

We performed Northern blot analysis of liver RNA from nuclear SREBP-1a, -1c, and -2 transgenic and wild-type mice to estimate the effects of nuclear SREBP on the expression of D5D as well as D6D. As shown in **Fig. 3**, the hepatic mRNA level of D5D was robustly increased by overexpression of nuclear SREBP-1a, -1c, and -2 at this order of magnitude. Hepatic D6D mRNA levels were also induced by SREBP overexpression in a pattern similar to that of D5D mRNA. A shorter mouse D6D transcript was barely detect-

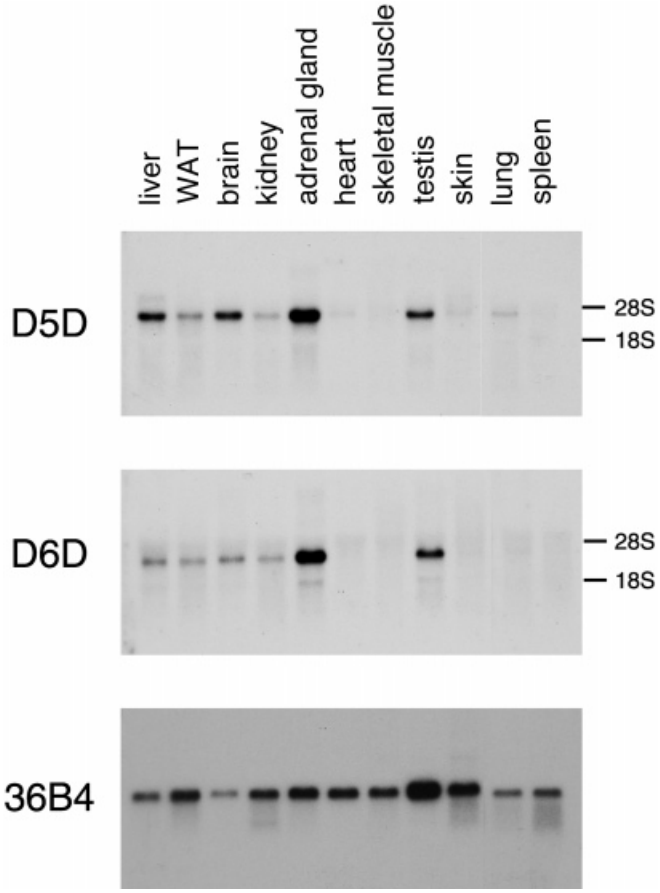

Fig. 2. D5D and D6D mRNA levels in mouse tissues. Northern blotting analysis was performed with 10 μg of total RNA isolated from a variety of mouse tissues with the indicated cDNA probes. WAT, White adipose tissue.

able from livers of fasted wild-type mice, but became prominent in SREBP transgenic livers. This minor, short transcript was identified in human D6D and was reported to be nutritionally regulated as well as the major transcript. The data indicate that every isoform of SREBP can activate the expression of both desaturase genes.

Nutritional regulation of D5D and D6D expression in SREBP-1-deficient mice

Nutritional regulation of hepatic D5D and D6D mRNA levels was estimated in wild-type and SREBP-1 deficient mice. As shown in **Fig. 4**, these mice were fasted and then refed with a high sucrose/fat-free diet. Interestingly, the wild-type mice did not show a marked change in D5D or D6D mRNA level between fasting and refeeding. In the fasted state, the SREBP-1-deficient mice showed hepatic expression levels of both D5D and D6D similar to those of wild-type mice. After refeeding, there was a considerable reduction in the expression of both desaturases in SREBP-1 null mice, showing a much lower level than in the refed wild-type mice. Expression of PPARα was induced by fasting and suppressed by refeeding, whereas the SREBP-1c mRNA level showed the opposite response. These data demonstrate that the hepatic expression of D5D and D6D

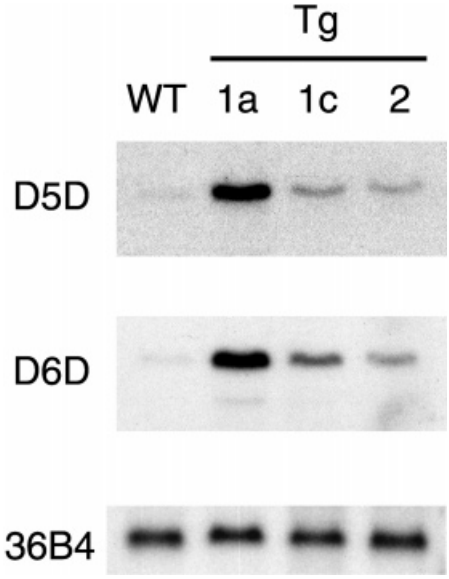

Fig. 3. Northern blot analysis of D5D and D6D mRNA in livers from wild-type (WT) mice and transgenic (Tg) mice overexpressing the nuclear form of SREBP-1a, -1c, and -2. Total RNA (15 μg) pooled equally from each group was subjected to Northern blotting, followed by hybridization with the D5D and D6D cDNA probes. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

is not upregulated by refeeding, but that SREBP-1 contributes strongly to their expression in the refed state. The nutritional regulation of these enzymes is distinct from that of regular lipogenic enzymes, which are suppressed by fasting and are markedly induced by refeeding, although both these desaturases and lipogenic enzymes are regulated by SREBP-1.

Effects of PUFAs on D5D and D6D expression

Numerous dietary studies indicate that the hepatic activity and expression of both desaturases are induced by diets low in essential fatty acids and suppressed by diets rich in PUFA such as vegetable or marine oils. We compared mouse hepatic D5D and D6D expression levels in mice fed a fat-free high carbohydrate diet with various kinds of fatty acids. **Figure 5** showed that stearate (C18:0) or oleate (C18:1) did not affect the expression of D5D and D6D. However, when a high carbohydrate diet was supplemented with linoleate or EPA, the expression of both desaturases was considerably suppressed, and the reduction was more marked with fish oils rich in EPA and DHA. These data were consistent with previous reports on the expression of human and rat desaturase genes. We also tested PUFA suppression of their expression in SREBP-1c transgenic mice (**Fig. 6**). This line of transgenic mice expresses amounts of nuclear SREBP-1c protein, derived from the transgene, similar to the physiological level of nuclear SREBP-1c in the livers of normal refed mice (27). The sustained level of nuclear SREBP-1c completely abolished PUFA suppression of D5D and D6D mRNA levels. These data suggest that endogenous SREBP-1c could

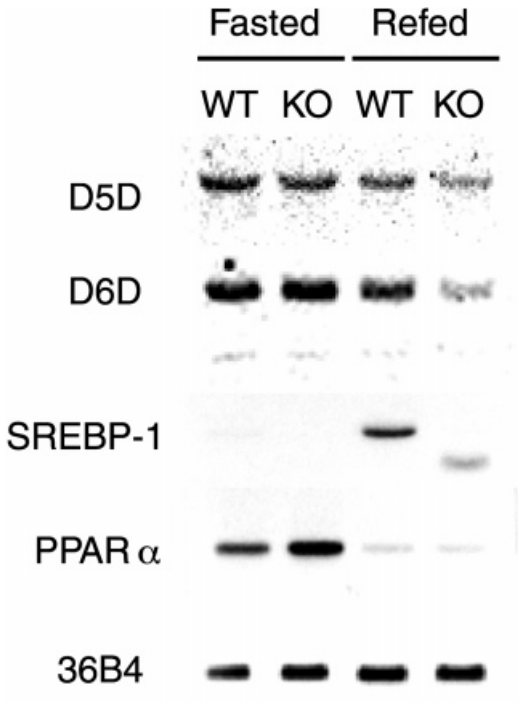

Fig. 4. Northern blot analysis of D5D and D6D mRNA in livers from fasted or refed wild-type (WT) and SREBP-1 knockout (KO) mice. Total RNA was extracted from the livers of the mice in each treatment group. Equal aliquots of 15 μg were pooled and subjected to electrophoresis and blot hybridization with the D5D, D6D, SREBP-1, and PPARα ^{32}P-labeled cDNA probes. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading. Note that SREBP-1c mRNA signal from refed KO mice was an aberrant transcript from the disrupted gene and its product is inactive (22).

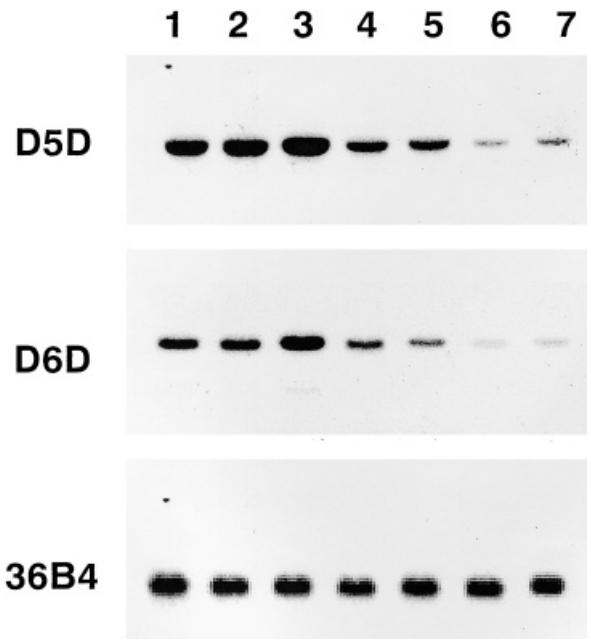

Fig. 5. Northern blot analysis of D5D and D6D mRNA from livers of mice fed a diet containing various fatty acids. Mice (three male C57BL/6J, 8 weeks old) were fed the indicated diet for 7 days and killed in a nonfasted state. Diets were as follows. Lane 1, a high carbohydrate fat-free diet; lane 2, a high carbohydrate diet with 20% tristearin (18:0); lane 3, 20% triolein (18:1); 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. Total RNA (10 μg) pooled equally from three mice was subjected to Northern blotting, followed by hybridization with the D5D and D6D cDNA probes. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

be involved in maintaining D5D and D6D expression in a fed state.

Effects of PPARα ligands on D5D and D6D expression

As shown in Fig. 4, fasting treatment induces expression of PPARα, crucial for fatty acid oxidation. To estimate the possible involvement of PPARα in D5D and D6D expression, we challenged mice with fibrate, a pharmacological PPAR ligand. Addition of fibrate to the diet increased both D5D and D6D mRNA levels (**Fig. 7**) whereas troglitazone, which induces PPARγ, had no effect, indicating that both desaturases can be induced by PPARα and not by PPARγ. These results are consistent with the previous report that peroxisome proliferators enhanced linoleic acid metabolism in rat liver (8).

DISCUSSION

D5D and D6D as potential SREBP-1 targets

The current study clearly demonstrates that SREBP activate the expression of both D5D and D6D in the liver. Both hepatic D5D and D6D mRNA levels were markedly increased by overexpression of SREBP, with preference for SREBP-1 relative to SREBP-2. Furthermore, the absence of SREBP-1 markedly impaired the hepatic expression of these desaturases in a refed state. All these data suggest that D5D and D6D are highly potential SREBP target genes and that SREBP-1 plays a pivotal role in the hepatic expression of these desaturases, at least under lipogenic conditions. The physiological relevance of SREBP activation of these desaturases is currently unknown. Under lipogenic conditions, when SREBP-1 is activated, newly synthesized fatty acids could be dominated by saturated or monounsaturated fatty acids, and availability of essential fatty acids might be limited. In this situation, activation of D5D and D6D might be necessary to meet cellular requirements for AA, EPA, and DHA. Cumulative evidence suggests that the cellular availability of PUFA is crucial for the regulation of lipogenesis. On the assumption that the PUFA that these desaturases synthesize can be used for inhibition of lipogenesis, SREBP-1 induction of these desaturases would contribute to efficient suppression of lipogenesis whenever essential fatty acids such as C18:2n-6 become available. It is consistent with the previous report that D6D activity is important for PUFA suppression of hepatic lipogenic gene expression. This is the first observation that there could be a feedback system for the hepatic SREBP-1c-lipogenesis system, which was thought to depend essentially on the supply of carbohydrates as discussed in the autoloop activation of the SREBP-1c pro-

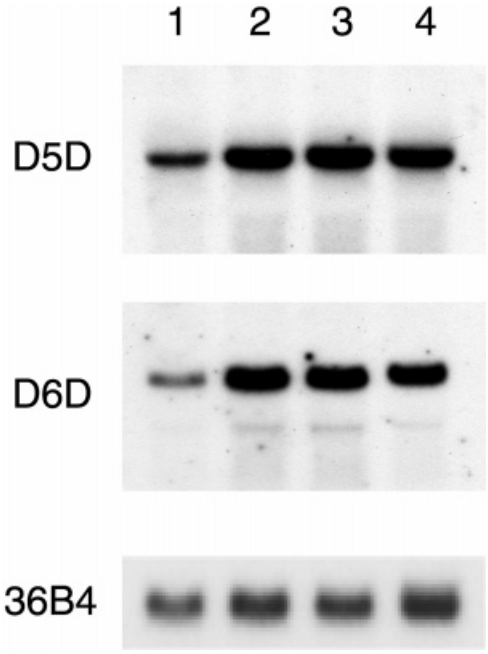

Fig. 6. Northern blot of D5D and D6D mRNA from livers of wild-type or TgSREBP-1c mice fed PUFA diets. Wild-type mice were fed a high carbohydrate fat-free diet (lane 1), and homozygous Tg-SREBP-1c mice were fed a high protein diet with 20% triolein (lane 2), 5% EPA ethyl ester plus 20% triolein (lane 3), or 20% sardine fish oil (lane 4) for 7 days and killed 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 D5D and D6D cDNA probes. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

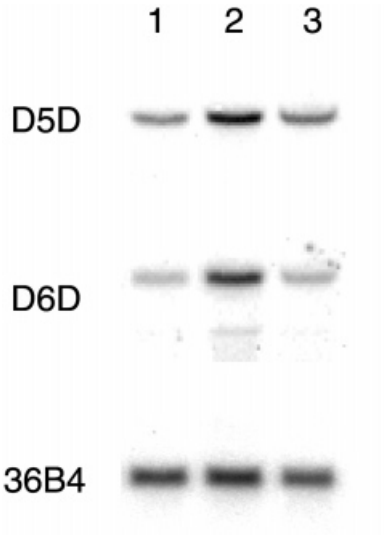

Fig. 7. Northern blot analysis of D5D and D6D mRNA from livers of mice administered fenofibrate or troglitazone. Mice (three male C57BL/6J, 8 weeks old) were fed a high carbohydrate fat-free diet without (lane 1) or with 0.5% fenofibrate (lane 2) or 0.1% troglitazone (lane 3) for 7 days. The mice were killed in a nonfasted state. Total RNA (10 μg) pooled equally from three mice was subjected to Northern blotting, followed by hybridization with the D5D and D6D cDNA probes. A cDNA probe for 36B4 (acidic ribosomal phosphoprotein P0) was used to confirm equal loading.

moter underlying the overshooting phenomenon in a refed state (28).

A mechanism for the feedback suppression of D5D and D6D by PUFA

Dietary PUFA have been reported to decrease both the activity and expression of D5D and D6D (9, 10), a finding supported by our current observations. PUFA were reported to inhibit SREBP-1 activity by multiple mechanisms (24–27, 33, 34). Considering our new finding that D5D and D6D are potential SREBP targets, it is likely that down-regulation of D5D and D6D expression by PUFA could be mediated through suppression of SREBP-1. This hypothesis is further supported by the observation that PUFA suppression of D5D and D6D was completely abolished by the sustained amount of nuclear SREBP-1c protein forcibly expressed in SREBP-1c transgenic mice.

Dual regulation of D5D and D6D expression by SREBP-1 and PPARα

This unique fashion of nutritional regulation (activation by SREBP-1c, but no change by a fasting-refeeding treatment) places D5D and D6D in a position distinct from regular lipogenic enzymes. In particular, in SREBP-1 knockout mice, D5D and D6D mRNA levels are considerably higher in fasted liver than in refed liver. These observations can be explained by the hypothesis that, in addition to SREBP-1c-mediated induction as observed in the refed state of normal mice, there is another regulatory mechanism that activates the expression of D5D and D6D in a fasted state. Our results from administration of fenofibrate, a PPARα ligand, indicate that PPARα is responsible for the fasting induction of these desaturases. Furthermore, we and others previously reported that starvation increases the expression of PPARα, which should enhance fasting-induced activation of D5D and D6D in addition to the ligand activation of PPARα (27, 35). In a fasted state, lipolysis from adipose tissue is enhanced and circulating free fatty acids enter the liver for β-oxidation that is controlled by PPARα. Many of these free fatty acids are essential fatty acids, and can be both ligands for both PPARα and substrates for D5D and D6D. Activation of D5D and D6D by PPARα in the liver might be important to produce required amounts of PUFA.

Taken together, the expression of D5D and D6D could be regulated by two distinct mechanisms, one through SREBP-1c as is shown by its induction during refeeding, and the other through PPARα that is inducible by fasting. Dual regulation of D5D and D6D by the two factors SREBP-1 and PPARα, which possess opposing activation properties depending on nutritional status, caused no remarkable change in the overall expression of these enzymes between fasting and refeeding as schematized in **Fig. 8**. This dual regulation of both desaturases contributes to a stable production of PUFA that are essential for cellular functions regardless of energy state.

Further analysis of the promoter regions of the D5D and D6D genes will be required. Interestingly, these genes

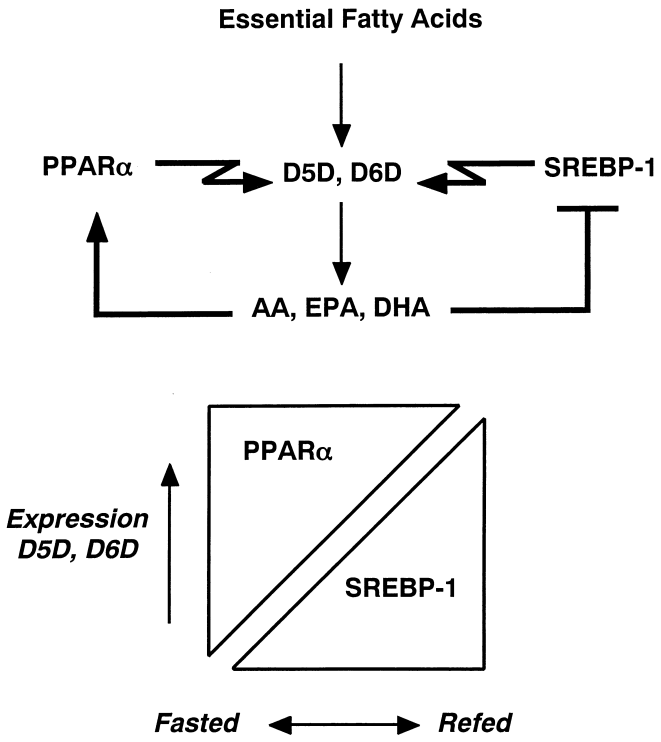

Fig. 8. Dual regulation of D5D and D6D expression by SREBP-1 and PPARα. AA, Arachidonic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

are located close to each other in an inverted orientation, sharing an 11-kb upstream region (9). It would be intriguing if D5D and D6D shared in this region enhancers that are important for nutritional regulation, such as an SRE responsible for SREBP activation and/or a peroxisome proliferator response element, which could explain PPAR ligand activation. The current study suggests that PUFA play an essential role in energy metabolism by regulating or modifying these lipid transcription factors and that D5D and D6D could be involved in this system by producing PUFA.

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