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Journal of Cellular Biochemistry 106:414–426 (2009)

Journal of Cellular Biochemistry

AMP-Activated Protein Kinase Suppresses LXR-Dependent Sterol Regulatory Element-Binding Protein-1c Transcription in Rat Hepatoma McA-RH7777 Cells

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ABSTRACT

AMP-activated protein kinase (AMPK) is an intracellular fuel sensor that plays a key role in regulating fatty acid synthesis in liver. Sterol regulatory element-binding protein (SREBP)-1c is a master regulator of hepatic lipogenic gene expression. It has long been documented that AMPK activation suppresses hepatic SREBP-1 mRNA and nuclear SREBP-1 protein. But the mechanism remains undefined. In this study we investigated the molecular mechanisms by which AMPK downregulates hepatic SREBP-1c mRNA using a novel model cell line McA-RH7777. We found that AMPK is robustly activated in rat hepatoma McA-RH7777 cells treated with two widely used AMPK activators, AICAR and metformin, and AMPK activation sharply suppresses SREBP-1c mRNA and nuclear SREBP-1c protein, but not SREBP-1a mRNA derived from the same gene. These inhibitory effects are reversed by the AMPK inhibitor Compound C or 8-BrAMP, demonstrating the requirement of AMPK in the suppression of SREBP-1c mRNA and nuclear SREBP-1c protein by AICAR and metformin. AMPK does not enhance SREBP-1c mRNA degradation in the presence of the general transcription inhibitor actinomycin D; instead it inhibits SREBP-1c promoter activity in a luciferase reporter assay. AMPK-mediated inhibition of SREBP-1c promoter activity can also be abrogated by the AMPK inhibitor Compound C. Furthermore AMPK activation significantly attenuates the synthetic liver X receptor (LXR) ligand T0901317-induced SREBP-1c promoter activity. AMPK also inhibits cleavage of LXR ligand-induced SREBP-1c precursor. We conclude that AMPK suppresses hepatic SREBP-1c mRNA expression by inhibiting LXR-dependent SREBP-1c transcription via inhibition of endogenous LXR ligand production and by inhibiting SREBP-1c processing in McA-RH7777 cells. J. Cell. Biochem. 106: 414–426, 2009.

KEY WORDS: AMPK; SREBP-1C; McA-RH7777 CELLS; TRANSCRIPTION; LIVER GENE EXPRESSION

The 5′ adenosine monophosphate (AMP)-activated protein kinase (AMPK) is a heterotrimeric enzyme complex consisting of a catalytic subunit (α) and two regulatory subunits (β and γ) [Hardie, 2007]. The α subunit has two isoforms (α1, α2), the β subunit has two isoforms (β1, β2), and the γ subunit has three isoforms (γ1, γ2, and γ3). Activation of AMPK requires phosphorylation of threonine 172 (T172) in the catalytic (α) subunit by an upstream kinase [Hardie, 2007]. Recently the protein kinase LKB1 [Hawley et al., 2003; Woods et al., 2003; Shaw et al., 2004], calmodulin-dependent protein kinase kinase (CaMKK)-β [Hawley et al., 2005; Hurley et al., 2005; Woods et al., 2005], and transforming growth factor-β (TGFβ)-activated kinase-1 (TAK1) [Momcilovic et al., 2006] were identified as the major upstream kinases that phosphorylate and activate AMPK. AMPK is activated by a number of physiologic and pathophysiologic signals such as exercise, hormones, and hypoxia [Hardie, 2007]. In addition,

Abbreviations used: AMPK, AMP-activated protein kinase; AICAR, 5′-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; ACC, acetyl CoA carboxylase, ABCG5, ATP-binding cassette transporter G5; TK, thymidine kinase; 8-BrAMP, 8-bromo-AMP; HMG CoA, 3-hydroxy-3-methylglutaryl coenzyme A; LXR, liver X receptor; NFY, nuclear factor Y; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; PKA, protein kinase A; SCAP, SREBP-cleavage activating protein; SREBP, sterol regulatory element-binding protein.

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: American Heart Association National Center; Grant number: #0635341N.

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Received 29 August 2008; Accepted 18 November 2008 • DOI 10.1002/jcb.22024 • 2009 Wiley-Liss, Inc.

Published online 5 January 2009 in Wiley InterScience (www.interscience.wiley.com).

anti-diabetes drugs metformin and thiazolidinediones (rosiglitazone and pioglitazone) activate AMPK [Zhou et al., 2001; Fryer et al., 2002; Hawley et al., 2002; Musi et al., 2002; Saha et al., 2004].

AMPK plays an important role in regulating hepatic lipid metabolism [Viollet et al., 2006]. In the short-term regulation, AMPK phosphorylates and inactivates acetyl CoA carboxylase (ACC) and 3-hydroxy-3-methylglutaryl (HMG) CoA reductase, thus inhibiting both cholesterol and fatty acid biosynthesis; at the same time, inactivation of ACC decreases malonyl CoA concentration, which in turn derepresses carnitine palmitoyltransferase (CPT1) and activates fatty acid oxidation [Hardie et al., 1998]. In the long-term, AMPK was thought to regulate hepatic lipogenic gene expression by inhibiting transcription factors [Ferre et al., 2003; Leff, 2003]. Multiple lines of evidence indicate that AMPK regulates sterol regulatory element-binding protein (SREBP)-1c [Lin et al., 2000; Zhou et al., 2001; Foretz et al., 2005; Shaw et al., 2005], a master regulator of hepatic lipogenic gene expression [Horton et al., 2002]. Activation of AMPK by metformin represses SREBP-1 mRNA and nuclear (n) SREBP-1 protein in liver [Lin et al., 2000; Zhou et al., 2001]; overexpression of a constitutively active (CA) AMPK-α2 in liver via adenoviral vector-mediated gene delivery inhibits SREBP-1c mRNA expression [Foretz et al., 2005]; and the loss of AMPK activity in the LKB1-deficient mouse liver leads to increased SREBP-1 mRNA level in the fasted state [Shaw et al., 2005]. Recently, we reported that SREBP-1c mRNA expression decreases in the liver of transgenic mice expressing CA-AMPK-α1 [Yang et al., 2008].

Despite these observations the mechanisms by which AMPK regulates SREBP-1c are not understood [Horton et al., 2002; Hardie, 2007]. SREBP-1c is one of the three SREBPs (1a, 1c, and 2) that are membrane-bound transcription factors [Brown and Goldstein, 1997, 1999]. SREBP-1c is regulated at both transcriptional and post-translational levels. At transcriptional level, SREBP-1c promoter contains at least four DNA elements (LXR responsive element or LXRE, NFY, SRE, and Sp1 element), which bind to liver X receptors (LXR), nuclear factor-Y (NF-Y), SREBP, and Sp1, respectively [Chen et al., 2004; Cagen et al., 2005]. SREBP-1c transcription is greatly stimulated by insulin [Horton et al., 1998]. Recent studies revealed that insulin activates SREBP-1c promoter primarily by increasing the activity of LXR, whereas SREBPs and NFY play permissive roles [Chen et al., 2004]. All SREBPs are synthesized as membrane-bound inactive precursors. When cells are depleted of cholesterol, newly synthesized SREBPs form a complex with SREBP-cleavage activating protein (SCAP) in the endoplasmic reticulum and are transported to the Golgi apparatus where they are processed by two distinct proteases, site-1 protease and site-2 protease, that release the NH_2-terminal transcription factor, which can then enter the nucleus. Once inside the nucleus, the transcription factor activates expression of genes encoding enzymes for cholesterol and fatty acid biosynthesis and uptake [Brown and Goldstein, 1997, 1999; Horton et al., 2002]. In contrast, when cells are overloaded with sterols, the SREBP–SCAP complex is retained in endoplasmic reticulum by the insulin-induced gene (Insig) and the proteolysis of the SREBPs is blocked, leading to inhibition of lipid synthesis [Goldstein et al., 2006]. High concentration of cholesterol completely suppresses SREBP-2 processing, whereas full suppression of SREBP-1 processing requires cholesterol as well as fatty acids [Hannah et al., 2001]. Interestingly, insulin signaling stimulates SREBP-1c processing [Hegarty et al., 2005].

In order to dissect the molecular mechanisms of AMPK downregulation of SREBP-1c mRNA expression, we employed a novel model cell line—rat hepatoma McA-RH7777 (ATCC Number CRL-1601) that mimics the behavior of the intact liver by producing high levels of SREBP-1c mRNA and protein [DeBose-Boyd et al., 2001]. In fed liver, SREBP-1c is the major isoform of SREBP-1 (mouse liver 1c-to-1a ratio is 9:1), whereas most cultured cell lines—including the HepG2 cell line that has been used as a standard cell culture model of the human liver—predominantly express SREBP-1a isoform [Shimomura et al., 1997]. Cultured McA-RH7777 cells contain SREBP-1c and 1a in a 3:1 ratio, which is close to the 9:1 ratio in the fed liver [DeBose-Boyd et al., 2001]. Subsequent characterization [DeBose-Boyd et al., 2001] demonstrated that the McA-RH7777 cells recapitulate the normal regulatory mechanism of the SREBP-1c mRNA expression by LXRs as previously observed in the intact liver [Repa et al., 2000]. Therefore, we chose this cell line as the cell culture model to study the regulation of SREBP-1c by AMPK. In this study we demonstrate that AMPK suppresses SREBP-1c mRNA expression by inhibiting LXR-dependent SREBP-1c transcription via inhibition of endogenous LXR ligand production and by inhibiting SREBP-1c processing in McA-RH7777 cells.

MATERIALS AND METHODS

MATERIALS

McA-RH7777 cell line (ATCC#CRL-1601) was purchased from American Type Culture Collection (ATCC, Manassas, VA). Fetal bovine serum (FBS, Cat.#16000-044) and first-strand cDNA synthesis kit were obtained from Invitrogen (Carlsbad, CA), delipidated FBS (Cat.#55-0116) from Cocalico Biologicals (Reamstown, PA), phRL-TK and Dual Luciferase Reporter Assay System (E1960) from Promega (Madison, WI), 5′-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR, Cat.#A611700) from Toronto Research Chemicals, metformin (Cat.#D5035), compactin (Cat.#M4667), actinomycin D (Cat.#A9415), 8-bromoadenosine 5′-monophosphate (8-BrAMP, Cat.#B3131), and 1× PBS/Tween-20 (PBST) from Sigma (St. Louis, MO), Compound C (Cat.#171260) and ALLN (Cat.#208719) from Calbiochem (La Jolla, CA), and the synthetic LXR ligand T0901317 (Cat.#71810) from Cayman Chemical (Ann Arbor, MI), RNA STAT-60 from TEL-TEST (Friendswood, TX), Hybond nitrocellulose membranes from Amersham Bioscience (Piscataway, NJ), Kodak X-Omat Blue XB-1 film, SuperSignal West Pico Chemiluminescent Substrate, and BCA assay kit from Fisher (Pittsburgh, PA), DNA-free™ kit from Ambion (Austin, TX), 2× SYBR Green Supermix (Cat.#1708880) from Bio-Rad (Hercules, CA), FuGENE 6 transfection reagent from Roche (Indianapolis, IN). Anti-AMPK-α, anti-phospho-AMPK-α, and anti-CREB antibodies were purchased from Cell Signaling (Beverly, MA), anti-phospho-ACC(ser79) antibodies from UpState Biotechnology (Lake Placid, NY), anti-calnexin antibody from Stressgen (Ann Arbor, MI), anti-β-actin antibody from Abcam (Cambridge, MA), and anti-LXRα/β antibody (R-20, sc-1206) from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-SREBP-1 and anti-SREBP-2 antibodies were kindly provided by Dr. Joseph Goldstein and

Dr. Michael Brown (UT Southwestern Medical Center at Dallas, TX). The secondary antibodies horseradish peroxidase-conjugated donkey anti-rabbit, donkey anti-goat, and donkey anti-mouse IgG were from Jackson ImmunoResearch (West Grove, PA).

PLASMIDS

The CA version of AMPK-α2 truncated at AA312 was generated by PCR from rat liver cDNA according to Stein et al. [2000] except that the protein has an HA epitope tag (YPYDVPDYA) at the N-terminus. The PCR primers were 5′-GGAATTCGCCATGTACCCATACGATGTTCCTGACTATGCGGGTGGCGGCGAGAAGCAGAAGCACGAC-3′ and 5′-AAAACTCGAGTTAGTATAAACTGTTCATCACTTC-3′. The PCR products were digested with *Eco*RI and *Xho*I and cloned into pcDNA3.1(+), yielding plasmid pCMV-CA-AMPK-α2. The plasmid pD containing the mouse SREBP-1c promoter linked to firefly luciferase reporter gene was previously described [Chen et al., 2004] and kindly provided by Dr. Guoxun Chen (University of Tennessee at Knoxville).

CELL CULTURE

McA-RH7777 cells were cultured and maintained in Medium A (DMEM + 100 units/ml of penicillin and 100 μg/ml streptomycin) supplemented with 10% FBS at 37°C in a 5% CO_2 incubator [DeBose-Boyd et al., 2001].

PREPARATION OF TOTAL CELL LYSATE

On day 0 McA-RH7777 cells were plated at the density of 7×10^5 cells per 100-mm dish and cultured in Medium A supplemented with 10% FBS at 37°C in a 5% CO_2 incubator as previously described [DeBose-Boyd et al., 2001]. On day 2 cells were washed with 1× phosphate-buffered saline (PBS) and cultured for 16 h in Medium A supplemented with 10% delipidated FBS and various compounds as indicated in the figure legends. Each treatment was performed in duplicate dishes. On day 3 the medium was aspirated and the cells in duplicate dishes were harvested with different methods for separate experiments as follows. For preparation of total cell lysates cells were lysed immediately by addition of 1 ml of boiling SDS-lysis buffer (10 mM Tris–HCl, pH 6.8, 100 mM NaCl, 1 mM EDTA, 1 mM EGTA and 1% SDS). Cell lysates were sonicated and proteins (50 μg/lane) were separated by 6 or 8% SDS–PAGE and transferred to nitrocellulose membranes which were blotted with antibodies as indicated. Protein concentration was determined by BCA assay using BSA as the standard.

For preparation of total cellular RNA cells were lysed and total cellular RNA was prepared with RNA STAT-60 according to manufacturer's instructions. For the time course experiment cells were set up on day 0 as above. On day 1 the medium was replaced with Medium A supplemented with 10% delipidated FBS. On day 2 cells were treated with AICAR or metformin at different times in a staggered manner so that all the cells were harvested at the same time. On day 3 cells were harvested with individual methods as described above.

CELL FRACTIONATION

For analysis of SREBP proteins McA-RH7777 cells were cultured and treated as above. Cells were homogenized, and the membrane and nuclear extracts were prepared and immunoblotting analysis was performed with anti-SREBP antibodies as previously described [DeBose-Boyd et al., 2001].

IMMUNOBLOTTING

Proteins (50 μg) were separated by 6 or 8% SDS–PAGE and transferred to Hybond nitrocellulose membranes. The membranes were blocked for 1 h at room temperature in the blocking buffer containing 1× PBS/Tween-20 (PBST) and 5% dry milk (or 5% BSA, depending on the antibodies used). Membranes were incubated with primary antibodies in the blocking buffer and incubated for 1 h at room temperature or overnight at 4°C with gentle shaking, followed by washing three times for 15 min in 1× PBST. The membranes were incubated with appropriate secondary antibodies in the blocking buffer for 30 min and washed three times for 15 min in 1× PBST. Immunoreactive proteins were visualized by SuperSignal West Pico Chemiluminescent Substrate. Membranes were exposed to Kodak X-Omat Blue XB-1 film at room temperature and proteins were quantified by Luminescent Image Analyzer (Model LAS-1000 CH, Fuji Photofilm Co., Japan).

REAL-TIME PCR

Real-time PCR was carried out as previously described [Yang et al., 2001; Liang et al., 2002]. Briefly, total cellular RNAs were prepared with RNA STAT-60, and treated with DNase I before cDNA synthesis. First strand cDNA was synthesized from 5 μg of DNase I-treated total RNA with random hexamer primers by using the first-strand cDNA synthesis system. Real-time PCR of samples was prepared in a final volume of 25 μl containing 50 ng of reverse transcribed total RNA, 167 nM forward and reverse primers, 12.5 μl of 2× SYBR Green Supermix. Real-time PCR was carried out in a 96-well plate on the iCycler iQ Real-Time Detection System (Bio-Rad). All reactions were done in triplicate. The relative amount of all mRNAs was calculated using the comparative C_T method (Applied Biosystem 2001. User Bulletin No.2, Applied Biosystems, Forster City, CA). Ribosomal phosphoprotein 36B4 mRNA was used as the invariant control. ABCG5 and ABCG8 mRNAs were quantified as described [Repa et al., 2002]. For the mRNA decay experiment, the relative amount of mRNA was calculated using the Equation $2^{-\Delta C_T}$, where $\Delta C_T = C_T$ at time X – C_T at time 0 (X = 0, 4, 8, 16 h). The amount of mRNA at time 0 was arbitrarily set as 1 because ΔC_T equals 0. Real-time PCR primer sequences are available upon request.

TRANSFECTION OF McA-RH7777 CELLS AND LUCIFERASE REPORTER ASSAY

On day 0, McA-RH7777 cells were plated at density of 5×10^4 cells per well in 24-well plates in Medium A supplemented with 10% FBS and incubated at 37°C in a 5% CO_2 incubator. On day 1 cells were washed with 0.5 ml PBS and 0.5 ml fresh Medium A was added to each well before transfection. Cells were cotransfected with 0.25 μg plasmids containing wild-type SREBP-1c promoter linked to the firefly luciferase reporter gene and 25 ng control plasmid phRL-TK containing the *Renilla* luciferase reporter gene by using FuGENE 6 transfection reagent. For the cotransfection experiment, 0.25 μg pCMV-CA-AMPK-α2 or the same amount of vector control plasmid pcDNA3.1 (+) was included. Each transfection was done in triplicate

wells. Six hours after transfection cells were washed with 0.5 ml PBS, switched to Medium A supplemented with 10% delipidated FBS and various compounds as indicated in the figure legends, and incubated for 16 h at 37°C and 5% CO_2. On day 2 cells were washed with 0.5 ml PBS and firefly and *Renilla* luciferase activities were measured using the Dual Luciferase Reporter Assay System and Turner Designs TD-20/20 Luminometer. Firefly luciferase activities in the transfected lysates were normalized by *Renilla* luciferase activity or protein concentration from the same tube.

STATISTICS

Experimental data were statistically analyzed using a two-tailed Student's *t*-test between two means. Results were expressed as mean ± SE. The difference between two groups with $P < 0.05$ was considered to be statistically significant.

RESULTS

AICAR AND METFORMIN ACTIVATE AMPK IN McA-RH7777 CELLS

To increase AMPK activity in the cultured McA-RH7777 cells, the cells were treated with two widely used AMPK activators, AICAR [Corton et al., 1995] and metformin [Zhou et al., 2001]. AICAR can be taken up by cells and converted into AICAR monophosphate, which mimics AMP, and thereby activates AMPK [Corton et al., 1995]. Metformin does not directly activate AMPK in vitro and the mechanism of activation of AMPK is not very clear [Hawley et al., 2002]. Figure 1A,B shows the dose-dependent activation of AMPK in

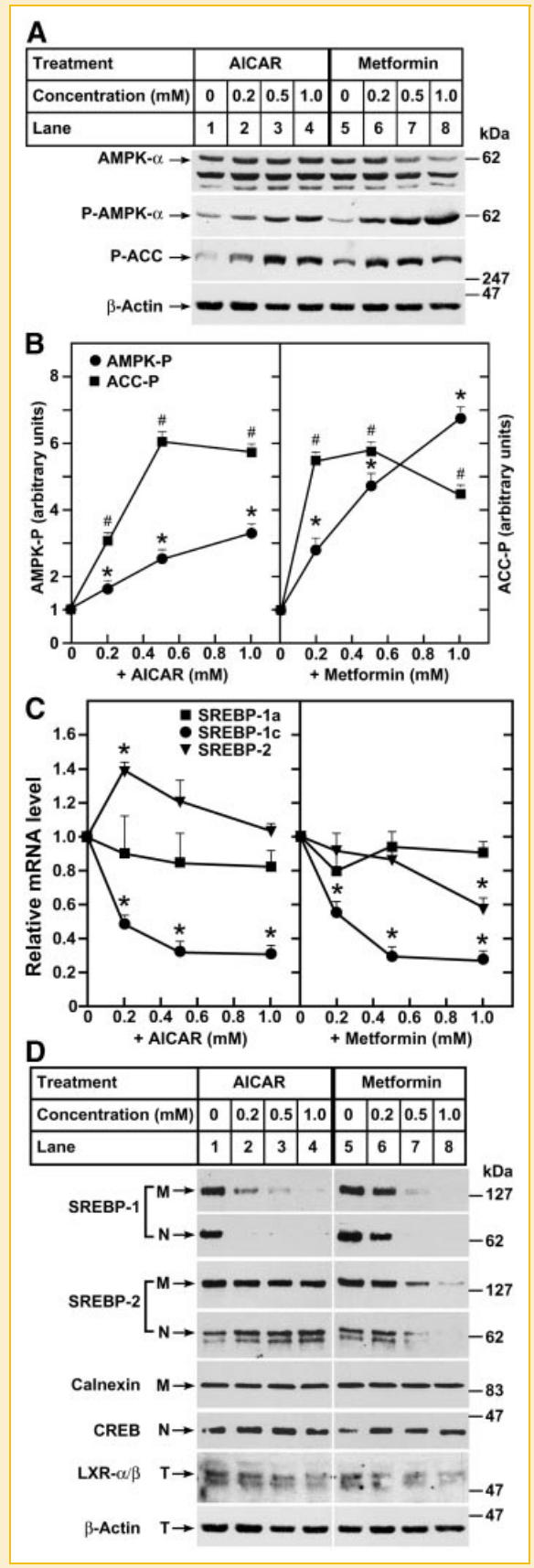

Fig. 1. AMPK activation suppresses SREBP-1c mRNA and nuclear SREBP-1c protein in the McA-RH7777 cells. A: Dose-dependent activation of AMPK in the McA-RH7777 cells by AICAR and metformin. On day 0 McA-RH7777 cells were plated at the density of 7×10^5 cells per 100-mm dish and cultured in Medium A (DMEM + 100 U/ml of penicillin and 100 μg/ml streptomycin) supplemented with 10% FBS at 37°C and 5% CO_2. On day 2 cells were washed with 1× PBS and cultured for 16 h in Medium A supplemented with 10% delipidated FBS and AICAR or metformin at various concentrations as indicated. On day 3 the medium was aspirated and the cells were lysed immediately by addition of 1 ml of boiling SDS-lysis buffer. Cell lysates were sonicated and proteins (50 μg/lane) were separated by 6% or 8% SDS–PAGE and transferred to nitrocellulose membranes, which were blotted with anti-AMPK-α, anti-phospho-AMPK-α, anti-phospho-ACC, and anti-β-actin antibodies as described in Materials and Methods Section. B: Quantitation of AMPK and ACC phosphorylation. Proteins were quantified by Luminescent Image Analyzer as described in Materials and Methods Section. The level of phosphorylated protein in the control was arbitrarily set as 1. $^{*,\#}P < 0.05$ versus control. C: Quantitation of SREBP mRNAs by real-time PCR. McA-RH7777 cells were set up and treated with AICAR and metformin as in A. Total cellular RNA was isolated with RNA STAT-60, treated with DNase I, reverse transcribed with the first-strand cDNA synthesis kit (Invitrogen), and analyzed by real-time PCR with gene-specific primers as described in Materials and Methods Section. The mRNA level in the control was arbitrarily set as 1. $^{*}P < 0.05$ versus control. D: Immunoblotting analysis. McA-RH7777 cells were set up and treated with AICAR and metformin as in A. Cells were fractionated into membrane and nuclear extracts. Membrane protein (M, 30 μg/lane), nuclear extracts (N, 40 μg/lane), and total cell lysate (T, 50 μg/lane) were separated by 8% SDS–PAGE and transferred onto nitrocellulose membranes, which were blotted with anti-SREBP-1, anti-SREBP-2, anti-calnexin, anti-CREB, anti-LXR-α/β (R-20), and anti-β actin antibodies as described in Materials and Methods Section.

the McA-RH7777 cells treated with AICAR and metformin. The concentrations (0.2–1.0 mM for both AICAR and metformin) used here are recommended and generally used by other investigator to increase AMPK activity in cultured liver cell lines [Davies et al., 1989; Zang et al., 2004] or primary hepatocytes [Zhou et al., 2001]. Since phosphorylation of the catalytic subunit AMPK-α at Thr172 position is essential for AMPK activation, AMPK activation was monitored by using a specific antibody that recognizes the phosphorylated AMPK-α at Thr172. As shown in Figure 1A and Figure S1, both AICAR and metformin stimulated phosphorylation of AMPK-α in the McA-RH7777 cells in a dose- and time-dependent manner, whereas the total amount of AMPK-α protein was not significantly changed as assayed by immunoblotting with the anti-AMPK-α antibody. Metformin (1.0 mM) stimulated AMPK phosphorylation at the 2-h time point and the increased amounts of phosphorylation of AMPK were also present at 4, 8, and 16 h. Stimulation of AMPK phosphorylation by AICAR (1.0 mM) was evident after 4 h and again at 8 and 16 h.

To further assess the activity of AMPK the phosphorylation status of ACC, a physiological target that is phosphorylated at Ser79 position by AMPK, was determined. A specific antibody that recognizes the phosphorylated ACC at Ser79 position was used. Figure 1A shows that metformin markedly induced phosphorylation of ACC, which correlated with the increased AMPK phosphorylation. AICAR also effectively increased ACC phosphorylation. In both cases ACC phosphorylation was robustly increased (Fig. 1B), indicating that AMPK was indeed activated within the cell.

AMPK ACTIVATION SUPPRESSES SREBP-1c mRNA AND nSREBP-1c PROTEIN

To determine whether activation of AMPK suppresses SREBP-1c mRNA in the McA-RH7777 cells, total cellular RNA was isolated from the AICAR- or metformin-treated cells and analyzed by real-time PCR. As shown in Figure 1C activation of AMPK with AICAR decreased SREBP-1c mRNA in a dose-dependent manner; SREBP-1c transcript declined 70% in the cells treated with 1 mM AICAR. In contrast, activation of AMPK had no effect on SREBP-1a mRNA, which is transcribed from the same gene encoding SREBP-1c, and had minimal effect on SREBP-2 mRNA. Similarly, AMPK activation by metformin had no effect on SREBP-1a mRNA but reduced SREBP-1c mRNA by 70%. Interestingly, SREBP-2 mRNA also declined 40% in the cells treated with 1 mM metformin.

To assess the SREBP-1c precursor and the nuclear form in the McA-RH7777 cells treated with AICAR or metformin, cell lysates were fractionated into separated membrane fraction where SREBP precursors reside and nuclear fraction where the active nSREBPs are located [DeBose-Boyd et al., 2001]. Figure 1D shows that SREBP-1 precursor decreases in the AICAR-treated cells in a dose-dependent manner and nSREBP-1 protein was barely detectable at all three concentrations. AICAR treatment had minimal effect on SREBP-2 precursor and its nuclear form, which correlated with its mRNA level. Calnexin and CREB were used as loading controls for membrane and nuclear proteins, respectively. Although the anti-SREBP-1 antibody does not distinguish between SREBP-1a and -1c, we reasoned that the dramatic reduction in both the precursor and the nuclear form of SREBP-1 was mainly ascribed to the decrease in SREBP-1c owing to the fact that (1) SREBP-1c is the major isoform of SREBP-1 (the ratio of 1c-to-1a is 3:1) in this cell line [DeBose-Boyd et al., 2001] and (2) SREBP-1c mRNA declined by 70%, whereas SREBP-1a mRNA fell only marginally (Fig. 1C). Metformin had similar effects on SREBP-1 precursor and nSREBP-1 protein as AICAR. Metformin treatment at high concentrations (1.0 mM) also reduced SREBP-2 precursor and its nuclear form.

AMPK ACTIVATION DOES NOT APPEAR TO CHANGE THE AMOUNT OF LXR PROTEIN

SREBP-1c mRNA expression is dominantly regulated by LXRs in McA-RH7777 cells [DeBose-Boyd et al., 2001] as well as in liver [Repa et al., 2000]. To determine whether AMPK activation changed the expression of LXR protein, an anti-LXR antibody (R-20) was used to monitor LXR expression in the McA-RH7777 cells treated with or without AMPK activators [Tobin et al., 2000]. As shown in Figure 1D, AMPK activation did not appear to significantly alter the amount of LXR proteins.

THE AMPK INHIBITORS COMPOUND C AND 8-BrAMP ATTENUATE SUPPRESSION OF SREBP-1c mRNA AND nSREBP-1c PROTEIN

To determine whether the suppression of SREBP-1c in the McA-RH7777 cells treated with AICAR and metformin was mediated by AMPK, the cells were pretreated with the AMPK inhibitor Compound C for 30 min before addition of the activators. Figure 2A shows that Compound C completely abolished AICAR-mediated reduction of SREBP-1c mRNA and significantly blocked metformin-mediated inhibition of SREBP-1c mRNA, suggesting that AMPK is required for suppression of SREBP-1c mRNA by AICAR and metformin.

To confirm the results with compound C another cell permeable AMPK inhibitor 8-BrAMP, an antagonist of AMP and ZMP, that inhibits AMPK activity and thereby blocks AICAR's action [Davies et al., 1989] was used. Figure 2B, shows that activation of AMPK by AICAR decreased SREBP-1c mRNA by 70% but had no effect on SREBP-1a and SREBP-2 mRNAs, thus validating the previous results (Fig. 1). Pretreatment of the cells with 8-BrAMP (100–200 μM) reversed the suppression of SREBP-1c mRNA and had minimal effect on SREBP-1a and SREBP-2 mRNAs. Moreover, the decline in SREBP-1 precursor and its nuclear form was readily blocked by pretreatment with 8-BrAMP (Fig. 2C). These data demonstrate that AMPK is required for the suppression of both SREBP-1c mRNA and nSREBP-1c protein by AICAR and metformin in McA-RH7777 cells.

AMPK DOES NOT ENHANCE SREBP-1c mRNA DEGRADATION

In principle AMPK could suppress SREBP-1c mRNA level by enhancing the rate of SREBP-1c mRNA degradation or by decreasing the rate of SREBP-1c transcription. To determine the effect of AMPK on SREBP-1c mRNA stability, McA-RH7777 cells were treated with the transcription inhibitor actinomycin D (1 μM) in the absence or presence of AMPK activators. Figure 3 shows that both AICAR and metformin treatments did not further decrease either SREBP-1a or SREBP-1c mRNA level in the presence of actinomycin D at early time points (4-or 8-h); on the contrary, activation of AMPK stabilized both SREBP-1a and -1c mRNAs at 16-h.

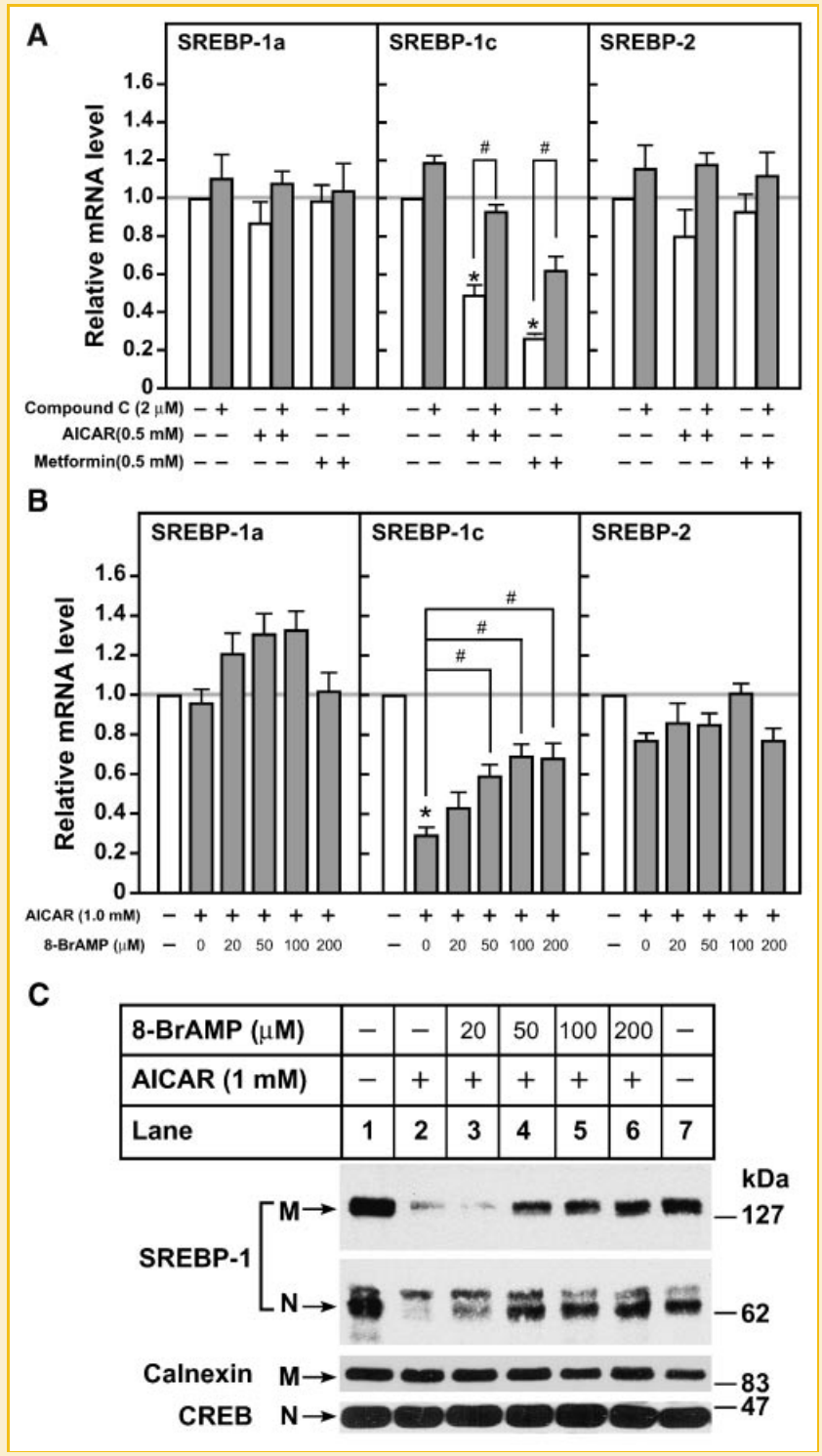

Fig. 2. The AMPK inhibitors Compound C and 8-BrAMP attenuate the suppression of SREBP-1c mRNA in the McA-RH7777 cells treated with AICAR and metformin. A: McA-RH7777 cells were set up and treated with AICAR (0.5 mM) and metformin (0.5 mM) as described in Figure 1, except that the cells were pretreated for 30 min with Compound C (2 μM). Real-time PCR was performed as described in Materials and Methods Section. The control mRNA level without treatment was arbitrarily set as 1.0 and indicated with a gray line. $^{*}P < 0.05$ versus control (no treatment); $^{\#}P < 0.05$ versus control (− Compound C, + AICAR or metformin). B: McA-RH7777 cells were set up and treated with AICAR (1 mM) as described in (A), except that cells were pretreated for 30 min with various concentration of 8-BrAMP as indicated. Real-time PCR was performed as described in (A). The control mRNA level without treatments was arbitrarily set as 1.0 and indicated with a gray line. $^{*}P < 0.05$ versus control (no treatment); $^{\#}P < 0.05$ versus control (− 8-BrAMP, + AICAR). C: McA-RH7777 cells were set up and treated with AICAR (1 mM) and various concentration of 8-BrAMP as described in (B). Cells were harvested and immunoblotting analysis was carried out as in Figure 1.

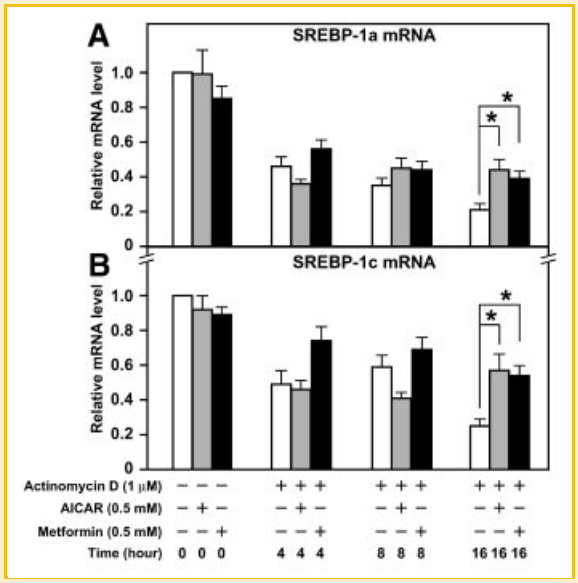

Fig. 3. The effects of AMPK activation on SREBP-1a and -1c mRNA stability in the McA-RH7777 cells. McA-RH7777 cells were set up and treated with different compounds (actinomycin D, 1 μM; AICAR, 0.5 mM; metformin, 0.5 mM) as described in Figure 1. Cells were harvested at different time points as indicated. RNA isolation and real-time PCR were carried out as described in Materials and Methods Section. The control mRNA level at time zero was arbitrarily set as 1.0. $^{*}P < 0.05$ versus control (-AMPK activator) at the same time point 0, 4, 8, or 16 h.

These data indicate that AMPK does not enhance SREBP-1c mRNA degradation.

AMPK ACTIVATION REPRESSES SREBP-1c PROMOTER ACTIVITY

To determine whether AMPK activation represses SREBP-1c transcription, McA-RH7777 cells were transfected with the firefly luciferase reporter Plasmid D that contains the wild-type mouse SREBP-1c promoter (Fig. 4A) [Chen et al., 2004] and a control plasmid phRL-TK encoding the synthetic *Renilla* luciferase gene. Six hours after transfection the cells were treated with or without AMPK activators for 16 h. Figure 4B, shows that AMPK activation by either AICAR or metformin sharply decreased SREBP-1c promoter activity by >75% and these inhibitory effects were readily reversed by the AMPK inhibitor Compound C. Interestingly, Compound C slightly increased the SREPB-1c promoter activity in the absence of AMPK activators, presumably by inhibiting the basal AMPK activity. Figure 4C, shows that expression of a CA-AMPK-α2 by cotransfection of pD with pCMV-CA-AMPK-α2 also inhibited SREBP-1c promoter activity by 70%.

AMPK INHIBITS LXR LIGAND-INDUCED SREBP-1c PROMOTER ACTIVITY

SREBP-1c transcription is dominantly regulated by LXRs in McA-RH7777 cells [DeBose-Boyd et al., 2001] and in liver [Repa et al., 2000], but AMPK activation did not alter the total amount of LXR proteins (Fig. 1D). It is anticipated that AMPK reduces LXR ligand production by inhibiting HMG CoA reductase and this in turn decreases SREBP-1c transcription [DeBose-Boyd et al., 2001]. If this is the case, supplementation of a synthetic LXR ligand should prevent the AMPK-mediated inhibition of SREBP-1c promoter activity. As shown in Figure 5A, SREBP-1c promoter activity dramatically decreased in McA-RH7777 cells treated with AICAR and metformin. Addition of a synthetic LXR ligand T0901317 increased SREBP-1c promoter activity by 4.2-fold in the absence of compactin and by 24-fold in the presence of compactin, but the induction was significantly attenuated by both AMPK activators. It should be noted that T0901317 was able to partially restore the promoter activity in the presence of AICAR or metformin. Similar results were observed in cultured FTO-2B cells (Fig. S2). This indicates that AMPK indeed reduced production of endogenous LXR ligand, which leads to decreased LXR transcriptional activity. However, SREBP-1c promoter activity was still severely inhibited by AICAR and metformin in the presence of T0901317, suggesting that AMPK may directly target LXR and inhibit its intrinsic activity. We also confirmed that AMPK inhibits LXR ligand-induced SREBP-1c mRNA expression in McA-RH7777 cells (Fig. 5B).

AMPK INHIBITS SREBP-1c CLEAVAGE

SREBP-1c is able to activate its own transcription for the SREBP-1c promoter contains an SRE [Amemiya-Kudo et al., 2000; Chen et al., 2004]. A direct inhibition of SREBP-1c cleavage by AMPK may also contribute to the AMPK-mediated suppression of SREBP-1c promoter activity. We were unable to directly test this hypothesis in the McA-RH7777 cell treated with AMPK activators because the inhibition of SREBP-1c transcription by AMPK led to dramatic reduction of SREBP-1c mRNA and this in turn diminished SREBP-1c precursor and nSREBP-1c (Fig. 1C,D). However, the LXR ligand T0901317 was able to partially restore SREBP-1c promoter activity (Fig. 5A) and SREBP-1c mRNA (Fig. 5B) in the presence of AMPK activators. We reasoned that the partially restored SREBP-1c mRNA by T0901317 will be translated to SREBP-1c precursor, which should be processed to nSREBP-1c as previously demonstrated [DeBose-Boyd et al., 2001], if AMPK does not inhibit SREBP-1c cleavage.

Figures 5C,D shows that the AMPK-caused decrease in SREBP-1c mRNA (Fig. 5B) was accompanied with a decline in SREBP-1 precursor and the nuclear form, either in the presence or absence of T0901317. As a control, compactin dramatically decreased SREBP-1 precursor and nSREBP-1 (lane 9, Fig. 5C,D), and T0901317 completely prevented the decline of SREBP-1c mRNA (Fig. 5B) and nSREBP-1 protein (compare lanes 9 and 10, Fig. 5C,D) in the presence of compactin. Compactin induces the cleavage of SREBP-2 and increases nSREPB-2 protein (compare lanes 1 and 9, Fig. 5C,D); SREBP-1 precursor decreases but nSREBP-1 increases despite the complete restoration of the SREBP-1 (1a plus 1c) mRNA by addition of T0901317 (compare lanes 1 and 10 in Fig. 5C,D), indicating that compactin also induced the cleavage of SREBP-1 in the presence of T0901317. AMPK is known to inhibit cholesterol synthesis and this should induce the cleavage of all three SREBPs. The nSREBP-1 protein was not increased in the AICAR-treated cells despite the presence of abundant SREBP-1 precursor that was partially restored by T0901317 (compare lanes 4, 6, 8 with lane 10, Fig. 5C), suggesting that activation of AMPK by AICAR inhibited the cleavage of

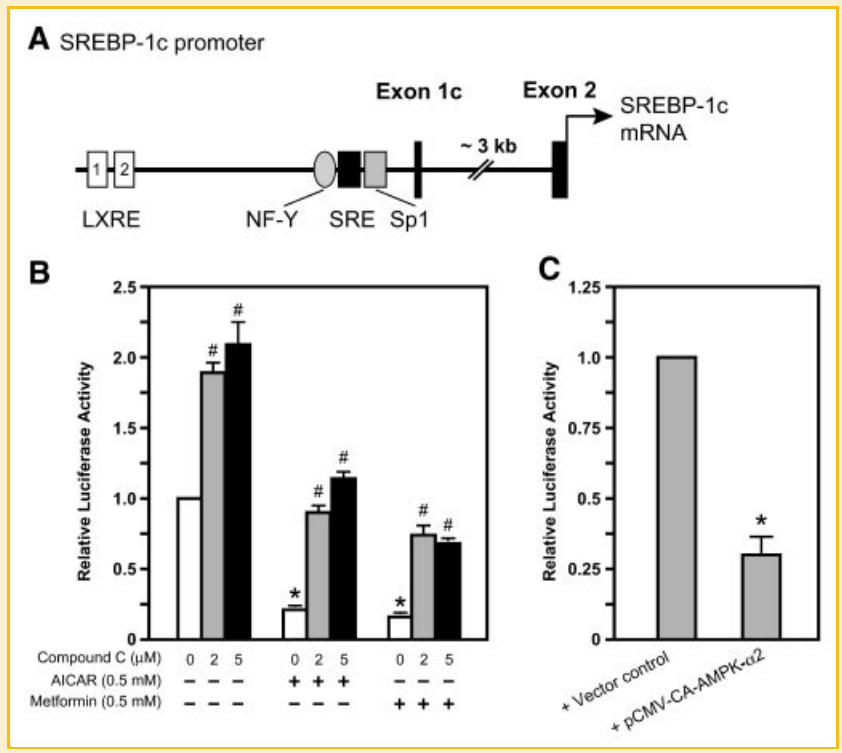

Fig. 4. AMPK activation suppresses SREBP-1c promoter activity and the AMPK inhibitor Compound C attenuates AMPK inhibition. A: Diagram of plasmid D that contains the wild-type mouse SREBP-1c promoter linked to the firefly luciferase reporter gene [see Chen et al., 2004]. B: Luciferase reporter assay. On day 0 McA-RH7777 cells were plated at density of 5×10^4 cells per well in 24-well plates in Medium A supplemented with 10% FBS and incubated at 37°C in a 5% CO_2 incubator. On day 1 cells were washed with 0.5 ml PBS and 0.5 ml fresh Medium A was added to each well before transfection. Cells were cotransfected with plasmid D and a control plasmid. Six hours after transfection, cells were washed with 0.5 ml PBS, switched to Medium A supplemented with 10% delipidated FBS and 0.5 mM AICAR or metformin in the absence or presence of compound C, and incubated for 16 h at 37°C and 5% CO_2. On day 2 cells were washed with 0.5 ml PBS and luciferase activities were measured as described in Materials and Methods Section. Normalized firefly luciferase activity without treatment was arbitrarily set as 1. $^*P < 0.05$ versus control (no treatment), $^\#P < 0.05$ versus control (-Compound C) in the same group. C: McA-RH7777 cells were set up and luciferase reporter assays were performed as in B except that 0.25 μg pCMV-CA-AMPK-α2 or the same amount of vector control plasmid pcDNA3.1 (+) was included in the transfection. $^*P < 0.05$ versus vector control.

SREBP-1c precursor. Interestingly, the amount of nSREBP-2 protein was not changed in the presence of AICAR at any of the three concentrations (Fig. 5C). Figure 5D shows that activation of AMPK by metformin also inhibited SREBP-1c cleavage (see lane 8, Fig. 5D). Again metformin treatment at high concentrations reduced the SREBP-2 precursor and the nuclear form, consistent with the results shown in Figure 1. Figure 5E shows that T0901317 did not affect AMPK and ACC phosphorylation induced by both AICAR and metformin. These data demonstrate that AMPK inhibits SREBP-1c cleavage.

AMPK SUPPRESSES mRNA EXPRESSION OF TWO LXR TARGETS ABCG5 AND ABCG8

To further confirm that AMPK inhibits LXR-dependent transcription, we analyzed the mRNA levels of two well-established LXR targets ATP-binding cassette transporter G5 (ABCG5) and ABCG8 in McA-RH7777 cells treated with AMPK activators. Figure 6 shows that the mRNA levels of ABCG5 and ABCG8 were significantly decreased by both AICAR and metformin treatments, similar to that of SREBP-1c (Fig. 1C). Since ABCG5 and ABCG8 are not regulated by SREBP-1c [Liang et al., 2002], these data strongly support the notion that AMPK activation suppresses LXR transcriptional activity.

DISCUSSION

SREBP-1c is a master regulator of lipogenic gene expression in liver [Horton et al., 2002] and its upregulation in the liver of animal models of obesity and type 2 diabetes increases the rate of fatty acid synthesis [Shimomura et al., 1999], further aggravating insulin resistance in these fatty liver diseases [Brown and Goldstein, 2008]. It has been known that the anti-diabetes drug metformin activates AMPK in liver, which suppresses SREBP-1 mRNA and nSREBP-1 protein, and this in turn decreases hepatic fatty acid synthesis [Lin et al., 2000; Zhou et al., 2001]. But the mechanism remains undetermined. Here we used a novel model cell line McA-RH7777 [DeBose-Boyd et al., 2001] to dissect the molecular mechanisms. We found that AMPK is robustly activated in McA-RH7777 cells treated with both AICAR and metformin and AMPK activation sharply suppresses SREBP-1c mRNA and nSREBP-1c protein, but not SREBP-1a mRNA derived from the same gene. These inhibitory

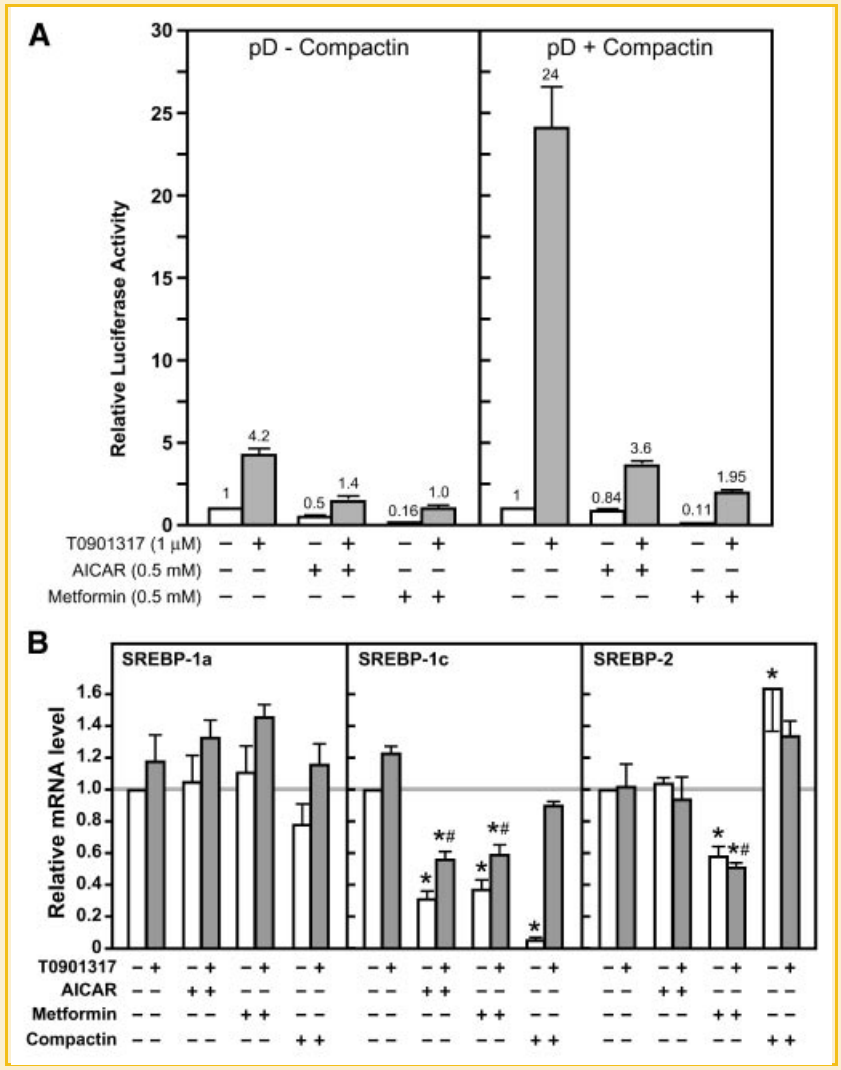

Fig. 5. AMPK activation suppresses LXR ligand-mediated induction of SREBP-1c promoter activity, SREBP-1c mRNA, and nSREBP-1c protein in the McA-RH7777 cells. A: AMPK activation represses LXR ligand-mediated induction of SREBP-1c promoter activity. On day 0 McA-RH7777 cells were plated at density of 5×10^4 cells per well in 24-well plates in Medium A supplemented with 10% FBS and incubated at 37°C in a 5% CO_2 incubator. On day 1 cells were washed with 0.5 ml PBS and 0.5 ml fresh Medium A was added to each well before transfection. Cells were cotransfected with plasmid D and a control plasmid as described in Figure 4. Six hours after transfection, cells were washed with 0.5 ml PBS, switched to Medium A supplemented with 10% delipidated FBS and 0.5 mM AICAR or metformin or 1 μM T0901317 in the absence or presence of compactin (50 μM compactin + 50 μM sodium mevalonate), and incubated for 16 h at 37°C and 5% CO_2. On day 2 cells were washed with 0.5 ml PBS and luciferase activities were measured as described in Materials and Methods Section. Normalized firefly luciferase activity without treatment was arbitrarily set as 1. B: AMPK activation represses LXR ligand-mediated induction of SREBP-1c mRNA in the McA-RH7777 cells. McA-RH7777 cells were set up and treated with compounds (T0901317, 10 μM; AICAR, 1 mM; metformin, 1 mM; compactin, 50 μM compactin + 50 μM sodium mevalonate) as described in Figure 1. Total cellular RNA was isolated with RNA STAT-60, treated with DNase I, reverse transcribed with the first-strand cDNA synthesis kit (Invitrogen), and analyzed by real-time PCR with gene-specific primers as described in Materials and Methods Section. The control mRNA level without treatment was arbitrarily set as 1.0 and indicated with a gray line. $^*P < 0.05$ versus control (-T0901317), $^\#P < 0.05$ versus control (+T0901317). C and D: AMPK activation suppresses cleavage of LXR ligand-induced SREBP-1c precursor. McA-RH7777 cells were set up and treated with various concentrations of AICAR (C) and metformin (D) and other compounds (T0901317, 10 μM; Compactin, 50 μM compactin + 50 μM sodium mevalonate) as described in Figure 1. Cells were fractionated into membrane and nuclear extracts as described in Materials and Methods Section. Membrane protein (30 μg/lane) and nuclear extracts (40 μg/lane) were separated by 8% SDS–PAGE and transferred onto nitrocellulose membranes, which were blotted with anti-SREBP-1, anti-SREBP-2, anti-calnexin, and anti-CREB antibodies as described in Figure 1. E: Cells were set up and treated as in (B and C). Cells were harvested and total cell lysates (50 μg/lane) were separated by 8% SDS–PAGE and transferred onto nitrocellulose membranes, which were blotted with anti-AMPK-α, anti-phospho-AMPK-α, anti-phosphoACC, and anti-β-actin antibodies as in Figure 1.

effects can be attenuated by the AMPK inhibitors Compound C and 8-BrAMP, demonstrating the requirement of AMPK in the suppression of SREBP-1c mRNA and nSREBP-1c protein by AICAR and metformin. AMPK does not enhance SREBP-1c mRNA degradation in the presence of the general transcription inhibitor actinomycin D; instead it inhibits SREBP-1c promoter activity in McA-RH7777 cells in a luciferase reporter assay. We demonstrated that AMPK activation significantly attenuates the synthetic LXR

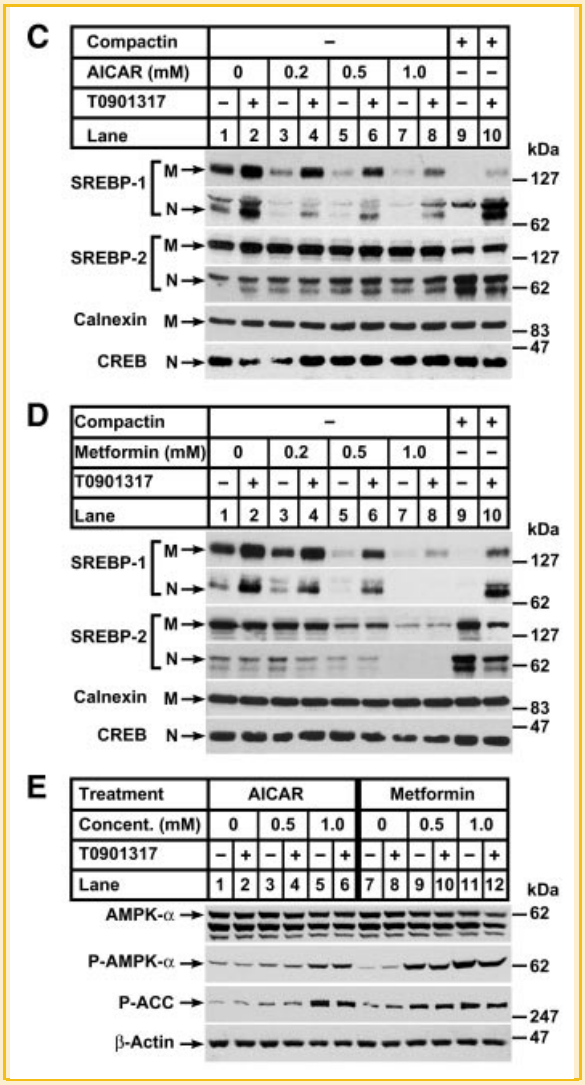

Fig. 5. (*Continued*)

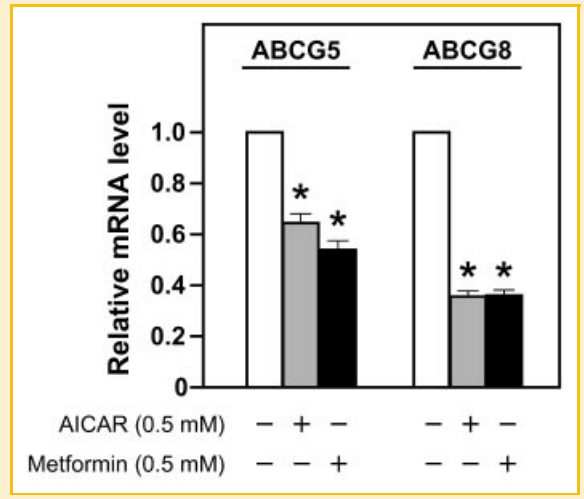

Fig. 6. AMPK suppresses mRNA expression of LXR targets ABCG5 and ABCG8 in the McA-RH7777 cells. The McA-RH7777 cells were set up and treated as described in Figure 1C. Total cellular RNA was isolated with RNA STAT-60, treated with DNase I, reverse transcribed with the first-strand cDNA synthesis kit (Invitrogen), and analyzed by real-time PCR as described in Materials and Methods Section. The mRNA level in the control was arbitrarily set as 1. $^{*}P < 0.05$ versus control.

ligand T0901317-induced SREBP-1c promoter activity in McA-RH7777 cells. We further demonstrated that AMPK inhibits SREBP-1c cleavage. Based on our findings, we propose a working model of how AMPK regulates SREBP-1c mRNA expression as illustrated in Figure 7. In this model, AMPK suppresses SREBP-1c transcription by at least two mechanisms: (1) AMPK inhibits cholesterol biosynthesis, leading to the reduced production of endogenous oxysterol ligands required for activation of LXRs; (2) AMPK inhibits SREBP-1c cleavage, blocking the feed-forward activation of SREBP-1c promoter activity by itself.

Both AICAR and metformin have other targets besides AMPK [Hardie, 2006]. However, similar inhibitory effects of both AMPK activators on SREBP-1c mRNA and nSREBP-1c protein and SREBP-1c promoter were observed (Figs. 1 and 4). These inhibitory effects can be attenuated or reversed by the AMPK inhibitor Compound C or 8-BrAMP (Figs. 2 and 4), clearly demonstrating the requirement of AMPK in the suppression of SREBP-1c mRNA expression by AICAR and metformin. A CA-AMPK-α2 also inhibited SREBP-1c promoter activity (Fig. 4C), further confirming the involvement of AMPK in the suppression of SREBP-1c transcription.

AMPK is known to regulate cellular mRNA turnover [Wang et al., 2002], but this does not appear to be the mechanism of AMPK downregulation of SREBP-1c mRNA (Fig. 3). The SREBP-1a and -1c transcripts are derived from the same gene with alternative promoters and alternate exon 1, designated 1a and 1c, each of which is spliced into a common exon 2, and thereafter they are the same [Shimomura et al., 1997]. The SREBP-1a and 1c mRNAs have different 5′ untranslated regions and different translational start sites. Information is scarce about the regulation of the degradation or decay of SREBP-1a and -1c mRNAs in liver cells. Several labs have reported that polyunsaturated fatty acids suppress SREBP-1c mRNA by enhancing its degradation in primary hepatocytes [Xu et al., 2001] and cultured HEK-293 cells [Hannah et al., 2001]. Activation of AMPK in the cultured McA-RH7777 cells did not facilitate early degradation of either SREBP-1a or -1c mRNA in the presence of a general transcription inhibitor actinomycin D (Fig. 3). Interestingly, prolonged activation of AMPK prevented further decline of SREBP-1c mRNA induced by actinomycin D (Fig. 3). It appears that AMPK exerts a general effect on cellular mRNAs when all transcription is inhibited by actinomycin D because it also stabilizes SREBP-1a mRNA. Actinomycin D is known to induce apoptosis in liver cells [Leist et al., 1994]; AMPK has both pro-apoptotic [Meisse et al., 2002] and anti-apoptotic activities [Shaw et al., 2004]. It is likely that AMPK inhibits actinomycin D-induced apoptosis and thus prevents further reduction of cellular mRNAs required for cell survival.

Activation of AMPK sharply decreases SREBP-1c promoter activity in McA-RH7777 cells (Figs. 4 and 5) and this correlates with

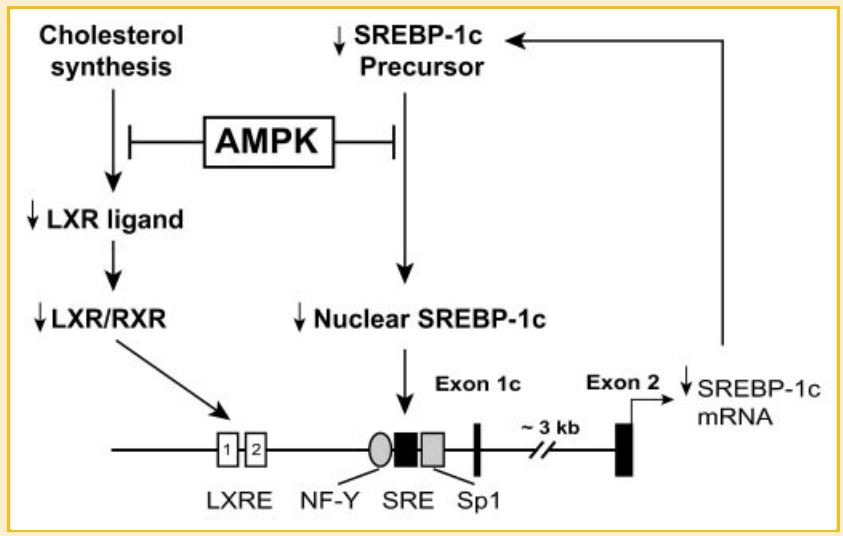

Fig. 7. A model of AMPK-mediated suppression of SREBP-1c transcription. As described in the text, AMPK suppresses SREBP-1c transcription by at least two mechanisms: (1) AMPK inhibits cholesterol biosynthesis, leading to reduction of endogenous oxysterol ligands required for activation of LXR; (2) AMPK inhibits SREBP-1c cleavage, blocking the feed-forward activation of SREBP-1c promoter activity by itself. Both events will lead to inhibition of SREBP-1c promoter activity and suppression of SREBP-1c mRNA expression.

the reduction of endogenous SREBP-1c mRNA (Fig. 1). The SREBP-1c promoter is highly conserved among different species and comprises four DNA elements (LXREs, NF-Y, SRE, and Sp1 element), which bind to the transcription factors LXRs, NFY, SREBPs, and Sp1, respectively [Chen et al., 2004; Cagen et al., 2005]. LXRs are the dominant regulators; SREBPs and NF-Y play permissive roles [Chen et al., 2004]. AMPK phosphorylates and inhibits HMG CoA reductase [Sato et al., 1993; Henin et al., 1995], which would decrease production of the ligands required for activation of LXRs [DeBose-Boyd et al., 2001; Chen et al., 2004]. AMPK may also inhibit cholesterol biosynthesis by inhibiting SREBPs (Figs. 1 and 5). As expected, addition of the synthetic LXR ligand T0901317 partially restored SREBP-1c promoter activity (Fig. 5A) as well as the endogenous SREBP-1c mRNA (Fig. 5B) in the presence of AMPK activators. The first mechanism in our model (Fig. 7) that AMPK inhibits LXR activity by inhibiting endogenous ligand production is well supported by our data (Fig. 5) and the published data [Sato et al., 1993; Henin et al., 1995]. However, LXR ligand-induced SREBP-1c promoter activity (Fig. 5) was still severely attenuated by AMPK activators in the absence and presence of compactin, a potent HMG CoA reductase inhibitor that blocks production of endogenous oxysterol ligands [DeBose-Boyd et al., 2001]. Furthermore, AMPK activation did not change LXR protein expression in McA-RH7777 cells (Fig. 1D), consistent with the published observation that the amount of LXR protein is not changed in the fasted liver [Yamamoto et al., 2007], while AMPK activity is increased [Munday et al., 1991; Witters et al., 1994; Assifi et al., 2005]. These data suggest that AMPK may directly target LXR or its heterodimeric partner RXR and inhibit their intrinsic activities. LXRs are phosphorylated in the nucleus, but the physiologic significance of LXR phosphorylation is not clear [Chen et al., 2006]. A recent study reported that protein kinase A (PKA) phosphorylates LXRs and inhibits LXR transcriptional activity, leading to decreased SREBP-1c transcription [Yamamoto et al., 2007]. One can postulate that AMPK, like PKA, directly phosphorylates LXRs and inhibits LXR transcriptional activity, and this in turn results in decreased SREBP-1c transcription. Further studies will be needed to test this hypothesis.

SREBP-1c transcription is positively regulated by itself via the regulatory element SRE, which is essential to the basal promoter activity [Amemiya-Kudo et al., 2000; Chen et al., 2004]. AMPK apparently inhibits SREBP-1c cleavage and decreases the active nSREBP-1c protein (Fig. 5). This effect appeared to be more specific for SREBP-1c because SREBP-2 cleavage was not significantly affected by AMPK activators (Figs. 1 and 5). It is also possible that AMPK facilitated degradation of nSREBPs. In either case, it would block the feed-forward activation of SREBP-1c transcription by SREBP-1c itself and result in decreased SREBP-1c transcription (Mechanism 2 in Fig. 7). SREBP-1c is one of the three SREBPs (1a, 1c, and 2) that require two-step proteolytic reactions for activation [Brown and Goldstein, 1997, 1999]. Exactly how AMPK inhibits SREBP-1c cleavage or enhances nSREBP-1c degradation remains to be determined.

Hepatic fatty acid synthesis is regulated by both nutritional and hormonal changes, which are manifested in the fast-fed cycle under normal physiological conditions. Recent research indicates that SREBP-1c plays a key role in mediating this regulation [Horton et al., 1998; Liang et al., 2002]. In the classical fasting experiments performed with mice [Horton et al., 1998; Liang et al., 2002], three major changes were observed on SREBP-1c mRNA and protein: first, the SREBP-1c mRNA declined; second, the SREBP-1c precursor declined; and third, notwithstanding that there was still plenty of SREBP-1c precursor present in the liver after a 6-h fast, the nuclear SREBP-1c protein was virtually undetectable at this time point. Importantly, transgenic mice that overexpress the nuclear SREBP-1c

failed to show the normally decreased mRNAs encoding for enzymes in the fatty acid synthesis pathway upon fasting, indicating that the reduced nuclear SREBP-1c upon fasting is responsible in part for the decrease in these mRNAs [Horton et al., 1998]. This notion is further supported from the studies with the SREBP-1c-deficient mice [Liang et al., 2002]. These experiments suggest that fasting induces a factor (or factors) that (1) inhibits SREBP-1c transcription and/or enhances SREBP-1c mRNA degradation, and (2) inhibits the cleavage of SREBP-1c precursor and/or enhances the degradation of the nuclear SREBP-1c. The AMPK activity in liver increases in the fasted state and rapidly declines after refeeding [Munday et al., 1991; Witters et al., 1994; Assifi et al., 2005], which correlates reciprocally with the changes of SREBP-1c mRNA and nuclear SREBP-1c protein in the fasting-refeeding cycle [Horton et al., 1998; Liang et al., 2002]. Based on this study, we speculate that AMPK is involved in the regulation of SREBP-1c and lipogenic gene expression in the normal fast-fed cycle in liver. Further studies in animal models will be required to address this important question.

In summary, we have demonstrated that AMPK suppresses SREBP-1c mRNA expression by inhibiting LXR-dependent transcription of *Srebp-1c* gene via inhibition of endogenous LXR ligand production and by inhibiting SREBP-1c cleavage in rat hepatoma McA-RH7777 cells. These novel findings expand the ever-increasing knowledge of AMPK biology and provide new information for understanding the therapeutic effects of the anti-diabetes drug metformin.

ACKNOWLEDGMENTS

We thank Dr. Joseph Goldstein and Dr. Michael Brown for providing the anti-SREBP antibodies and intellectual support, Dr. Guoxun Chen (University of Tennessee at Knoxville) for the SREBP-1c promoter construct, and Dr. Thomas Lincoln and Dr. Michael Cohen for critical review of the manuscript. Fuichi Yap provided excellent technical assistance. Sharon Hong was supported in part by the Summer Medical Student Research Program at University of South Alabama College of Medicine. This work was supported by National Scientist Development Grant #0635341N from American Heart Association National Center (JY).

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