Mediator, a large multisubunit protein complex, plays a pivotal role in gene transcription by linking gene-specific transcription factors with the preinitiation complex and RNA polymerase II. In the liver, the key subunit of the Mediator complex, Med1, interacts with several nuclear receptors and transcription factors to direct gene-specific transcription. Conditional knock-out of Med1 in the liver showed that hepatocytes lacking Med1 did not regenerate following either partial hepatectomy or treatment with certain nuclear receptor activators and failed to give rise to tumors when challenged with carcinogens. We now report that the adenosinovirally driven overexpression of Med1 in mouse liver stimulates hepatocyte DNA synthesis with enhanced expression of DNA replication, cell cycle control, and liver-specific genes, indicating that Med1 alone is necessary and sufficient for liver cell proliferation. Importantly, we demonstrate that AMP-activated protein kinase (AMPK), an important cellular energy sensor, interacts with, and directly phosphorylates, Med1 in vitro at serine 656, serine 756, and serine 796. AMPK also phosphorylates Med1 in vivo in mouse liver and in cultured primary hepatocytes and HEK293 and HeLa cells. In addition, we demonstrate that PPARα activators increase AMPK-mediated Med1 phosphorylation in vivo. Inhibition of AMPK by compound C decreased hepatocyte proliferation induced by Med1 and also by the PPARα activators fenofibrate and Wy-14,643. Co-treatment with compound C attenuated PPARα activator-inducible fatty acid β-oxidation in liver. Our results suggest that Med1 phosphorylation by its association with AMPK regulates liver cell proliferation and fatty acid oxidation, most likely as a downstream effector of PPARα and AMPK.

Mediator, a large multisubunit protein complex consisting of nearly 30 subunits, plays an essential role in the transcription of all RNA polymerase II-transcribed genes in eukaryotic cells (1–3). In metazoans, the transcriptional activation or repression signals, generated as a result of transcription factors and nuclear receptors binding to upstream gene promoter sequences, converge on the Mediator complex. The Mediator then integrates these diverse upstream signals and delivers them to the RNA polymerase II transcription machinery (1, 2). The Mediator, therefore, is essential for the regulation of all protein-coding genes including those responsible for homeostasis, growth, differentiation, and development (1–3). It is likely that the nuclear receptor regulation of different metabolic pathways may depend on the specificity of the Mediator subunit interaction with different nuclear receptors. For the past several years, we have been studying the role of Mediator subunit Med1 (also known as PBP/TRAP220/DRIP205/CRSP1/RB18A) (4–8) in the regulation of metabolic pathways responsible for the normal liver functions and in the development of liver diseases. The Med1 subunit was first cloned as the nuclear receptor peroxisome proliferator-activated receptor (PPAR)α-binding protein and was characterized as a transcription coactivator (4). In earlier studies, we and others have shown that Med1 null mutation in mice results in embryonic lethality between E11.5 and E12.5 (5, 6). In later studies, we and others have shown that Med1 null mutation in mice results in embryonic lethality between E11.5 and E12.5 days, thus establishing that Med1 plays a fundamental role in...
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In mammals, the liver is a major organ that maintains energy homeostasis by monitoring both the short- and long-term whole body energy needs (17, 18). Hepatic fuel metabolism is a tightly regulated process in that the synthesis, utilization, and storage of carbohydrates and lipids are under constant surveillance by the metabolic needs of the cell (17, 18). Med1 is critical to energy homeostasis because of its role in fatty acid oxidation and glucose metabolism (13, 19). This is attributed to the coactivator function of Med1 for PPARα and PPARβ, the two nuclear receptors involved in energy expenditure (13, 19–21).

Recent studies have identified several catabolic processes including glucose uptake and fatty acid synthesis and stimulates their overexpression in these cancer cells (28, 29). Thus Med1 may play a pivotal role in neoplastic and non-neoplastic cell proliferation.

Adenosine monophosphate-activated protein kinase (AMPK), a heterotrimeric protein kinase composed of a catalytic α-subunit and two regulatory subunits (β and γ), plays a key role in energy homeostasis (30–33). AMPK acts as a conserved molecular sensor for AMP levels in cells and closely monitors the ATP/AMP ratio. The kinase is activated by an elevated AMP/ATP ratio, when AMP levels rise in cells as a result of ATP consumption due to cellular processes that utilize ATP (33). The activated AMPK reduces glucose production and lipid synthesis by decreasing the expression of gluconeogenic and lipogenic enzymes (32–36). In liver, AMPK phosphorylates its targets to inhibit the anabolic processes including gluconeogenesis and cholesterol and fatty acid synthesis and stimulates several catabolic processes including glucose uptake and fatty acid oxidation (32, 33). Recent studies have identified several proteins as AMPK targets including PGC-1α, a coactivator of nuclear receptors, and several other transcription factors (37–41). Evidence indicates that Med1 plays a major role in integrating the upstream transcriptional control in liver and orchestrates metabolic pathways of carbohydrate, fat, and protein metabolism. These observations prompted us to examine whether Med1 is a target of AMPK. In this study, we show that Med1 alone is sufficient for the induction of hepatocyte proliferation. We present evidence that Med1 associates with AMPK in vivo and that it is phosphorylated by AMPK both in vitro and in vivo. Using in vitro kinase assays, we established that Med1 is a substrate for AMPK and identified three AMPK phosphorylation sites, serines 656, 756, and 796. The chemical compound AICAR, an AMP analog and a widely studied activator of AMPK (42), stimulated Med1 phosphorylation in vivo, indicating that Med1 is a target of AMPK in vivo. We further show that PPARα activators fenofibrate and Wy-14,643 phosphorylated Med1 in vivo, presumably by enhancing AMPK activation. Our data suggest that AMPK phosphorylation of Med1 may be an important mechanism by which liver controls cell proliferation and maintains energy homeostasis.

**EXPERIMENTAL PROCEDURES**

**Reagents**—Recombinant, human AMPK (α1, β1, and γ1) holoenzyme was purchased from Millipore (Billerica, MA, catalog No. 14-840). The AMPK activator AICAR was purchased from Tocris Bioscience (Minneapolis, MN), and the specific inhibitor compound C and fenofibrate were obtained from Sigma. Wy-14,643 was custom-synthesized gift from Dr. Reddy’s Laboratories, Ltd., Hyderabad, India. Radioisotopes, [γ-32P]ATP (catalog No. BLU0025000UC) and [32P]orthophosphate (catalog No. NEX054025MC) were purchased from PerkinElmer Life Sciences.

**cDNA Constructs and Antibodies**—GST-Med1 constructs Med1-A (AA 440–740), Med1-B (AA 740–1130), and Med1-C (AA 980–1370) were made previously (25). Two fragments of Med1-B, designated Med1-B1 (AA 670–790) and Med1-BII (AA 770–950), were subcloned into the BamHI/EcoRI sites of the pGEX-5X-1 expression vector using PCR. These fragments were generated based on the presence of consensus AMPK phosphorylation sites, and each of them contains one phosphorylation site for the AMPK. Three mutants, S656A, S756A, and S796A, were generated by site-directed mutagenesis (QuikChange™ kit, Stratagene, La Jolla, CA) according to the manufacturer’s directions, and the resulting constructs were verified by DNA sequencing in their entirety to show that no additional mutations were introduced during the PCR or mutagenesis steps. The oligonucleotides used for the generation of wild-type and mutant clones are listed in supplemental Table S1A. The generation of other GST fusion fragments used in this study, such as PPAR-binding protein (PPB/Med1)-1–6, and the full-length mouse Med1 containing a His tag at the N terminus and cloned into pShuttle-CMV vector (pShuttle-His-Med1) have been described previously (25). FLAG-AMPKα plasmid was a kind gift from Dr. Hong-Gang Wang (Penn State College of Medicine, Hershey, PA) (43). Anti-Med1 (sc-8998) and anti-His (sc-803) were from Santa Cruz Biotechnology, Santa Cruz, CA. M2 mouse monoclonal FLAG antibody (F1804) was purchased from Sigma. Antibodies against fatty acyl-CoA oxidase1 (ACOX1),...
peroxisomal ω-bifunctional enzyme (ω-PBE/Ehhadh), peroxi- 
somal thiase (PTL), peroxisomal δ-bifunctional enzyme (δ-PBE),
medium chain acyl-CoA dehydrogenase, and catalase were from
Prof. Takashi Hashimoto, Matsumoto, Japan.

Adeno-Med1 Virus—Adenovirus expressing His-tagged Med1
(Ad-Med1) was generated and amplified using standard adeno-
ivirus preparation techniques. Briefly, mouse Med1 (His-Med1)
cloned into pShuttle-CMV was co-transformed with Pacllin-
earized AdEasy vector (Quantum Biotechnologies, Inc., Mon-
treal, Canada) into Escherichia coli strain B/J5183. Positive
recombinant plasmid Ad/His-Med1 was then used for the ade-
novirus generation as described previously (44). Ad-LacZ was a
kind gift from Professor W. El-Deiry (Penn State College of
Medicine, Hershey, PA).

Mice and Treatment—Wild-type (C57BL/6), Med1+/−, Med1ΔN
(13), and PPARα−/− (45) mice ages 4–6 weeks were used in this
study. Mice were maintained on a 12-h light/dark cycle and had
ad libitum access to water and pelleted chow. For Med1 over-
expression studies, mice were injected via the tail vein with 4 ×
1013 Ad-Med1 particles in a volume of 200 μl and killed 3 or 5
days after injection. Ad-LacZ-injected mouse liver served as
the control. For liver cell proliferation analysis, mice were admin-
istered bromodeoxyuridine (BrdUrd) (0.5 mg/ml) in drinking
water and were killed at the end of 3 or 5 days. Liver sections
(4-μm thick) were fixed in 10% neutral buffered formalin or 4%
paraformaldehyde and embedded in paraffin. BrdUrd nuclear
labeling indices were obtained by analyzing immunohisto-
chemically stained liver sections as described (13). Images were
acquired by a light microscope adapted to a high resolution
camera (AxioCam, Carl Zeiss) and analyzed by the computer
using AxioVision Rel 4.8 software (Carl Zeiss). All procedures
of animal handling were approved by the Institutional Animal
Care and Use Committees of Northwestern University.

Cell Culture—HEK293T and HeLa cells were grown in Dul-
becco’s modified Eagle’s medium (DMEM) (Cellgro, Mediat-
ecch, Inc., Manassas, VA) containing 10% heat-inactivated fetal
bovine serum and 100 units/ml penicillin plus 100 μg/ml strep-
tomycin (HyClone, Logan, UT) at 37 °C with 5% CO₂. Primary
mouse hepatocytes were isolated from 5–7-week-old C57BL/6
mice by a two-step collagenase perfusion method and sub-
sequently purified by three rounds of low speed cen-trifugation
(46). The viability of hepatocytes was ~95% as determined by
trypan blue exclusion. Isolated hepatocytes were plated onto
100-mm dishes (BD Bioscences) using DMEM, which included
10% fetal bovine serum. Four hours later, the culture medium
was replaced with fresh medium to remove dead or unattached
cells and kept overnight for 12 h before starting the experiment.
All cells were grown at 37 °C in a humidified atmosphere of 5%
CO₂.

AMPK in Vitro and in Vivo Phosphorylation Analyses—GST-
Med1 fragments, wild type, and mutants were expressed in bac-
teria (BL21 strain; Invitrogen) and purified as described else-
where (47). Recombinant GST-Med1 protein (5 μg) bound to
glutathione beads was used as a substrate for the in vitro
phosphorylation reaction when incubated with 30 ng of active
AMPK in kinase buffer (20 mM HEPES (pH 7.4), 10 mM magne-
sium acetate, 0.5% glycerol, 0.1% Triton X-100, and 1 mM dithio-
threitol) containing either 2 μCi of [γ-32P]ATP or nonradioac-
tive ATP (0.1 mM) for 30 min at 30 °C and processed as
described previously (25). For AMPK-dependent in vivo phos-
phorylation of Med1, HEK293 cells, HeLa cells, and mouse pri-
mary hepatocytes were infected with Ad-Med1 (3 × 109 virus
particle) or transiently transfected with 24 μg of pShuttle-His-
Med1 plasmid using Lipofectamine (Invitrogen). Cells washed
twice in phosphate-free buffer (containing in mM: 140 NaCl, 3
KCl, 1 MgSO4·7 H2O, 10 glucose, and 10 HEPES (pH 7.4) with
1 mM Tris base) were labeled at 37 °C for 6 h in 4 ml of phosphate-
free medium with 2% serum (DMEM, high glucose, and no
phosphate; catalog No. 11971-025) containing 0.5 μCi/ml
32P]orthophosphate (PerkinElmer Life Sciences, catalog No.
NEX054025MC). Cells were treated with 1 μM AICAR, an
AMPK activator, or 1 mM compound C for 4 h. Ad-Med1-in-
fected cells, not treated with AICAR but incubated with
32P]orthophosphate, served as the basal control. Metabolic
labeling using PPARα activator Wy-14,643 (100 μM) or fenofi-
bate (100 μM) was performed in HeLa cells, and primary hepa-
tocytes were infected with Ad/Med1.

After metabolic labeling, cells were washed once in ice-cold
phosphate-free buffer before lysis in ice-cold radioimmuno-
precipitation assay buffer containing 1% (w/v) Nonidet P-40, 1%
(w/v) sodium deoxycholate, 0.1% (w/v) SDS, 0.075 M sodium
phosphate; catalog No. 11971-025) containing 0.5 mCi/ml
32P]orthophosphate, served as the basal control. Metabolic
labeling using PPARα activator Wy-14,643 (100 μM) or fenofibrate
(100 μM) was performed in HeLa cells, and primary hepa-
tocytes were infected with Ad/Med1.

LC-MS/MS—Med1 protein was phosphorylated with AMPK
in the presence of 100 μM cold ATP and run on Mini-Protean
TGX gel (4–20%; Bio-Rad, Catalog No. 456-1093S). Specific
bands were excised and processed by the Proteomic Core Facility
at the University of Arkansas for Medical Sciences for LC-MS/MS
analysis. Briefly, gel slices were dehydrated in acetonitrile (Fisher).
Peptide products were then reduced in 10 mM Tris [2-carboxyethyl]
phosphine (Pierce) and alkylation in 50 mM iodoacetamide (Sigma-Aldrich) followed by
reduction in 10 mM Tris [2-carboxyethyl]phosphine (Pierce) and
alkylation in 50 mM iodoacetamide (Sigma-Aldrich). Gel slices
were then dehydrated in acetonitrile (Fisher) followed by the addi-
tion of 100 ng of porcine trypsin (Promega, Madison, WI) in 100
μM ammonium bicarbonate (Sigma-Aldrich) and incubation.
For proteinase K (Promega, catalog No. V3021) digestion, incub-
ation was done at 37 °C for 2–4 h. Peptide products were then
acidified in 0.1% formic acid (Fluka). Tryptic peptides were sep-
parated by reverse phase Jupiter Proteo resin (Phenomenex,
Torrance, CA) on a 100 × 0.075-mm column using a nano-
ACQUITY UPLC system (Waters). Eluted peptides were ionized by electrospray (1.9 kV) followed by MS/MS analysis using col-
cision-induced dissociation on an LTQ Orbitrap Velos mass
spectrometer (Thermo Fisher Scientific). Proteins and modifi-
cations were identified by a database search using Mascot
(Matrix Science, Boston, MA).

Immunoprecipitation and Immunoblotting—Lysates were
pre pared in Nonidet P-40 lysis buffer (25 mM Tris-HCl (pH 7.5),

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150 mM NaCl, 1 mM Na2EDTA, 1 mM EGTA, 1% Nonidet P-40, 1% sodium deoxycholate, 1 mM PMSF, and 1 mM Na3VO4) 293T cells transfected with FLAG-AMPK/H9251 and infected with Ad/His-Med1. The clarified lysates were subjected to immunoprecipitation overnight with 2.5 g of anti-Med1 (sc-8998) or anti-FLAG (F1804) antibody in the presence of protein G-Sepharose beads (catalog No. 17-0618-01, GE Healthcare). The resulting complexes were washed, denatured, separated by SDS-PAGE, transferred onto nitrocellulose membrane (Bio-Rad), immunoblotted with the indicated antibodies, and visualized with Super-Signal West Pico chemiluminescent substrate (Pierce Chemicals).

Quantitative Real-time PCR and Microarray Analysis—Total RNA isolated from mouse liver with TRIzol® reagent (Invitrogen) was reverse-transcribed to make cDNA using the Superscript III first strand synthesis system for RT-PCR (Invitrogen) The primers used for the real-time PCR are listed in supplemental Table S1B. Quantitative expression of genes was checked using SYBR Green (Applied Biosystems, Foster City, CA) in triplicates and normalized with 18S ribosomal RNA. The relative gene expression changes were measured using the comparative CT method, 

\[ \frac{C_{	ext{Target}}}{C_{	ext{Reference}}} = 2^{-\frac{\Delta C_{T}}{\Delta C_{R}}} \]

Microarray analysis was done on a MouseWG-6 BeadChip (Illumina) containing 45,281 genes as described elsewhere (22).

Multiple Sequence Alignment—To determine the AMPK phosphorylation motif surrounding the serine residues on Med1 across different species, multiple sequence alignments were done using the Web-based ClustalW2 tool.

RESULTS

Exogenously Introduced Med1 Induces Hepatocyte Proliferation—Our previous work showed that upon partial hepatectomy, livers from the Med1<sup>Liv</sup> mice do not regenerate, indicating that the Mediator complex without Med1 inhibits liver cell proliferation (13–16). Because the Mediator complex plays an important role in establishing the cell type-specific transcriptional networks (1, 2), we sought to determine whether overexpression of Med1 in wild-type mouse liver or reintroduction of Med1 alone into Med1<sup>Liv</sup> mouse liver. Control animals were injected with an adenovirus vector expressing β-galactosidase (Ad-LacZ; Fig. 1B, left panel). Mice were then labeled with BrdUrd in drinking water for 5 days and then killed. Liver sections ~4 μm thick and processed immunohistochemically revealed that nuclear incorporation of BrdUrd in Ad-LacZ–injected livers was negligible,
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whereas a large number of nuclei in Ad-Med1-injected livers (∼35% of the total number of nuclei examined) were positive for BrdUrd nuclear staining (Fig. 1C). A quantitative PCR assay of RNA samples prepared from livers that overexpress Med1 showed that Med1 mRNA levels increased progressively beginning at day 1 after injection (Fig. 1D). Western blot analysis of nuclear extracts prepared from livers that express exogenously introduced Med1 at 3 days after injection showed increased expression of Med1 protein (Fig. 1E). Note that with an equal amount of protein loaded onto this gel (Fig. 1E, 20 μg in lanes 1 and 2), a striking band specific to Med1 is visible in the Ad-Med1 lane (lane 2) as compared with the control lane (lane 1). Ad-Med1 overexpressed in Med1ΔLiv mouse liver also showed an increase in nuclear BrdUrd labeling (Fig. 2, A–D). Thus, we conclude that Med1 alone is capable of inducing a significant proliferative response in liver. In Ad-Med1-injected wild-type (Med1+/+I) mice, a ∼15% increase in the liver weight/body weight ratio was noted at 5 days (not shown).

**PPARα Null Mice Respond to Med1-induced Liver Cell Proliferation**—The above described observations indicate that Med1 by itself can induce cell proliferation in wild-type (Med1+/+) and Med1ΔLiv livers. We showed previously that PPARα signaling in liver is dependent on the Med1 subunit of the Mediator complex, as all effects of PPARα activation were abrogated in Med1ΔLiv livers (13). Because activators of nuclear receptor PPARα induce cell proliferation and require Med1 for this action, we asked whether the cell proliferative response that occurs in Med1ΔLiv livers due to the reintroduction of Ad-Med1 is dependent on PPARα. As illustrated in Fig. 2 (see panels E–H), PPARα−/− mice injected with Ad-Med1 virus by the tail vein revealed liver cell proliferation similar in magnitude to that noted in wild-type mice given Ad-Med1. These observations indicate that the induction of liver cell proliferation by Med1 is not dependent on PPARα.

**Med1 Overexpression Leads to Induction of a Wide Spectrum of Genes**—Because Med1 alone was capable of inducing liver cell proliferation, it was important to determine which genes were induced in Med1 overexpressing liver and whether any liver-specific genes were transcriptionally targeted by Med1. To this end, total RNA was prepared from the control and the Ad-Med1-overexpressing livers at 3 and 5 days after Ad-Med1 injection. The RNA samples were then subjected to microarray analysis as described previously (22). The results showed that a vast array of genes was induced in these livers (≥2-fold, p < 0.05), including those belonging to initiation and elongation of DNA replication and cell cycle progression, *i.e.* the genes related to cell growth and mitosis. Increased expression of nuclear receptors was observed, including many that are specific for hepatocytes, liver-specific nuclear receptor-regulated genes, co-activators, Wnt signaling pathways, and genes related to NF-κB regulation. A full list of the genes induced by Med1 at 3 and 5 days after Ad-Med1 injection was deposited in the Gene Expression Omnibus (accession number GSE48950); a partial list of the genes induced by Med1 at 3 and 5 days after Ad-Med1 injection is shown in supplemental Table S2. Using quantitative real-time PCR assays, we confirmed the induction of 18 of these genes (Table 1). The magnitude of induction of these genes is close to the induction levels observed by microarray analysis. Examination of the microarray data revealed a number of interesting aspects of the Med1-induced gene expression profile. For example, almost all of the genes involved in initiation of DNA replication, namely Orc6L, Cdt1, MCM helicases, Dbf4, Rpa1, and PCNA, were induced at robust levels, indicating a vigorous initiation of DNA replication. Similarly, many E2F family members (E2f1, E2f4, and
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TABLE 1
Genes up-regulated by Med1

Real-time PCR values obtained from the Ad-Med1-injected liver RNA were normalized with Ad-LacZ-injected liver samples. Values are means ± S.D. obtained from three independent experiments.

| Pathway                              | Genes                     | 3 days        | 5 days        |
|--------------------------------------|---------------------------|---------------|---------------|
|                                       |                           | -Fold induction ± S.D. |
| Transcriptional co-activator          | Med1                      | 15 ± 1.25     | 10.5 ± 0.13   |
|                                       | FoxO1                     | 6.5 ± 0.33    | 1.8 ± 0.18    |
| Cell cycle and proliferation          | Cyclin B1                 | 5.5 ± 0.21    | 2.1 ± 0.02    |
|                                       | Cyclin D1                 | 2.7 ± 0.37    | 1.3 ± 0.38    |
|                                       | Cyclin E1                 | 5.5 ± 0.33    | 3.6 ± 0.07    |
|                                       | Cd20                      | 18 ± 1.41     | 3.7 ± 0.1     |
|                                       | Pkl1                      | 15.5 ± 0.83   | 2.05 ± 0.23   |
|                                       | Auerb                     | 4.5 ± 0.22    | 0.83 ± 0.03   |
|                                       | Auerka                    | 19 ± 1.24     | 3.6 ± 0.15    |
|                                       | Cdk1                      | 4.5 ± 0.27    | 1.5 ± 0.09    |
|                                       | Cdk2                      | 6.6 ± 0.31    | 5.4 ± 0.32    |
|                                       | Cdk4                      | 3.1 ± 0.37    | 6 ± 0.41      |
|                                       | Cenpa                     | 3.41 ± 0.06   | 0.88 ± 0.04   |
|                                       | E2f1                      | 6.84 ± 0.5    | 4.8 ± 0.53    |
|                                       | Mad21I                    | 5 ± 0.23      | 3.32 ± 0.16   |
| Mitotic arrest and apoptosis          | Igfbp1                    | 4 ± 0.09      | 16 ± 0.25     |
|                                       | Gadd45                    | 38 ± 0.33     | 24 ± 0.3      |
|                                       | Survivin                  | 5.7 ± 0.2     | 4.33 ± 0.06   |

* List of selected genes induced at 3 and 5 days after overexpression of Med1 in Med1<sup>−/−</sup> mouse liver.

E2f6), cyclins (cyclins D1, D3, and E1) and Cdk (Cdk2, Cdk4, and Cdc8), were also induced at significant levels, indicating a coordinated progression of cells from G<sub>1</sub> to S phase. Some of the DNA repair and DNA damage response-related genes, including Rad1, Rad23b, Rad51, Rad52, Rad54b, Fen1, and Ddb1, were also induced, raising the possibility that there may be some aberrant DNA replication activity. Of importance was the induction of the FoxM1, FoxO1, ChREBP, and C/EBP genes that are related to liver function. Eight essential peroxisomal proteins involved in the biogenesis of the peroxisome (peroxins) (48), including Pex5 and Pex7, were also induced significantly. Surprisingly, genes encoding 16 subunits of the Mediator complex (1–3) were induced, and their range of induction varied from 2 (Med25)- to ∼5-fold (Med1). Thus, it would appear that the composition (and the activity) of the Mediator complex may have been altered (see “Discussion”). Finally, two early response genes, Fos and c-jun, that are critical for cell proliferation, were also induced to significant levels. Interestingly, induction of c-Myc was not detected, whereas a variant of Myc (MycL) that was reported to be expressed in lung cancers was induced by more than 2-fold.

AMPK Phosphorylates Med1 in Vitro—Because Med1 is central to the transcriptional regulation of both catabolic and anabolic genes, we reasoned that AMPK might control the activity of Med1 in liver by phosphorylation either to stimulate or inhibit transcription of these genes depending on the physiological context. The 1560-amino acid mouse Med1 protein contains at least four putative AMPK sites (consensus AMPK recognition site LRRVVXxxX; see Fig. 3A for ClustalW alignment and Refs. 30 and 34) surrounding serines at 656, 756, 796, and 1345, which can potentially be phosphorylated by AMPK. To determine whether Med1 is a substrate for AMPK, we carried out an in vitro phosphorylation assay using the GST-Med1 fusion protein and purified AMPK. Because Med1 is too large to obtain as GST fusion protein in sufficient quantities, we subcloned the Med1 protein coding sequences into three fragments that covered AA 440–740 containing Ser-656 (Med1-A), AA 740–1130 containing both Ser-756 and Ser-796 (Med1-B), and AA 980–1370, which contains Ser-1345 (Med1-C). The GST-Med1 subfragments were affinity-purified and then incubated with purified AMPK in a kinase buffer in the presence of [γ<sup>32</sup>P]ATP as described (25). The in vitro radiolabeled proteins were analyzed on SDS-PAGE, and the bands were visualized by autoradiography. All three fragments incorporated γ<sup>32</sup>P to significant levels in the presence of AMP and purified AMPK, whereas the control GST protein was not labeled (Fig. 3B).

These results indicate that each of these fragments served as AMPK substrates in vitro and confirms that Med1 is a substrate for AMPK under in vitro conditions.

Mutational and Tandem Mass Spectrometric Analyses Identify Phosphorylation of Serine at Positions 656, 756, and 796 by AMPK—Because the above results show that Med1 is an AMPK substrate and because the optimum AMPK sites were distributed in three different fragments used in the above mentioned kinase assays, it was important to determine whether the key serines of the potential AMPK sites are phosphorylated. Thus, we mutagenized the serines at positions 656 (in fragment Med1-A), 756 (fragment Med1-B1, AA 670–790), and 796 (fragment Med1-B1I, AA 770–950) to alanines and then assayed them in vitro for phosphorylation by AMPK as described above. The Med1B fragment (AA 740–1130) above was subcloned into Med1-BI and Med1-BII to separate Ser-756 and Ser-796. The autoradiograph shown in Fig. 3C, top panel (the Coomassie-stained gel shown in the bottom panel indicates that equal amounts of wild-type and mutant protein were used), indicates that the Med1 subfragments containing the Ser > Ala mutations of the putative AMPK sites are not phosphorylated. These results confirm that at least three AMPK sites are phosphorylated by AMPK in vitro.

To provide additional evidence that the serines identified above are indeed phosphorylated in vitro, the Med1 fragments were phosphorylated in vitro using unlabeled ATP. The phosphorylated Med1 fragments were subjected to tandem mass spectrometry as described under “Experimental Procedures.” The mass spectrometry profiles show an m/z 80-dalton shift in tryptic digests of Med1-A and Med1-BI fragments indicative of
phosphorylation of Ser-656 and Ser-756 (Fig. 3, D and E). The phosphopeptide 649SPLERQNSSGSGPR662 from Med1-A was observed with an m/z value of 791.36, indicating the presence of a phosphate group at Ser-656 (Fig. 3D), whereas the phosphopeptide 754LSSEDGSRGPDVSIDK773 from Med1-B1 was observed with an m/z value of 1265.58, confirming phosphorylation at Ser-756 (Fig. 3E). For reasons that we cannot explain at present, the mass spectrometry did not identify the phosphorylation of Ser-796 (not shown). However, we repeatedly observed phosphorylation of the Med1-BII fragment in vitro kinase reactions, which was abolished after S796A mutation (Fig. 3C). Nonetheless, the mutational analysis combined with mass spectrometry data confirms that AMPK phosphorylates three AMPK sites in Med1 as identified by the Clustal alignment.

AMPK Forms a Complex with Med1 in Vivo and Binds Directly to Med1 in Vitro—Because AMPK phosphorylates Med1 in vitro, we considered it possible that AMPK might form a complex with Med1 in vivo. To test this possibility, 293T cells were co-transfected with plasmids expressing the FLAG epitope-tagged AMPKα2 and His-tagged Med1, and the cell extracts were immunoprecipitated using anti-His antibody. Immuno precipitation (IP) and immunoblotting (IB) analyses using FLAG-tagged AMPKα2 and His-tagged Med1 revealed interaction of Med1 with AMPK (see “Experimental Procedures” for details).

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3 h, and then $[^{32}P]_{\text{orthophosphate}}$ was added. Two hours after the addition of the label (6 h before harvesting), the AMPK stimulator AICAR (1 mg/ml) was added; both the control and AICAR-treated cells were harvested and lysed, and the cell lysates were immunoprecipitated using anti-Med1 antibody. The immunoprecipitates were then subjected to 10% SDS-PAGE, and the radiolabeled Med1 was detected by autoradiography. As shown in Fig. 4A, a faint radiolabeled band due to phosphorylated Med1 was evident in the control lane. The intensity of this band increased dramatically with AICAR treatment. Because AICAR stimulates AMPK specifically, we concluded that Med1 is phosphorylated in vivo. To further confirm Med1 phosphorylation by AMPK in vivo, hepatocytes prepared from the livers of Med1$^{fl/fl}$ mice were first infected with Ad-Med1 for 24 h and then labeled with $[^{32}P]_{\text{orthophosphate}}$ in the presence and absence of AICAR. Phosphorylation of Med1 was assessed by immunoprecipitation/SDS-PAGE as described above. Phosphorylated Med1 was detected only in AICAR-treated cells, indicating that Med1 was phosphorylated by AMPK in cultured hepatocytes in vivo (Fig. 4B). The reason that Med1 was not labeled in the absence of AICAR in hepatocytes may be insufficient levels of active AMPK, as studies have shown that AMPK needs to be phosphorylated at its $\alpha$-subunit by upstream kinases before it phosphorylates the substrates (32, 43). Med1 phosphorylation was also observed in HeLa cells (data not shown). Overall, these results are in agreement with the data presented above showing that purified AMPK phosphorylates Med1 in vitro (Fig. 3) and Med1 complexes with AMPK in vivo (Fig. 3, F and G).

PPAR$\alpha$ Activators Fenofibrate and Wy-14,643 Induce Med1 Phosphorylation in Vivo—In the results presented above, we showed that Med1 is phosphorylated by AMPK both in vitro and in vivo (Figs. 3 and 4, A and B). We showed in earlier work that PPAR$\alpha$ cannot function in Med1$^{\Delta\text{iso}}$ livers consistent with an integral role for Med1 in PPAR$\alpha$-induced transcriptional activation of enzymes involved in fatty oxidation (13, 49, 50). Given these observations, it was important to ask whether Med1 is phosphorylated in hepatocytes treated with the PPAR$\alpha$ activators fenofibrate and Wy-14,643. We reasoned that PPAR$\alpha$ ligands that are known to activate AMPK (51, 52) would also phosphorylate Med1 (and perhaps increase its activity) during ligand-induced transcriptional activation. For this purpose, primary hepatocytes and HeLa cells treated with the aforementioned ligands for 24 h were labeled with $[^{32}P]_{\text{orthophosphate}}$ for 6 h. Incorporation of $^{32}P$ into Med1 in cell extracts prepared from these cells was assessed by immunoprecipitation and radiographic visualization (Fig. 4, C and D). These data indicate that the Med1 phosphorylation in primary hepatocytes is dramatically increased in the presence of fenofibrate and moderately increased in the presence of Wy-14,643 (~2-fold). Similar results were also obtained using HeLa cells (data not shown). Collectively, these results show that Med1 is phosphorylated when AMPK is activated by PPAR$\alpha$ ligands.

AMPK Inhibitor Compound C Attenuates Med1-inducible Liver Cell Proliferation—As described above, when Med1 is overexpressed in wild-type mice, a remarkable transcriptional amplification of genes related to DNA synthesis and cell cycle progression takes place (Table 1 and supplemental Table S2). The induction of DNA replication, the hallmark of cell proliferation response, consumes considerable amounts of ATP. We reasoned that in such cells, because of ATP hydrolysis, AMP levels would rise. As a consequence, Med1-overexpressing cells would activate AMPK to influence liver function. To test this prediction, we used a chemical, compound C, which specifically.

![FIGURE 4. In vivo phosphorylation of Med1 by AMPK activator AICAR and PPAR$\alpha$ activators fenofibrate and Wy-14,643. A, HEK293T cells transfected with His-tagged Med1 plasmid in the presence or absence of the AMPK activator AICAR. Lysates prepared were immunoprecipitated (IP) with anti-Med1, and the precipitates were run on SDS-PAGE, transferred to filter paper, and autoradiographed. ß-Actin immunoblot (IB) visualized using alkaline phosphatase shows protein content. B, AMPK phosphorylates Med1 in primary hepatocytes. Primary mouse hepatocytes infected with His-tagged Ad-Med1 were treated with the AMPK activator AICAR. Lysates were immunoprecipitated with anti-Med1 or anti-His to pull down phosphorylated Med1. C and D, PPAR$\alpha$ activators fenofibrate and Wy-14,643, known to activate AMPK, phosphorylate Med1 in primary hepatocytes (C) and HeLa cells (D). Cells were infected with Ad-Med1 in the presence or absence of the PPAR$\alpha$ activator and immunoprecipitated with anti-Med1 or anti-His.](image-url)
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inhibits AMPK activation. Accordingly, Med1 was overexpressed in the livers of Med1fl/fl mice by Ad-Med1 as described above. Controls included mice with tail vein injected with Ad-LacZ. To monitor the DNA replication, BrdUrd was given in drinking water from the start of the experiment, and all mice were killed 3 days post-tail vein injection. To assess the effect of inhibition of AMPK on Med1-initiated liver cell proliferation, we administered compound C by intraperitoneal injection daily for 3 days after Ad-Med1 injection. Increased BrdUrd nuclear labeling was noted in the liver of Ad-Med1-injected mice as compared with Ad-LacZ controls (Fig. 5, A–C). Of interest, the Ad-Med1-inducible hepatocyte proliferation was diminished markedly when these mice were given Ad-Med1 along with compound C (Fig. 5D). Quantification of the immunostained nuclei from these livers revealed low levels of BrdUrd incorporation in that it decreased by more than 50% in hepatocytes expressing Ad-Med1 in the presence of compound C. Our data also indicated that rapid cell proliferation in hepatocytes induced by Med1 overexpression is sensitive to AMPK inhibitor compound C (Fig. 5E). We also examined whether inhibition of AMPK by compound C attenuates hepatocyte proliferation induced by PPARα activator Wy-14,643, presumably by affecting Med1 phosphorylation. The data shown in Fig. 6 clearly indicate a profound inhibition of BrdUrd labeling in the livers of mice treated with both Wy-14,643 and compound C when compared with Wy-14,643 alone (Fig. 6, A–C and G). These results are consistent with the hypothesis that the AMPK may be monitoring cell proliferation by influencing Med1 and Med1 is a new signaling target of AMPK in vivo.

AMPK Inhibition by Compound C Reduces PPARα Ligand-induced Fatty Acid Oxidation—We then examined whether the livers of mice fed with a diet containing PPARα ligands such as fenofibrate and W-14,643 are known to induce peroxisomal proliferation with a concomitant induction of peroxisomal, mitochondrial, and microsomal fatty acid oxidation systems (49, 50). In addition to their function as PPARα activators, these PPARα agonists also induce AMPK phosphorylation in some cell types (51–54). To determine whether AMPK activation is involved in PPARα ligand-induced fatty acid oxidation enzymes, mice were fed with a diet containing Wy-14,643 for 3 days, and they also received compound C injections intraperitoneally once daily for 3 days (Fig. 6). The animals were killed 24 h after the last injection. Expression of the t-PBE/Ehhadh in liver sections was determined by immunohistochemistry, and the selected fatty acid oxidation enzymes were assayed by Western blotting (Fig. 6H). Immunostaining of t-PBE revealed noticeable inhibition in livers of mice co-treated with Wy-14,643 and compound C (Fig. 6, D–F). As expected, the levels of all three classical peroxisomal β-oxidation enzymes, namely ACOX1, t-PBE/Ehhadh, and PTL, increased by 2- to >5-fold in the livers of mice treated with Wy-14,643 (Fig. 6D). Increased levels of d-PBE and mitochondrial medium chain acyl-CoA dehydrogenase were also evident with Wy-14,643 treatment. However, the levels of these enzymes in compound C-treated mice fed with Wy-14,643 decreased significantly, and ACOX1 isoform A and PTL decreased to basal levels. Further, the enzyme levels in those mice treated only with compound C dropped to almost undetectable levels. These results suggest that ligand-stimulated PPARα-inducible fatty acid oxidation enzymes are controlled by AMPK, possibly by regulating Med1. These observations clearly establish that Med1, which is vital for PPARα-activated induction of fatty oxidation, is also a tar-
get of AMPK and may be an important link in the AMPK-PPARα-based lipid reduction process in hepatocytes.

DISCUSSION

In this study, we have described two novel functional aspects of Med1 in liver. First, when overexpressed in the livers of Med1\textsuperscript{fl/fl} or Med1\textsuperscript{ΔLiv} mice, Med1 alone can induce a rapid liver cell proliferative response. Second, we showed that Med1 is a substrate for AMPK \textit{in vitro} and \textit{in vivo} and that the AMPK phosphorylation of Med1 \textit{in vivo} has biological consequences, including the down-regulation of hepatocyte proliferation induced by Med1 overexpression and PPARα ligand-induced fatty acid oxidation. Both of these findings, to the best of our knowledge, are the first reports of Med1 properties in liver.

The role of Med1 of the Mediator complex in integrating diverse upstream signals and linking them to context-dependent transcriptional activation programs, combined with our earlier observations that the Med1 gene is amplified in a number of breast cancers (27), prompted us to ask whether Med1 by itself has the capacity to induce cell proliferation. Analysis of the Med1-induced gene expression profile described in this study showed a robust induction of a wide spectrum of genes in liver. Genes related to cell proliferation, DNA repair and damage, apoptosis, nuclear and other types of receptors, signal transduction, transcriptional activation, Wnt signaling, NF-κB activation, translational regulation, and mRNA biogenesis are all induced when Med1 is overexpressed. We also observed induction of a number of genes involved in peroxisome biogenesis, transport mechanisms, inflammatory response, immune regulation, and metabolism. Thus, it appears that high levels of Med1 in hepatocytes exert a somewhat global effect on transcription but most notably on cell proliferation-related genes, some of which are liver-specific. Additional investigations are essential to define the sequence of events that lead to the induction of DNA synthesis and cell cycle progression upon Med1 overexpression.

Liver cells are normally quiescent, and the liver is bestowed with a remarkable capacity to regenerate after injury (55–60). Regenerating liver after a partial hepatectomy is a time-honored model to study the molecular mechanisms involved in hepatocellular proliferation (55–59). Studies from a number of laboratories suggest that during liver regeneration, paracrine and endocrine signals from nonparenchymal hepatic cells first induce transcription factors NF-κB, Stat3, and AP1, each of which has a number of downstream targets (59, 60). This sets the stage for the induction of early response genes (Myc, Gadd45, Fos, JunB, and Egr1) followed by the induction of genes related to DNA replication, cell cycle progression, and mitosis (60, 61). On closer examination, our microarray data show that a majority of the genes involved in liver regeneration are also induced in hepatocytes overexpressing Med1 (supplemental Table S2). For example, the early response genes Fos, junD, JunB, and EGR1 are induced to significant levels. Although c-Myc was not induced, a variant of Myc, known as Mycl, is up-regulated in Med1-expressing livers. We do not know whether this Myc variant, found at high levels in lung cancer, can substitute for c-Myc functions or whether Med1-overexpressing cells bypass the Myc requirement in cell cycle progression (62). Most pro-growth E2F genes are also activated in these Med1-overexpressing livers as are cyclins and Cdk, which promote the transition of cells from G\textsubscript{1} to S and G\textsubscript{2}/M phase. Several mitosis genes are also found elevated in Med1-overexpressing livers as evaluated by counting colchicine-arrested metaphases (data not shown). Overall, these data indicate that the normal cell cycle progression occurs in at least some of the Med1-overexpressing liver cells.

It is well known that activation of PPARα in rat and mouse liver induces the proliferation of hepatocytes and that this requires Med1 (13, 15, 63). However, our results indicate that the proliferation induced by Med1 is not dependent on PPARα (Fig. 2). In addition to PPARα, activation of other nuclear receptors such as CAR and TR (thyroid hormone receptor) can also induce hepatocyte proliferation (14, 60). Accordingly, Med1 could induce hepatocyte proliferation by amplifying the signaling of nuclear receptors and transcription factors (64). Consistent with this hypothesis, our microarray data revealed that several target genes of CAR (60) are induced by overexpression of Med1. Mutational analysis of Med1 that would specifically abolish the interaction between Med1 and nuclear receptors could eventually clarify this important issue as to whether nuclear receptor signaling plays a role in Med1-initiated hepatocyte proliferation. Studies show that after partial hepatectomy, hepatocytes move synchronously through different cell cycle phases and cell cycle terminates after mitosis (55, 56). Our microarray data also show a number of apoptosis-related genes activated in Med1-overexpressing cells, raising the possibility that some of the Med1-overexpressing cells may undergo apoptosis. The presence of elevated levels of many DNA damage response genes is also relevant here, as DNA damage response is a part of the apoptotic response.

The mechanism by which Med1, by itself, induces transcription of a large number of genes that belong to various metabolic pathways remains to be elucidated. One possibility is that although liver cells are quiescent, genes that belong to various pathways in these cells always remain primed or poised for transcription and waiting for the proper functional Mediator complexes to start transcription. Exogenously introduced or endogenously overexpressed Med1 in the nucleus of such cells may rapidly attract other subunits to form functional Mediator complexes, thus assembling the preinitiation complex to start transcription. It is important to note here that elevated Med1 levels not only induce a variety of genes belonging to diverse metabolic pathways but also induces the expression of 16 other members of the Mediator complex. Therefore, it is conceivable that the Mediator complexes formed in these cells may have been altered with respect to their capacity to activate transcription. This may have contributed to the amplified induction of a myriad of genes in Med1-overexpressing cells.

As stated above, we reported earlier that Med1 is amplified in a significant number of breast cancers and cancer cell lines (27). Our findings of the relevance of Med1 in neoplastic growth were further substantiated by several recent reports (27–29). For example, elevated expression of Med1, Med24, and Med30 is reported in various breast cancer cell lines, and reduced expression of Med1 and Med24 leads to reduced DNA synthesis and cell proliferation (27, 29). Med1 is overexpressed in
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human prostate cancers, and overexpression of Med1 correlates with elevated cell proliferation (28, 65–68). In humans, HER2-positive breast tumors also demonstrated Med1 gene amplification (65, 66). Tissue microarray analysis of breast cancer tissues positively correlate Med1 levels with HER2-mediated tamoxifen resistance in breast cancer cells (Med1 is a key estrogen receptor coactivator), suggesting an important role for Med1 in the development HER2 resistance (65). Interestingly, another study reports that the loss of Med1 correlates with an increased rate of invasion and metastasis in human non-small cell lung cancer progression, suggesting that a Med1-containing submodule may also negatively regulate expression of some genes (69). In summary, these and other results indicate an important transcription regulatory role of Med1 in the maintenance of neoplastic cell transformation and suggest that Med1 may prove useful as a therapeutic target in cancer treatment.

The second important finding described in this article relates to the phosphorylation of Med1 by AMPK. By making a comparison with previously reported optimum AMPK sites in other proteins, we could identify six potential AMPK sites on Med1 amino acid sequence that are also conserved across a wide variety of species (Fig. 3). Using an in vitro kinase assay, we showed that at least three of these sites (Ser-656, Ser-756, and Ser-796) are bona fide AMPK sites. Using tandem mass spectrometry we confirmed that Ser-656 and Ser-756 are phosphorylated in vitro. The inability to detect phosphorylation of Ser-796 may be due to insufficient quantities of in vitro phosphorylated protein in the preparation used in mass spectrometry analysis. We also presented evidence in this report that Med1 is phosphorylated in vivo, as AICAR, which is specific for AMPK, stimulated the phosphorylation of Med1 in both hepatocytes and 293T cells. In this context, we also established that AMPK interacts with Med1 and phosphorylates Med1 both in vivo and in vitro. Its physiological significance is underlined by our observation that Med1-mediated cell proliferation and PPARα-induced response in liver are compromised when AMPK activities are inhibited. Identification and analysis of the additional phosphorylation sites in Med1 and elucidation of the contribution of individual phosphorylated sites to their functions will be the focus of future studies.

We believe that phosphorylation of Med1 plays a central role in AMPK-mediated energy homeostasis. The mechanism by which AMPK maintains energy homeostasis is complex and continues to evolve. In the liver AMPK phosphorylates multiple targets to inhibit or increase their activities, which finally results in the down-regulation of anabolic pathways to conserve energy and turning on the catabolic pathways to generate ATP. AMPK phosphorylates acetyl-CoA carboxylase (ACC), an important regulator of lipid metabolism, and inhibits its activity (30–33). The enzyme ACC is a key player in promoting fatty acid synthesis and decreasing mitochondrial fatty acid oxidation (30, 33). Thus, phosphorylated ACC negatively controls fatty acid synthesis while promoting fatty acid oxidation. Several other hepatic AMPK targets involved in lipid homeostasis have also been identified in which activities are either up- or down-regulated (30–33). In this regard, we showed here that treatment of cells with the PPARα ligands fenofibrate and Wy-14,643, which are strong stimulators of fatty acid oxidation, by inducing PPARα transcriptional activity also induce phosphorylation of Med1 (Fig. 5). PPARα activators fenofibrate and Wy-14,643 activate the AMPK signaling pathway (53, 54, 70, 71). We reported here the attenuation of hepatocyte proliferation in the liver of wild-type mice by compound C; they were fed a diet containing the PPARα activator Wy-14,643, suggesting that AMPK is also involved in the PPARα pathway. We speculated that activation of AMPK by these agonists may directly phosphorylate Med1, which then potentiates the transcriptional activity of PPARα on the promoter of the genes involved in fatty acid oxidation in mouse liver. Elevated fatty acid oxidation contributes to oxidative DNA damage and increased hepatocellular proliferation (49). This suggests that in addition to several targets described above (30–33), when cells are under metabolic stress to conserve energy, AMPK also phosphorylates Med1. Although we do not know the targets of the Mediator complexes under these conditions, AMPK modifies Med1, and presumably the modified Mediator complex would affect the synthesis of some of the proteins involved in fatty acid oxidation at the transcriptional level. A combination of ChIP-seq and mRNA-seq approaches along with the phosphospecific antibodies specific for the individual AMPK sites on Med1 should facilitate the unraveling of the Med1 functions under these conditions.

We also presented evidence that compound C, an inhibitor of AMPK, can inhibit cell proliferation induced by Med1 overexpression. Med1 has been shown to increase fatty acid oxidation, an anabolic process that consumes a significant amount of ATP as well as phosphorylation and dephosphorylation of many proteins. For example, during the elongation step of protein synthesis, 4 ATP equivalents/peptide bond formations are utilized. The mechanism of DNA synthesis and cell cycle progression involves de novo synthesis and cyclic degradation of numerous proteins. Therefore, one would expect ATP levels to drop in these cells, which would lead to the activation AMPK, and such cells would be sensitive to compound C. It is also worth noting here that one of the main AMPK targets during cell proliferation is the mammalian target of rapamycin (mTOR; phosphorylation inhibits its activity), a protein kinase known to positively regulate cell growth by modulating protein translation, cell survival, and autophagy (31, 34, 37). It will be interesting to determine whether mTOR-mediated effects are in some way linked to AMPK-mediated Med1 phosphorylation.

Phosphorylation of Med1 by kinases such as PI3K/AKT and MAPK has shed some light on the effects of Med1 phosphorylation on its transcriptional activation activity. In an earlier study we showed that Med1 phosphorylation by Raf/MEK/MAPK cascade induces its coactivator function (25). Subsequently, it was shown that phosphorylation of Med1 by steroid and thyroid hormones via MAPK/ERK stimulated its interaction with the Mediator complex (26, 29). Another study showed that phosphorylation of Med1 at Thr-1032 induces its interaction with proteins bound to promoter proximal elements (FoxA1, RNA polymerase II, and TATA-binding protein) of the UBE2C oncogene with the far upstream enhancer (chromatin looping) to stimulate transcription (28). It was suggested that the stimulation of chromatin looping by this mechanism may be the basis of overexpression of the UBE2C oncogene in sev-
several types of solid tumors including prostate cancers (28). Mitogen-activated protein kinase phosphorylation of Med1 has also been shown to enhance its interaction with androgen receptor (29). It remains to be determined what effects the AMPK phosphorylation of Med1 has on its functions in maintaining energy homeostasis (72). In summary, in this study we have presented results on two novel roles for Med1 in cell proliferation and energy homeostasis.

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