Mutations in the *HNF4α* gene are responsible for type 1 maturity-onset diabetes of the young (MODY1), which is characterized by a defect in insulin secretion. Hepatocyte nuclear factor (HNF)-4α is a transcription factor that plays a critical role in the transcriptional regulation of genes involved in glucose metabolism in both hepatocytes and pancreatic β-cells. Recent evidence has implicated AMP-activated protein kinase (AMPK) in the modulation of both insulin secretion by pancreatic β-cells and the control of glucose-dependent gene expression in both hepatocytes and β-cells. Therefore, the question could be raised as to whether AMPK plays a role in these processes by modulating HNF-4α function.

In this study, we show that activation of AMPK by 5-amino-4-imidazolecarboxamide riboside (AICAR) in hepatocytes greatly diminished HNF-4α protein levels and consequently downregulates the expression of HNF-4α target genes. Quantitative evaluation of HNF-4α target gene expression revealed diminished mRNA levels for HNF-1α, GLUT2, L-type pyruvate kinase, aldolase B, apolipoprotein (apo)-B, and apoCIII. Our data clearly demonstrate that the MODY1/HNF-4α transcription factor is a novel target of AMPK in hepatocytes. Accordingly, it can be suggested that in pancreatic β-cells, AMPK also acts by decreasing HNF-4α protein level, and therefore insulin secretion. Hence, the possible role of AMPK in the physiopathology of type 2 diabetes should be considered. *Diabetes* 50:1515–1521, 2001
of apolipoprotein (apo)-AII, apoB, and apoCIII (10,11). Very recently, the liver-specific knockout of HNF4α has confirmed the central role of HNF-4α in lipid metabolism via the control of apo gene expression (12).

The AMP-activated protein kinase (AMPK) has been defined as a metabolic master switch, phosphorylating key target enzymes that control flux through various metabolic pathways (13,14). It is the mammalian homologue of the sucrose nonfermenting-1 protein kinase, the key regulator of glucose-dependent gene repression in yeast (15–17). It is the mammalian homologue of the glucose-dependent gene repression in yeast (15–17). It is the mammalian homologue of the glucose-dependent gene repression in yeast (15–17). It is the mammalian homologue of the glucose-dependent gene repression in yeast (15–17). It is the mammalian homologue of the glucose-dependent gene repression in yeast (15–17). It is the mammalian homologue of the glucose-dependent gene repression in yeast (15–17). It is the mammalian homologue of the glucose-dependent gene repression in yeast (15–17). It is the mammalian homologue of the glucose-dependent gene repression in yeast (15–17). 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RESULTS

AMPK activation decreases HNF-4α protein levels in a time-dependent manner. We have previously shown that AMPK activation by 200 μmol/l AICAR in hepatocytes in primary culture inhibited glucose-dependent gene transcription (18). This result was confirmed by others using either AICAR (20) or a constitutively active form of the protein kinase (21), thereby indicating that the use of AICAR is relatively safe in terms of specificity for AMPK activation. Figure 1A shows that AMPK is efficiently activated in primary hepatocytes in response to 200 μmol/l AICAR.

To test whether AMPK plays a role in transcriptional repression by modulating HNF-4α function, we first assessed HNF-4α binding activity after AMPK activation in a primary culture of hepatocytes. For that purpose, we performed EMSAs using nuclear extracts prepared at different incubation times with 200 μmol/l AICAR. As shown in Fig. 1B (upper panel), we observed a diminution of the HNF-4α-retarded band after 6 h and a complete loss of HNF-4α DNA-binding activity after 12 h of AICAR treatment. Similar results were obtained by using either the HNF-4α binding site from the rat L-PK promoter (Fig. 1B) or the human α1-antitrypsin gene promoter (not shown) as DNA probes. As a control, we monitored DNA-binding activity for the ubiquitously expressed transcription factor nuclear factor Y (NF-Y) and showed that it was not significantly affected by AICAR treatment over time (Fig. 1B). Competition studies using excess of unlabeled oligonucleotides corresponding to consensus HNF-4α binding site and supershift assays using specific HNF-4α antibodies were performed to confirm the identity of the HNF-4α–retarded band (not shown).

The observed loss of the HNF-4α–retarded band could be caused by changes in protein levels. To test this possibility, we performed Western blot analysis to monitor the level of HNF-4α protein in response to AICAR treatment. As shown in Fig. 1B (lower panel), we observed that the HNF-4α signal decreases after 6 h and practically disappears after 12 h of incubation with AICAR. In contrast, the level of the control protein annexin V was not affected by AICAR treatment over time. Thus, activation of AMPK in hepatocytes results in a complete loss of HNF-4α DNA-binding activity that was strictly parallel to a pronounced time-dependent disappearance of HNF-4α protein levels.

AMPK activation does not modify HNF-4α mRNA levels. Considering the diminution of HNF-4α protein after AMPK activation, it was tempting to speculate that the AMPK effect could be accounted for by suppression of HNF4α gene transcription. To examine this issue, we evaluated HNF-4α mRNA levels using a semiquantitative RT-PCR assay. As shown in Fig. 2A, HNF-4α mRNA was constitutively expressed in the absence and presence of 200 μmol/l AICAR, suggesting that AMPK activation did not affect transcription of the HNF4α gene. As a control, we monitored the mRNA levels for the L-PK and albumin genes, which have previously been found to be sensitive and insensitive, respectively, to AMPK transcriptional repression (18,20). As expected, the expression of the L-PK gene was highly decreased in the presence of AICAR, whereas the expression of the albumin gene was not significantly affected by AICAR treatment (Fig. 2A).

In addition, to confirm that the AMPK effect was not mediated by direct transcriptional repression of the HNF4α gene promoter, we transiently transfected hepatocytes with a luciferase construct driven by the murine HNF-4α promoter (27,28). Overnight incubation of these hepatocytes with 200 μmol/l AICAR caused no changes in the luciferase reporter enzyme activity when compared with untreated hepatocytes (Fig. 2B). Therefore, the sensitivity of HNF-4α protein abundance to AMPK activation was not related to a direct transcriptional effect on the HNF-4α promoter.

AMPK activation represses transcription of HNF-4α–dependent constructs. To determine whether HNF-4α might be a bona fide transcriptional target of AMPK, we considered the possibility that AMPK might affect expression of an HNF-4α–dependent gene by repression of transcription. As a candidate target of AMPK repression, we selected the rat L-PK promoter DNA probe (5′-GGGTTAGGAAACCATT-GAAATGAAAGGTTA-3′), which has been shown to be highly sensitive to AMPK activation (30,31). We first examined whether the L-PK promoter is repressed by modulating HNF-4α activity.
that AMPK can modulate HNF-4α incubation with 200 μmol/l AICAR. Mouse hepatocytes were transfected with a luciferase construct driven by the murine HNF-4α promoter in the presence of AICAR. Mouse hepatocytes were transfected with the following constructs: −183PK/Luci, (L3)−54PK/Luci, or (H4)−105TK/Luci. At 24 h after transfection, 200 μmol/l AICAR (white bars) was added and left overnight before luciferase assay.

**DISCUSSION**

It is now well established that AMPK, in addition to its role in fuel partitioning (14), is also involved in the transcriptional control of glucose-regulated genes (18–22). The present study further elucidates the molecular targets involved in transcriptional repression by AMPK. Here, we demonstrated that the MODY1/HNF-4α transcription factor is a novel target of AMPK. The extent of decrease in transcription rate induced by AICAR treatment essentially correlates with the decrease in HNF-4α protein levels. This effect is most likely not related to a transcriptional repression of the HNF4α gene promoter because no significant modification of HNF-4α mRNA can be detected by semiquantitative RT-PCR analysis (Fig. 2A) and the murine HNF-4α promoter appears to be insensitive to AICAR treatment in transfection studies (Fig. 2B).

Cellular processes that limit the transcriptional activity, subcellular location, and abundance of transcription factors play an important role in regulating gene expression. Proteolysis is a prominent mechanism regulating transcription factor, which makes transcriptional activation and activator degradation closely coupled events (37,38). Several proteolytic processes have been implicated in transcription factor destruction, including ubiquitin-mediated proteolysis (39) and cleavage by calpains (40) and lysosomal proteases (41). In the case of transcription factor turnover by the ubiquitin-proteasome pathway, phosphorylation has often been associated as a positive signal

**FIG. 2.** Effect of AMPK activation on HNF-4α gene transcription. A: Quantification of HNF-4α mRNA levels after AMPK activation. Hepatocytes were incubated overnight with 200 μmol/l AICAR, and mRNA levels for HNF-4α, L-PK, and albumin were analyzed by semiquantitative RT-PCR analysis. B: Transcriptional activity of the HNF-4α promoter in the presence of AICAR. Mouse hepatocytes were transfected with a luciferase construct driven by the murine HNF-4α promoter, and then 200 μmol/l AICAR was added 24 h after transfection and left overnight before luciferase assay.

**FIG. 3.** The activity of HNF-4α-dependent promoters after AMPK activation. Hepatocytes were transfected with the following constructs: −183PK/Luci, (L3)−54PK/Luci, or (H4)−105TK/Luci. At 24 h after transfection, 200 μmol/l AICAR (white bars) was added and left overnight before luciferase assay. *P < 0.05 for the effect of AICAR.
resulting from proteolytic cleavage by cellular proteases. Efforts to identify the exact mechanisms implicated in this process are underway.

The rapid and controlled adjustment in the amount of transcription factors permits the attenuation of cellular responses to extracellular cues by rapidly modifying key signaling proteins. This provides a fail-safe mechanism against unbridled changes in the level of transcription and allows the transcriptional apparatus to be quickly reprogrammed in the case of unexpected change in the cellular state. AMPK is activated in response to ATP depletion and leads to the switching-off of ATP-consuming pathways and the switching-on of ATP-regenerating pathways. It plays a major role in lipid metabolism and in inhibiting fatty acid, cholesterol, and triglyceride synthesis; it also plays a major role in promoting fatty acid oxidation. This switch from anabolism to catabolism restores the energy status of the cell (25). By decreasing HNF-4α protein levels (as in this study), AMPK decreases the apo synthesis and stops lipid export by the liver, thus preserving this carbon source for ATP repletion at the cellular level. Recently, HNF-4α mutations have been associated with decreased levels of circulating apoAII, apoB, apoCIII, and triglycerides in MODY1 patients and in mice carrying a HNF-4α-null mutation in the liver (10,11).

Genetics studies have shown that heterozygous mutations in HNF-4α result in MODY1, an autosomal-dominant form of type 2 diabetes. MODY1 mutants included nonsense and frameshift mutations that give rise to truncated HNF-4α proteins and missense mutations, which affect different domains of the HNF-4α protein. These mutations impair protein activity by decreasing its binding to target DNA sequences or by affecting protein dimerization and/or the transactivation potential. Surprisingly, none of the MODY1 mutants described so far have exhibited any signs of a dominant-negative effect in functional studies (43). It is speculated that dominant-negative HNF-4α mutants are not compatible with human development, as is the case with HNF4α inactivation in mice (6). Hence, haploinsufficiency or reduced gene dosage may explain the mechanism leading to the MODY1 phenotype. Expression of HNF-4α at a relatively low amount in endocrine pancreatic cells (5) appears to be a limiting factor for efficient transcription of HNF-4α target genes. A 50% reduction in the amount of functional HNF-4α protein would be deleterious in the context of pancreatic β-cells, whereas in hepatocytes it would have less dramatic effects on gene transcription.

The MODY1 phenotype is characterized by a defect in nutrient-induced insulin secretion by the pancreatic β-cells (3) and by a diminution in serum triglycerides, and it accounts for a diminution in apoAII, apoB, and apoCIII synthesis by the liver (10,11). Target genes of HNF-4α in pancreatic β-cells include genes involved in glucose metabolism, e.g., GLUT2, L-PK, and aldolase B (44). Overexpression of wild-type HNF-4α in INS1 β-cells is associated with an increase in insulin secretion in response to glucose, whereas the overexpression of a dominant-negative form of HNF-4α causes a decrease in glucose-induced insulin secretion (44). Endogenous HNF-4α protein levels in islet β-cells must therefore be tightly regulated to maintain normal insulin secretion and euglycemia. This observation suggests that reduced levels of HNF-4α in the

for the ubiquitination and targeting of proteins to the 26S proteasome (42). Therefore, we verified that HNF-4α is indeed a target of AMPK phosphorylation in cell-free extracts (not shown) and anticipated that this pathway would be involved in HNF-4α degradation. To test this hypothesis, we treated hepatocytes concomitantly with AICAR and the proteasome inhibitor MG132. We observed that the diminution of HNF-4α protein was not prevented under these conditions (not shown) suggesting that HNF-4α disappearance was independent of the proteasome pathway. Alternatively, it is possible that phosphorylation of HNF-4α by AMPK may introduce a conformational change that makes the protein more susceptible to degradation.
pancreas could contribute to the development of β-cell dysfunction. It is noteworthy that AMPK activity is regulated by glucose in pancreatic β-cells and has been implicated in the control of glucose-stimulated insulin secretion (19,24). Whether HNF-4α is also a target of AMPK in islet β-cells is of major interest because any inappropriate activation of the AMPK signaling pathway would down-regulate HNF-4α protein levels. Consequently, this would have deleterious effects on glucose-induced insulin secretion and could contribute to the development of type 2 diabetes.

In conclusion, our results add the transcription factor MODY1/HNF-4α to the list of AMPK targets. In addition to its role in the regulation of hepatic gene expression, HNF-4α also regulates the expression of genes that are involved in glucose transport and glucose metabolism in the pancreatic β-cells. This suggests that the modulation of HNF-4α protein levels by AMPK could have important metabolic effects. The potential importance of the loss of HNF-4α protein after AMPK activation is reinforced by the fact that only a minor perturbation of HNF-4α activity results in MODY1 (43). Therefore, we propose that modulation of HNF-4α function by AMPK activation could be of importance in the physiopathology of type 2 diabetes. This issue is an interesting area for future investigation.

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