AMP-activated protein kinase (AMPK) is a sensor for maintaining energy balance (1). We have previously reported that overexpression of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR; an AMPK activator)-responsive element binding protein (AREBP) represses transcription of phosphoenolpyruvate carboxykinase (PEPCK), a gluconeogenic enzyme gene, in response to AICAR treatment (AMPK activation) in cultured cells. It is caused by phosphorylation of Ser470 of AREBP by activated AMPK. Phosphorylated AREBP induces transcriptional repression of PEPCK gene expression (2). Moreover, overexpression of AREBP represses not only PEPCK but also the expression of other gluconeogenic genes in response to AICAR treatment in mice. These results suggest that AREBP plays an important role in gluconeogenesis. In fact, the blood glucose level was significantly reduced after AICAR injection in AREBP-overexpressing mice (3). Therefore, a combination of pharmacological and/or nutritional induction of AREBP expression is expected to reduce blood glucose levels under insulin resistance conditions such as diabetes. In this report, promoter analysis was conducted in order to explore the method of induction of AREBP gene expression.

Results and Discussion

To identify transcriptional regulatory elements associated with AREBP gene expression, functional analysis was performed using the ~1.3 kb upstream region of the AREBP promoter and a series of promoter deletion mutants in transient transfection assays. Chimeric gene constructs were made in which the human AREBP gene promoter and deletion mutants were inserted into the pGL3 Luciferase reporter plasmid (Invitrogen) (4). AREBP is mainly expressed in the liver, kidney and brain (3). Therefore Fao (derived from liver cancer), 293T (derived from embryonic kidney) and SH-SY5Y (derived from neuroblast-like) cells were used as target cells. When we used a 1.3 kb promoter-luc construct as a reporter plasmid, transcriptional activation was observed in all cells examined (Fig. 1, lanes 1 to 3). However, transcriptional activation disappeared when we used 1.2 kb promoter-luc deletion constructs (lanes 4 to 6). These results suggest that a transcriptional activation element is located between −1.2 kb and −1.3 kb of the AREBP gene promoter. It is noteworthy that, when the Fao cell line derived from rat liver, which is a main organ of glucosegenesis was used, transcriptional activation was relatively low compared to that for the other cell lines (lane 3). In contrast, a 1.0 kb promoter-luc construct activated transcription only in Fao cells (lane 12). Although not statistically significant, luciferase activity was detectable when we used a 0.38 kb promoter-luc construct (lane 24). Similar results were obtained using the HepG2 cell, which is derived from human hepatocellular carcinoma (data not shown). Thus, the transcriptional activation mechanisms of AREBP in liver may be more complicated than in other tissues.

To identify the transcriptional regulatory element interacting with regulatory proteins, we conducted DNase I protection analysis (5). The AREBP gene promoter region (including −1.2 kb to −1.3 kb) was labelled with [γ-32P]ATP and used in a footprint reaction with nuclear extract from several cell lines (6). Although clear protected regions were not observed, regions with thin bands were observed in the presence of nuclear extracts (Fig. 2, arrow heads). Since there are
some DNase I hypersensitive sites (closed circles) around the thin bands, this may indicate a structural change induced by DNA-protein interaction (7, 8). These results suggest that there are nuclear factors which can bind to the element associated with transcriptional activation of the AREBP gene.

To further investigate the DNA-protein interaction, we performed an electrophoresis mobility shift assay (EMSA) using the identified DNA sequence (21259 to 21284, 26 bp) as a probe. We detected DNA-protein complexes whose mobility shifted upon incubation with the nuclear extracts (Fig. 3). The complexes disappeared when cold competitor was added in a dose-dependent manner, indicating the sequence-specific binding of nuclear factors. Interestingly, at least two shifted bands were observed. This may suggest that certain nuclear factors form monomers and/or dimers which bind to the DNA. Indeed, the repetitive sequence (TACAAAmnnnnnnnTACAAA) found within the probe is a typical DNA motif (tandem-repeat) for dimer formation (Fig. 2, boxed). We’re trying to clone a nuclear factor using a yeast one-hybrid system.

In this report, the AREBP gene promoter was analyzed by transient transfection assay. Systematic deletion analysis showed the transcriptional activation element to be located between −1.2 kb and −1.3 kb from the transcriptional start site of the AREBP gene promoter. Protein-DNA binding analysis showed that a nuclear factor binds to a short region within this element (−1259 to −1284, 26 bp) in a sequence-specific manner.

Our previous results indicated that the blood glucose curve of AREBP-overexpressing mice did not differ from that of wild-type mice after insulin administration (3). These results suggest that the AMPK-AREBP pathway is independent of the insulin signal. Even though the blood glucose level is high, gluconeogenesis is increased in insulin resistance. Therefore, the AMPK signaling pathway may have a crucial role for control of glucose production in insulin resistance (9). Pharmacological and/or nutritional control of AREBP expression may therefore improve insulin resistance.

**Acknowledgments**

This work was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS Kakenhi Grant Number 16780106 and 19580158).
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