A Novel Factor Binding to the Glucose Response Elements of Liver Pyruvate Kinase and Fatty Acid Synthase Genes*

(Received for publication, August 12, 1998, and in revised form, October 14, 1998)

Jun-ichi Hasegawa, Kiyoshi Osatomi, Ru-Feng Wu, and Kosaku Uyeda‡

From Research and Development, Dallas Veterans Affairs Medical Center and Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, Texas 75216

Transcription of the liver type pyruvate kinase and lipogenesis enzyme genes is induced by high carbohydrate in liver. We have found a novel factor involved in rat liver nuclei that binds to the glucose response element (CACGTG motifs) of the pyruvate kinase gene (Liu, Z., Thompson, K. S., and Towle, H. C. (1993) J. Biol. Chem. 268,12787–12795) and the “insulin response element” of fatty acid synthase gene. The amounts of this DNA-binding protein, termed “glucose response element binding protein” (GRBP) in the nuclear extract, were increased in liver by a high carbohydrate diet and decreased by starvation, high fat, and high protein diet. GRBP also occurs in cytosols of liver and is dependent on carbohydrate. Both the nuclear and the cytosolic GRBP showed similar properties, except the former was more resistant to thermal inactivation than the latter. Kinetics of glucose activation of the cytosolic GRBP in a primary culture of hepatocytes indicated that a half-maximum activation was achieved after 2 h, and glucose concentration required for the maximum activation of the GRBP was approximately 12 mM. Dibutyryl-cAMP, okadaic acid, and forskolin inhibited glucose activation of both GRBP and liver pyruvate kinase transcription. These results suggested that GRBP may be a factor that recognizes the glucose response motif site and may be involved in mediating carbohydrate response of the pyruvate kinase gene.

The liver, the principal site of lipogenesis, is responsible for conversion of excess dietary carbohydrate to triglycerides. A high carbohydrate diet induces the synthesis of several key enzymes involved in glycolysis and lipogenesis, including pyruvate kinase, ATP citrate lyase (ACL), ACC, and FAS (reviewed in Refs. 1 and 2). This increased enzyme synthesis is correlated to increased mRNA, resulting from glucose-induced expression of the corresponding genes.

Glucose-stimulated liver type pyruvate kinase (LPK) gene expression in liver is mediated through the glucose response element (GRE) that is located within the region −183 to −96 base pairs upstream from the cap site of the LPK gene (Fig. 1). (3). Vaulont et al. (4) found three protein-binding sites within this segment which they designated MLTF-like, HNF4, or LF-A1 and NF-1 as binding sites for the transcription factors. The MLTF-like site contains a palindromic sequence, CACGGG (underlined in Fig. 1), separated by 5 bases that corresponds to the consensus binding site (CACGTG) for MLTF/USF, c-Myc, and its related family members, and TFE3, SREBP/ADD1 (5–11). Among these factors, upstream stimulatory factor (USF) is the predominant factor of hepatic nuclear extracts. Electrophoretic mobility shift assays demonstrated that USF does bind to the carbohydrate or GRE of LPK which is “super-shifted” by antibodies against USF (12–14). However, several lines of evidence rule out USF being the GRE-binding protein as follows: (a) the USF-binding site from adenovirus major late promoter was unable to replace the carbohydrate-responsive element of LPK (15); (b) when dominant negative forms of USF, which are able to form a heterodimer with endogenous USF, but are unable to bind to DNA, are expressed in hepatocytes, they do not block LPK induction by glucose (16); (c) mutant GRE constructs are able to retain glucose responsiveness of chloramphenicol acetyltransferase transcription yet lose ability to bind USF (16). Thus, these results rule out USF as the specific carbohydrate-responsive factor that activates LPK gene expression, and thus far such a factor has not been found.

Little is known about the nature of the signal transduction pathway for the glucose stimulation of transcription of any of these genes. The possible role of various glucose metabolites, including glucose-6-P (17), 3-P-glycerate (18), and P-enolpyruvate (18) etc., has been suggested but not proved. More recently, Doiron et al. (19) showed that incubation of hepatocyte-derived transformed cells (At3F) with xylitol results in induction of LPK, and they suggest that xylitol is converted to xylulose-5-P (Xu-5-P) inside the cells, which in turn activates LPK transcription. This idea for xylitol feeding is based on the earlier demonstration that Xu-5-P activates specific protein phosphatase (PP2A), which in turn activates the synthesis of hepatic Fru-2,6-P2, the most potent activator of phosphofructokinase, in response to increased glucose (20, 21). However, this Xu-5-P activation of PP2A by glucose happens rapidly, within a few minutes, whereas glucose activation of gene transcription is considerably slower, taking hours. Thus, it is questionable whether the same mechanism of the glucose signaling applies to both short and long term regulation. More recently Mourrieres et al. (22) demonstrated that the effect of xylitol (on FAS and S14 genes) could be explained by increased glucose-6-P by conversion of Xu-5-P to glucose-6-P. Attempts to identify a signaling metabolite in whole cells where numerous metabolic intermediates are formed from a major substrate such as glucose are futile without knowing the individual steps involved in glucose activation of LPK gene expression. A possibility that Xu-5-P-activated PP2A might be involved in glucose-dependent activation of the LPK transcription prompted us to

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Research (151B), Dallas VA Medical Center, 4500 S. Lancaster Rd., Dallas, TX 75216. Tel.: 214-372-7028; Fax: 214-372-9534; E-mail: kuyeda6400@aol.com.

1 The abbreviations used are: ACL, ATP citrate lyase; GRBP, glucose response element binding protein; LPK, liver pyruvate kinase; DTT, dithiothreitol; FINSF, phenylmethylsulfonyl fluoride; GRE, glucose response element; FAS, fatty-acid synthase; ACC, acetyl-CoA carboxylase; PP2A, protein phosphatase; USF, upstream stimulatory factor; PEG, polyethylene glycol; Xu-5-P, xylulose-5-P; IRS, insulin-responsive sequence.
search for a transcription factor responsible for the glucose signaling. In the present study we report that we found a factor binding to the GRE of LPK in rat liver which is induced by high carbohydrate diet. We examined its DNA binding characteristics and regulation of its activity using cultured hepatocytes.

**EXPERIMENTAL PROCEDURES**

**Materials**—[γ-32P]ATP (3,000 Ci/mmol) was purchased from American Pharmacia Biotech and poly(dI-dC) from Pharmacia Biotech (Uppsala, Sweden). Okadate acid was purchased from Calbiochem, and dibutyryl-cAMP and forskolin were from Sigma. Restriction enzymes and T4 polynucleotide kinase were purchased from New England Biolabs (Beverly, MA). The Mutagen-Gen M13 in vitro mutagenesis kit was from Bio-Rad. Antibody against USF1, USF2, Sp1, c-Myc, and HNF4 was fromSanta Cruz Biotechnology (Santa Cruz, CA). All other chemicals were reagent grade and were obtained from commercial sources.

**Plasmid Constructions and Oligonucleotides**—The promoter region between positions −206 and −7 of the LPK gene (3) was obtained by polymerase chain reaction amplification using rat kidney genomic DNA as a template with outside primers containing engineered XhoI and HindIII sites. The primers used were CCCTGAGGCGCTGCTGACAGCGCCAAAG and CCAAGCTTGGTCTGGTGCTGGTTATATAC. The plasmid p−206/−7LPK was constructed by digesting pGEM-T vector (Promega/Madison, WI) and was digested with XhoI and HindIII. The digested fragment was ligated into the XhoI and HindIII sites of the luciferase expression plasmid, plg3-Base vector (Promega). Oligonucleotide-directed in vitro mutagenesis was performed as described by Kunkel (23) using the Muta-Gen M13 in vitro mutagenesis kit. Oligonucleotides used for the mutagenesis as well as DNA binding experiments are listed in Tables I and II.

**Animals**—Animals were starved for 48 h and then fed 24 h with NIH lab chow, high sucrose (20% casein, 60.2% sucrose, 15% cellulose, 2.25% minerals, and 2.25% vitamins) (24), high fat without starch (31% casein, 30.5% cellulose, 74% minerals, 1.5% vitamins, 1.5% corn oil, 1.5% minerals, and 2.25% vitamins) (25), and containing either 5.5 or 27.5 mM glucose. Cells were then cultured in Dulbecco’s modified Eagle’s medium supplemented as described above for 3 h to remove the insoluble matrix. The protein concentration was determined using the Bradford method (29).

**DNA Binding Assay Method**—Gel mobility shift assay was performed as described by Liu et al. (13). Double-stranded oligonucleotides were prepared by mixing equal amounts of the complementary single-stranded DNAs in 50 mM NaCl, heating to 70 °C for 15 min, and then cooling to room temperature. The annealed oligonucleotides were labeled with 32P in the presence of [γ-32P]ATP and poly(dI-dC). Gel mobility shift assay was performed as described above.

**RESULTS**

**Changes in GRE-binding Protein with Diet**—Following 48 h starvation, rats were re-fed NIH lab chow, high sucrose, high fat, or high protein diet for 12 and 24 h. The livers were quickly removed, nuclear and cytosolic extracts prepared, and GRE-binding proteins in the extracts analyzed by gel shift assay. The results showed that a specific binding factor (“band 1”) was present in the nuclear extract in low concentration and was difficult to detect, especially since an intense band later identified as USF was present in these extracts which migrated more slowly than band 1 protein. After precipitation of USF with PEG and subsequent concentration of the remaining extract, band 1 was detected more clearly. The results demonstrated that the nuclear extract contained two GRE-binding proteins (bands 1 and 2, Fig. 2, Liver), and only the band 1 protein changed with diet, i.e., the protein was extremely low upon starvation, induced by high sucrose diet in 12 and
between these sequence motifs also was altered by one base. Constructs of mutant oligonucleotides were the same as those used previously by Kaytor et al. (16) to examine the GRE-binding sites in the expression of S14 and LPK. The results (Fig. 3A) showed that band 1 binding to most of the mutants was decreased, compared with the wild type DNA, except for M5, M3/5, and M1. Competitive binding assays also were performed in which unlabeled oligonucleotide DNAs were incubated with the labeled, wild type oligonucleotide in order to test the ability of mutant oligonucleotides to compete for the DNA-binding site. The results were similar to those of the binding experiments in that M5, M3/5, and M1 were the most effective in competing for the binding site (data not shown).

In order to test the biological activities of the mutants, the 5′-flanking region containing the LPK promoter (−197 to +12; Fig. 1) was prepared by polymerase chain reaction using rat genomic DNA as a template with oligonucleotide primers based on the rat LPK gene sequence (30, 31). A HindIII site at −7 and XhoI site at −206 were introduced by site-directed mutagenesis using a synthetic oligonucleotide. The LPK promoter was then subcloned into the polylinker of a luciferase reporter vector, pGL3-Basic Vector (Promega). LPK promoters containing the wild type and various mutant GREs were constructed using oligonucleotide-directed in vitro mutagenesis, confirmed by DNA sequencing, and the activity of each was determined in primary cultured hepatocytes. The results, as depicted in Fig. 3B, demonstrated that all the mutant promoters except M5 and M3/5 showed significant loss of ability to activate LPK transcription by high concentration of glucose. M5, containing the perfect consensus CACGTG sequence, showed the highest activity. These results were generally in agreement with the previously published results of Kaytor et al. (16) for LPK transcription using chloramphenicol acetyltransferase transcription system in which only the MLTF site sequence (from −170 to −145) of the LPK promoter DNA was linked to −96 of the rest of the promoter DNA, thus lacking the HNF4 and NF1 sites (Fig. 1). One significant difference was the result of M6 in which they (16) showed that this mutant responds to glucose, whereas our result indicated no response. This discrepancy could be due to the lack of the accessory sites such as HNF4 and NF1 sites in the promoter they employed, which may influence the glucose response, since these accessory proteins may regulate the transcriptional activity. Thus, the above results demonstrated that bases 1–4 and 6, as well as the spacing between the consensus GRE sequences, were critical for the glucose response, but base 5 may not be. These transcriptional activities were, in general, similar to those of the DNA binding characteristics of band 1 protein, although the effect on the latter was not as striking as the former activities. Important differences were exhibited by M6 and M1 in which the glucose-activated transcription was completely inhibited by the mutation, but the DNA binding activity was unaffected. This discrepancy suggested that an additional factor(s) may be required to confer a higher degree of specificity of LPK transcription induced by glucose. We have termed the band 1 protein as glucose response element binding protein (GRBP) throughout the paper.

Specificity of GRBP for GRE Elements of Other Genes—In order to further examine the specificity of GRBP, DNA binding activities of GRBP for the carbohydrate response elements of S14, FAS, ACC, and ACL genes were determined. All these enzyme promoters contain the consensus CACGTG motifs (Table II). Both the nuclear and cytosolic GRBP showed similar binding characteristics (Fig. 4); GRBP bound FAS/IRS nearly as well as the LPK GRE elements but S14 less strongly. It did not bind to the GREs of ACC, ACL, or FAS. The binding


TABLE I

| LPK MLTF (wild type) (B) | 5’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’ (Ref. 33) |
|-------------------------|---------------------------------------------------|
| LPK MLTF (U)            | 3’-CCCGGCTCGCCCGTGAGGACCCACAG-5’                  |
| E box 1                 | 5’-CATGGGCCACCGCGCACTCCCGCTCCGCTGTTCC-3’         |
| E box 2                 | 3’-GGGCGCACCCCGCGCGGACCCCAAG-5’                  |
| S1                      | 5’-GTACCGGGCGGACCCCAAG-5’                         |
| M1                      | 3’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M2                      | 5’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M3                      | 3’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M4                      | 3’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M5                      | 5’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M6                      | 3’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M3/5                    | 3’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M/2                     | 3’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M1/2                    | 3’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |
| M1/3                    | 3’-GGGGGAGGAGCTCCGGCCATCCGCTCCGCTGTTCC-3’        |

Glucose-stimulated Gene Expression

Identity of GRBP—The transcription factors thus far suspected to bind to the glucose-responsive promoter sites of LPK and other genes include USF, Sp1, and c-Myc families of proteins. In order to see if GRBP is related to any of these factors, the nuclear and the cytosolic extracts were reacted with specific antibodies and subjected to gel mobility shift assay. The results showed that USF1 and Sp1, and c-Myc affected the electrophoretic mobility of GRBP, suggesting that GRBP does not belong to any of these families of factors.

Heat Lability of GRBP—Previously Liu et al. (13) described a hepatic nuclear factor that binds to the GRE site (MLTF-like site) of LPK and is resistant to heating (7 min at 70 °C). In contrast, both nuclear and cytosolic GRBP were completely inactivated by the heat treatment as discussed below. The results also ruled out GRBP as a member of NF1 family which binds to the −149- to −126-base pair region (Fig. 1) of the LPK promoter, since it has been shown to be resistant to heating at 90 °C for 5 min (32). Moreover, the heat inactivation studies revealed that the cytosolic GRBP was more sensitive to heating, since a half-maximum inactivation occurred at 40 °C, whereas the nuclear GRBP was more resistant, with a half-maximum at 56 °C (data not shown), suggesting there was a structural difference between the GRBPs in the cytosol and the nuclei.

Kinetics of GRBP Activation by Glucose in Hepatocytes—To gain insight into the glucose signaling mechanism, kinetics of glucose activation of GRBP in cytoplasm of hepatocytes were followed using the DNA binding assay. The primary hepatocytes, isolated from rats starved for 24 h, were incubated in a medium containing 10 mM lactate for 4 h. At 0 time the medium was switched to glucose (27.5 mM), and at the indicated time intervals the cells were harvested, and changes in the DNA binding activity of cytosolic GRBP were determined. The activity of GRBP increased linearly with time up to 12 h and reached a half-maximal value in 6 h and the maximum in 12 h (Fig. 7A). The activity in the hepatocytes incubated in lactate remained constant during the same period.

Effect of Varying Glucose Concentration on GRBP Activity—Hepatocytes isolated from rats starved for 48 h were incubated with varying concentrations of glucose for 12 h, and cytosolic GRBP activity was determined. The cells incubated in 10 mM lactate served as a control. The results showed that half-maximum activation of GRBP was achieved at approximately 12 mM and maximum activation at approximately 30 mM glucose (Fig. 7B), which is comparable to the range of glucose concentration required for LPK mRNA synthesis (34).

Specificity for Sugar—None of the sugars examined, fructose, ribose, and xylitol, at 20 mM were as effective as glucose in activating GRBP (Table III). 2-Deoxyglucose, which is known to be phosphorylated in vivo but not metabolized further (35), also...
activated GRBP, suggesting that 2-deoxyglucose-6-P could serve as an activator. 3-O-Methylglucose, which is not phosphorylated, failed to activate GRBP, suggesting that a phosphorylated sugar is an activator. These results of the glucose analogs were similar to the effect on LPK (34), ACC, and FAS gene transcription (35). Unfortunately, we were unable to obtain nuclei from these hepatocytes to examine the effect of these sugars and sugar analogs on the nuclear GRBP.

**Inhibition of GRBP by cAMP, Okadaic Acid, and Forskolin**

Transcriptional and DNA binding activities of the cytosolic GRBP were sensitive to inhibition by dibutyryl-cAMP (Bt2cAMP), okadaic acid, a potent inhibitor of PP2As, and forskolin, activator of adenylcyclase, in hepatocytes cultured in the presence of lactate or glucose prior to glucose administration (data not shown). A half-maximum inhibition of GRBP activity was elicited with approximately 0.1 mM Bt2cAMP and 90% inhibition with 1 mM, and the transcription activity showed comparable inhibition with the same concentrations of Bt2cAMP. Forskolin also inhibited both GRBP and the transcriptional activities, suggesting that increased cAMP in the hepatocytes is responsible for the inactivation of both activities. Okadaic acid at 1 mM inhibited the GRBP and the transcriptional activities 40 and 20%, respectively, and at 10 mM over 90 and 65%, respectively. Phorbol ester (1 mM) showed no inhibition of GRBP under these conditions (data not shown), suggesting that protein kinase C was not responsible for inactivation. Cycloheximide (10 μM) had no effect on the glucose activation (data not shown), suggesting that protein synthesis was not involved in increased GRBP in the hepatocytes. These results further suggest that GRBP may be involved in glucose stimulation of LPK transcription. Moreover, the observed inhibitory effects of cAMP and okadaic acid together imply that phosphorylation/dephosphorylation may be involved in the regulation of activity of GRBP, and a phosphorylation of the binding protein inactivates the activity.

**DISCUSSION**

The presence of two CACGTG-type E box motifs within the GREs of LPK promoter suggests that a member of the basic/helix-loop-helix/leucine zipper family of transcription factors binds to the site (36, 37) and is involved in carbohydrate activation of transcription. Among the potential protein factors binding to the site, USF appears to be a predominant hepatic nuclear factor. USF in hepatic nuclear extracts does bind to the site (12, 13, 38) but has been excluded as the right factor of the LPK GRE in **in vivo** based on several lines of evidence (12, 16), as discussed in the Introduction. We report herein a novel factor which bound to the “MLTF-like” sequence as demonstrated by electrophoretic mobility shift assay, and which depended on carbohydrate diet **in vivo**, and thus may play an important role in carbohydrate regulation of LPK expression. The evidence in support for this suggestion is as follows: (a) the factor occurred in the nuclei of rat livers and was induced by high carbohydrate diet, no other glucose responsive factor found; (b) the factor showed specificity for the MLTF-like sequence of the LPK promoter but not CACGTG-like E box of other genes of ACC, ACL, or FAS; (c) the factor showed specificity, although weak, toward palindromic CACGTG sequence; (d) the kinetics and the concentration of glucose required for the activation of GRBP were near physiological range; and (e) both the DNA binding and the transcription activities were inhibited to comparable degree by Bt2cAMP, forskolin, and okadaic acid consistent with the previous observation that glucose-induced expression of LPK gene is inhibited by cAMP (39) and also suggests that these activities are regulated by phosphorylation/dephosphorylation.

It was somewhat surprising to find the similar GRBP in the cytosolic extracts of rat livers. Other factors such as USF, HNF4, or the band 2 protein were absent in the cytosols. The cytosolic and nuclear GRBPs appear to be related since all the characteristics thus far uncovered were similar. The similar properties include electrophoretic mobility. DNA sequence binding specificity, immunoreactivity, solubility in salt and
The only difference between the nuclear and cytosolic factors was that the former was considerably more heat-resistant than the latter, suggesting they have different structures.

We can exclude GRBP from a number of known families of transcription factors. GRBP was not HNF4 which is capable of binding to the adjacent site just 3' to the GRBP-binding site (Fig. 1) and appears to serve as an accessory factor in the glucose activation (4, 13, 40). GRBP was not USF, Sp1, or c-Myc since it failed to react with any of the antibodies against those transcription factors that are known to interact with MLTF-like sequence (41). These results suggested that GRBP may be a new and unique factor.

The GRE and the insulin response element of FAS gene have been localized +283 to +303 nucleotides (12) and −71 to −50 nucleotides (42), respectively (Table II), and USF family of transcription factors have been shown to bind to both sites (43, 44). We found that GRBP did not bind to the GRE of the FAS gene even though it contains a perfect E box sequence CACGTG. However, GRBP did bind to the "insulin response element" of the FAS gene which contains an E box with a sequence CATGTG as tightly as to the GRE site of the LPK gene (Fig. 7). Wang and Sul (43) demonstrated that USF1 and USF2 as the major factors binding to the FAS-IRS site and the same complex also bind the E box sequence of S14 and LPK GREs. Thus, it is not surprising that GRBP was able to bind to the FAS-IRS site, even though the GRBP is not USF, and the E-box is not palindromic. The results suggested that GRBP bound not only to the consensus CA XX TG core but also the 3' extension of the FAS gene, and the latter region may be equally important for strong interaction.

The observation that cAMP and forskolin inhibited the activation of cytosolic GRBP may suggest that phosphorylation of

**Fig. 4.** Binding activities of nuclear and cytosolic GRBP to carbohydrate response elements of S14, FAS, ACC, and ACL genes. Nuclear (7.5 mg) and cytosolic (10 mg) extracts were incubated with 32P-labeled oligonucleotides (Table II) and subjected to electrophoresis in 4.5% nondenaturing polyacrylamide gel. The DNA binding activities were quantified as described under "Experimental Procedures."

**Fig. 5.** Gel supershift assay of nuclear and cytosolic GRBP binding to LII and HNF4 and immunoreactivity with anti-HNF4 antibody. Gel mobility shift assays were performed after incubation with or without HNF4 antibody. Three different 32P-labeled oligonucleotides (MLTF (U), L-II, HNF4; Tables I and II) were used as probes in the binding assay. Crude nuclear and cytosolic GRBP extracts were incubated with (+) or without (−) the antibody against HNF4 (2 μg) for 30 min at 25 °C prior to the DNA binding reaction. Lanes 1 and 2, crude nuclear extract (5 μg) reacted with MLTF; lanes 3 and 4, crude nuclear extract (5 μg) with LII; lanes 5 and 6, crude nuclear extract (5 μg) with HNF4; lanes 7 and 8, partially purified nuclear GRBP (12 μg) with MLTF; and lanes 9 and 10, cytosolic GRBP (1 μg) with MLTF.

**Fig. 6.** Gel shift assay of nuclear and cytosolic GRBP reacted with anti-USF1, anti-Sp1, and anti-c-Myc antibodies. The nuclear and the cytosolic extracts were reacted with indicated antibodies (2 μg) for 30 min at 25 °C and then incubated with 32P-labeled MLTF (U) oligonucleotide for the binding assay. Experimental conditions and procedures were the same as in Fig. 2. Lanes 1 and 2, crude nuclear extract (5 μg) and + anti-USF1 antibody; lanes 3 and 4, partially purified nuclear GRBP (10 μg) ± anti-USF-1 antibodies + anti-Sp1 (lane 5), and anti-c-Myc (lane 6) antibodies; lanes 7 and 8, cytosolic GRBP (20 μg) ± anti-USF1 + anti-Sp1 (lane 9), and + anti-c-Myc (lane 10) antibodies.
in vivo inhibition of a PP2A, thus maintaining GRBP in a phospho inactive form of the phosphorylated GRBP. The protein kinase(s). The activation of the cytosolic GRBP probably involves dephosphorylation of phosphorylated GRBP which is supported by the fact that okadaic acid, a potent inhibitor of PP2As, may inhibit dephosphorylation and activation of the GRBP may be involved in the inactivation. This is further supported by the fact that okadaic acid, a potent inhibitor of PP2As, may inhibit dephosphorylation and activation of the inactive form of the phosphorylated GRBP. The protein kinase involved in the phosphorylation of GRBP could be cAMP-dependent protein kinase, but a few preliminary results indicated that it was not the case. Thus, the effect of cAMP may not be direct but probably more complex and may require additional enzyme(s). The activation of the cytosolic GRBP probably involves dephosphorylation of phosphorylated GRBP which is stimulated by glucose. Evidence in support of the suggestion was the observation that okadaic acid in nanomolar concentrations inactivated cytosolic GRBP, which can be explained by its inhibition of a PP2A, thus maintaining GRBP in a phospho form.

Doiron et al. (19) showed that the LPK transcription in hepatocytes and mhAT3F hepatoma cells was increased when these cells were incubated in xylitol, and they suggested that the same xylulose-5-P (Xu-5-P)-mediated mechanism may apply for the LPK transcription as that originally demonstrated for short term regulation of glucose signaling in Fru-2,6-P2 formation (20). Unfortunately, Doiron et al. (19) failed to demonstrate the formation and the concentration of Xu-5-P in their cells under the conditions of LPK transcription; consequently, the rates of Xu-5-P formation and LPK induction cannot be correlated. We found that the activation of the LPK transcription in hepatocytes took at least 3–6 h (Fig. 7), while the rate of Xu-5-P formation is less than 5 min (in perfused liver) (20). Thus, these two rates are vastly different and do not seem to support the Xu-5-P-mediated mechanism and Xu-5-P as the glucose signaling compound.

In summary, we have found a protein factor binding to the glucose response elements of LPK and FAS/IRS genes in both nuclear and cytosolic extracts of rat liver. The nuclear and cytosolic factors exhibited similar activities and appeared to be related but not identical. The DNA binding activity was inhibited by low glucose in vitro and by cAMP in hepatocytes and was activated by high glucose in vivo. Purification, characterization, and determination of their roles in regulation of LPK are under way.

Acknowledgments—We thank Dr. Sarah McIntire for critical review of this manuscript and Drs. Steven L. McKnight and Richard Gaynor for their advice.

REFERENCES

1. Goodridge, A. G. (1987) Annu. Rev. Nutr. 7, 157–185
2. Granner, D., and Pilkis, S. J. (1990) J. Biol. Chem. 265, 10173–10176
3. Thompson, K. S., and Towle, H. C. (1991) J. Biol. Chem. 266, 8679–8682
4. Vaulont, S., Puzenat, N., Levrat, F., Cognet, M., Kahn, A., and Raymondjean, M. (1989) J. Mol. Biol. 206, 205–219
5. Blackwell, T. K., Kretzner, L., Blackwood, K. E., Eisenman, R. N., and Weintraub, H. (1990) Science 250, 1149–1151
6. Beckmann, H., Su, L.-K., and Kadesch, T. (1990) Genes Dev. 4, 167–179
7. Carr, C. S., and Sharp, P. A. (1989) Mol. Cell. Biol. 10, 4384–4388
8. Ogawa, N., and Oshima, Y. (1990) Mol. Cell. Biol. 10, 2224–2236
9. Yokoyama, C., Wang, X., Briggs, M. R., Admon, A., Wu, J., Hua, X., Goldstein, J. L., and Brown, M. S. (1993) Cell 75, 187–197
10. Tontonoz, P., Kim, J. B., Graves, R. A., and Spiegelman, B. M. (1993) Mol. Cell. Biol. 13, 4753–4759
11. Gregor, P. D., Sarwadouy, M., and Roeder, R. G. (1990) Genes Dev. 4, 1730–1740
12. Shih, H.-M., and Towle, H. C. (1994) J. Biol. Chem. 269, 9580–9587
13. Liu, Z., Thompson, K. S., and Towle, H. C. (1993) J. Biol. Chem. 268, 12787–12795
14. Shih, H.-M., Liu, Z., and Towle, H. C. (1995) J. Biol. Chem. 270, 21991–21997
15. Shih, H.-M., and Towle, H. C. (1992) J. Biol. Chem. 267, 13222–13228
16. Kayter, E. N., Shih, H., and Towle, H. C. (1997) J. Biol. Chem. 272, 7525–7531
17. Prip-Buus, C., Perdereau, D., Peaule, F., Maury, J., Ferre, P., and Girard, J. (1995) Eur. J. Biochem. 236, 309–315
18. Kang, R., Yamada, K., Tanaka, T., Lu, T., and Noguchi, T. (1996) J. Biochem. (Tokyo) 119, 162–166
19. Doiron, B., Cuff, M.-H., Chen, R., and Kahn, A. (1996) J. Biol. Chem. 271, 5321–5324
20. Nishimura, M., Fedorov, S., and Uyeda, K. (1994) J. Biol. Chem. 269, 26100–26106

**TABLE III**

| Sugar              | GRBP activity |
|--------------------|---------------|
| Lactate            | 1             |
| Glucose            | 10.5 ± 3.4    |
| Fructose           | 4.4 ± 0.8     |
| Ribose             | 7.4 ± 1.9     |
| Xylitol            | 7.5 ± 2.7     |
| 2-Deoxyglucose     | 6.4 ± 0.9     |
| 3-O-Methylglucose  | 2.0 ± 0.8     |

**FIG. 7.** A, kinetics of activation of cytosolic GRBP by glucose and lactate. Primary hepatocytes were isolated from rats starved for 48 h and incubated in 6-cm culture plates at a density of 3.6 × 10⁶ cells/well in glucose-free medium containing 10 mM lactate for 6 h. The medium was switched to 27.5 mM glucose (●) or 10 mM lactate (▲), and at the indicated time intervals the cells are collected. Cytosolic extracts of the cells were prepared and subjected to gel mobility shift assay as in Fig. 2. B, effect of varying concentrations of glucose on cytosolic GRBP activity in hepatocytes. Hepatocytes were cultured for 12 h in the presence of the varying concentrations of glucose. Cytosolic extracts were prepared as described under “Experimental Procedures” and subjected to gel shift assays as in Fig. 2.
Glucose-stimulated Gene Expression

21. Nishimura, M., and Uyeda, K. (1995) *J. Biol. Chem.* 270, 26341–26346
22. Mourrieras, F., Foufelle, F., Foretz, M., Morin, J., Bouche, S., and Ferré, P. (1997) *Biochem. J.* 326, 345–349
23. Kunkel, T. A. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 488–492
24. Casazza, J. P., and Veech, R. L. (1986) *Biochem. J.* 236, 635–641
25. Fraucone, O. L., Griffaton, G., and Kalopissis, A. D. (1992) *Am. J. Physiol.* 236, E615–E623
26. Berry, M. N., and Friend, D. S. (1969) *J. Cell Biol.* 43, 506–520
27. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M., and Danielsen, M. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 7413–7417
28. Hattori, M., Tugores, A., Veloz, L., Karin, M., and Brenner, D. A. (1990) *DNA Cell Biol.* 9, 777–781
29. Bradford, M. M. (1976) *Anal. Biochem.* 72, 248–254
30. Cognet, M., Lone, Y. C., Vaulont, S., Kahn, A., and Marie, J. (1987) *J. Mol. Biol.* 196, 11–25
31. Noguchi, T., Yamada, K., Inoue, H., Matsuda, T., and Tanaka, T. (1987) *J. Biol. Chem.* 262, 14366–14371
32. Yamada, K., Tanaka, T., and Noguchi, T. (1997) *Biochem. J.* 324, 917–925
33. Matsuda, T., Nogushi, T., Yamada, K., Takenaka, M., and Takenaka, T. (1999) *J. Biochem. (Tokyo)* 108, 778–784
35. Foufelle, F., Gouhot, B., Péquier, J.-P., Perdereau, D., Girard, J., and Ferré, P. (1992) *J. Biol. Chem.* 267, 20543–20548
36. Prendergast, G. C., and Ziff, E. B. (1991) *Science* 251, 186–189
37. Murre, C., McGaw, P. S., Vaessin, H., Caudy, M., Jan, L. Y., Lan, Y. N., Cabrera, C. V., Buskin, J. N., Hauchek, S. D., Lassar, A. B., Weintraub, H., and Baltimore, D. (1989) *Cell* 58, 537–544
38. Diaz Guerra, M.-J., Burgof, M. O., Martinez, A., Cuif, M. H., Kahn, A., and Raymondjean, M. (1993) *Mol. Cell. Biol.* 13, 7725–7733
39. Decaux, J. F., Antoine, B., and Kahn, A. (1989) *J. Biol. Chem.* 264, 11584–11590
40. Viellet, B., Kahn, A., and Raymondjean, M. (1997) *Mol. Cell. Biol.* 17, 4208–4219
41. Liu, Z., Thompson, K. S., and Towle, H. C. (1993) *J. Biol. Chem.* 268, 12787–12795
42. Moustaid, N., Sakamoto, K., Clarke, S., Beyer, R. S., and Sul, H. S. (1993) *Biochem. J.* 292, 767–772
43. Wang, D., and Sul, H. S. (1995) *J. Biol. Chem.* 270, 28714–28722
44. Foufelle, F., Lepetit, N., Bose, D., Delzenne, N., Morin, J., Raymondjean, M., and Ferré, P. (1995) *Biochem. J.* 308, 521–527