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Commentary

Carbohydrate responsive element-binding protein (ChREBP): a key regulator of glucose metabolism and fat storage

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Abstract

Feeding a high carbohydrate diet induces transcription of more than 15 genes involved in the metabolic conversion of glucose to fat. A new transcription factor binding to a glucose response element of the pyruvate kinase and lipogenesis enzyme genes was discovered recently. This factor, termed carbohydrate responsive element-binding protein (ChREBP), is activated in response to high glucose and up-regulates these genes. Cyclic AMP and a high fat diet inhibit ChREBP and slow down glucose utilization. ChREBP is able to control transcription of lipogenic enzyme genes in response to nutritional and hormonal inputs, and may play an important role in disease states such as diabetes, obesity, and hypertension. Published by Elsevier Science Inc.

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1. Introduction

Evolutional pressures have favored the ability to efficiently store nutrients as fat during abundant food supply as a safeguard against occasional famine [1]. Due to the abundance of the food supply and dramatic changes in modern lifestyle, these ''thrifty genes'' may now contribute to a major epidemic of obesity, especially in the United States where over half of the population is overweight. This is predicted to be a major public health problem in the very near future.

The liver is the principal organ responsible for the conversion of excess dietary carbohydrate into triglycerides. Ingestion of a high carbohydrate diet leads to activation of several regulatory enzymes of glycolysis and lipogenesis, including L-type pyruvate kinase (LPK), phosphofructokinase (PFK) via increased fructose 2,6-P_2 (Fru 2,6-P_2), acetyl CoA carboxylase (ACC), and fatty acid synthase (FAS) [2]. Excess carbohydrate also results in post-translational activation as well as transcription of at least 15 genes encoding the same key enzymes involved in carbohydrate metabolism and lipogenesis, thereby promoting long-term storage of carbohydrates as triglycerides [3,4]. Until recently, it was thought that insulin and glucagon regulate the transcription of these genes. However, it has been shown using cultured primary hepatocytes that nutrients themselves play an important role in the regulation of transcription, independent of these hormones (reviewed in [5,6]). LPK gene expression is stimulated by glucose, independent of insulin, in cultured hepatocytes expressing glucokinase [7].

The mechanism by which excess carbohydrate generates a signal to induce the transcription of lipogenesis enzyme genes is not known. Previous studies have shown that many lipogenic genes contain ChREs within their promoters that mediate glucose responsiveness [4,8–11]. These sequences have been used to characterize several ChRE-binding factors that mediate glucose responsiveness [12–15]. However, the identity of the carbohydrate responsive transcription factor remains elusive, since expression/activity of these proteins does not correlate with glucose activation regulated by the ChRE. Because the transcription factor is not known, the nature of the glucose-signaling compound cannot be determined, although it has been established that a metabolite of glucose, not glucose *per se*, is responsible for the signal (reviewed in [16]). Several metabolites of glucose have been proposed as candidates [17–19], but

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Abbreviations: LPK, L-type pyruvate kinase; Fru 2,6-P_2, fructose 2,6-P_2; ChREs, carbohydrate responsive elements; ChREBP, carbohydrate responsive element-binding protein; b/HLH/Zip, basic helix-loop-helix-leucine zipper; PKA, protein kinase A; AMPK, AMP-activated protein kinase; cAMP, cyclic AMP; PP2A, protein phosphatase 2A; and SREBP, steroid responsive element-binding protein.

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since these studies were performed in whole cells where many metabolites are formed from glucose, it is impossible to identify any specific compound as the signaling substance.

2. Discovery of a transcription factor

Several years ago we attempted to purify and identify the transcription factor as well as the glucose-signaling compound. The LPK gene was selected because the glucose response element of the gene has been firmly established previously by others (reviewed in [20,21]). The glucose response element consists of two imperfect E boxes (CACGGG and CCCGTG) separated by 5 bases (reviewed in [20,21]. Synthetic oligonucleotides containing the sequence were used in detection of the transcription factor in rat liver nuclei. Our initial attempts to detect the DNA-binding protein in nuclear extracts from rat liver were unsuccessful. This failure was attributed to the preparation of the nuclear extract by a conventional method [22] using ammonium sulfate to precipitate the proteins in nuclear extracts followed by dialysis. This method resulted in complete denaturation of the transcription factor as well as many other proteins. Use of polyethylene glycol as a precipitation agent stabilized the factor (the procedure was first described in [13]), eliminated the time-consuming dialysis, and made it possible to assay nuclear extracts for the DNA-binding activity within a few hours. This improvement in the extract preparation led us to detect a new transcription factor by ''gel shift assay''. The active protein was responsive to diet, i.e. activated by a high carbohydrate diet, and inhibited by high fat or starvation [23]. A number of other tests provided evidence in support of the factor as the long-sought glucose response element-binding protein. This transcription factor was purified to apparent homogeneity and identified. It was designated as ChREBP. ChREBP is a large protein (864 amino acids and $M_r = 94{,}600$) (Fig. 1) and contains several domains including a nuclear localization signal (NLS) near the N-terminus, polyproline domains, a basic helix-loop-helix-leucine zipper (b/HLH/Zip), and a leucine-zipper-like (Zip-like) domain. In addition, it contains several potential phosphorylation sites for cAMP-dependent protein kinase (PKA) and AMPK.

3. Regulation of ChREBP by glucagon and nutrients

The regulation of ChREBP by cAMP/glucagon, high glucose, and high fat was described recently [24]. It is well known that glucagon and a high fat diet inhibit the transcription of glycolytic enzyme and lipogenesis enzyme genes and inhibit gluconeogenic enzyme genes (reviewed in [3,25]).

3.1. Nuclear import/export of ChREBP

High glucose is able to overcome these inhibitions. We have determined how ChREBP is regulated by these diets and glucagon/cAMP in hepatocytes [24]. Using ChREBP fused with a green fluorescent protein (GFP), we demonstrated that ChREBP is localized in the cytosol if the hepatocytes are incubated in low glucose (5.5 mM), but imported into the nucleus under high glucose conditions (27.5 mM). We also demonstrated that this translocation is controlled by the phosphorylation/dephosphorylation of Ser196, which is located near the NLS site. The phosphorylation of this site by PKA was confirmed since mutation of Ser196 to Ala permits translocation of ChREBP to the nucleus, but a Ser196Asp mutant remains in the cytosol. Glucose is able to overcome the cAMP inhibition and activates the import of ChREBP into the nucleus. Thus, the mechanism of glucose activation appears to involve the import of ChREBP into the nucleus probably by dephosphorylation of the phospho-Ser196 of ChREBP. This is confirmed, since a PKA inhibitor (H-89) results in activation of nuclear localization, but an inhibitor of protein phosphatase (canthradic acid) results in complete inhibition. The observation that the effects of the inhibitor and activator are nearly complete suggests that the phosphorylation/dephosphorylation is the only factor controlling the nuclear and cytosolic partitioning process.

3.2. Regulation of DNA-binding activity

There is a second tier of regulation of ChREBP by excess glucose. ChREBP contains additional phosphorylation sites for PKA, namely Ser626 and Thr666. Ser626 is situated a few amino acids toward the N-terminus of the b/HLH/Zip domain, while Thr666 is within the basic residues that bind the DNA. When a recombinant C-terminal region of ChREBP containing the b/HLH/Zip with a single

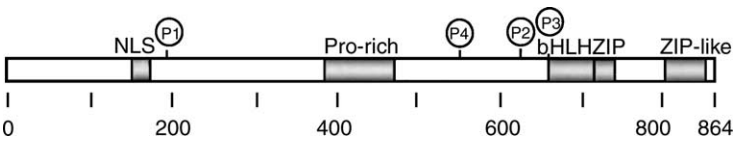

Fig. 1. Schematic representation of the functional domains and phosphorylation sites for cAMP-dependent protein kinase and AMPK of ChREBP. The locations of three phosphorylation sites of PKA are indicated as P1, P2, and P3. The location of a putative AMPK phosphorylation site is indicated as P4. NLS, nuclear localization site; Pro-rich, high proline contents; b/HLH/Zip, basic helix-loop-helix-leucine zipper; and Zip-like, leucine zipper-like structure.

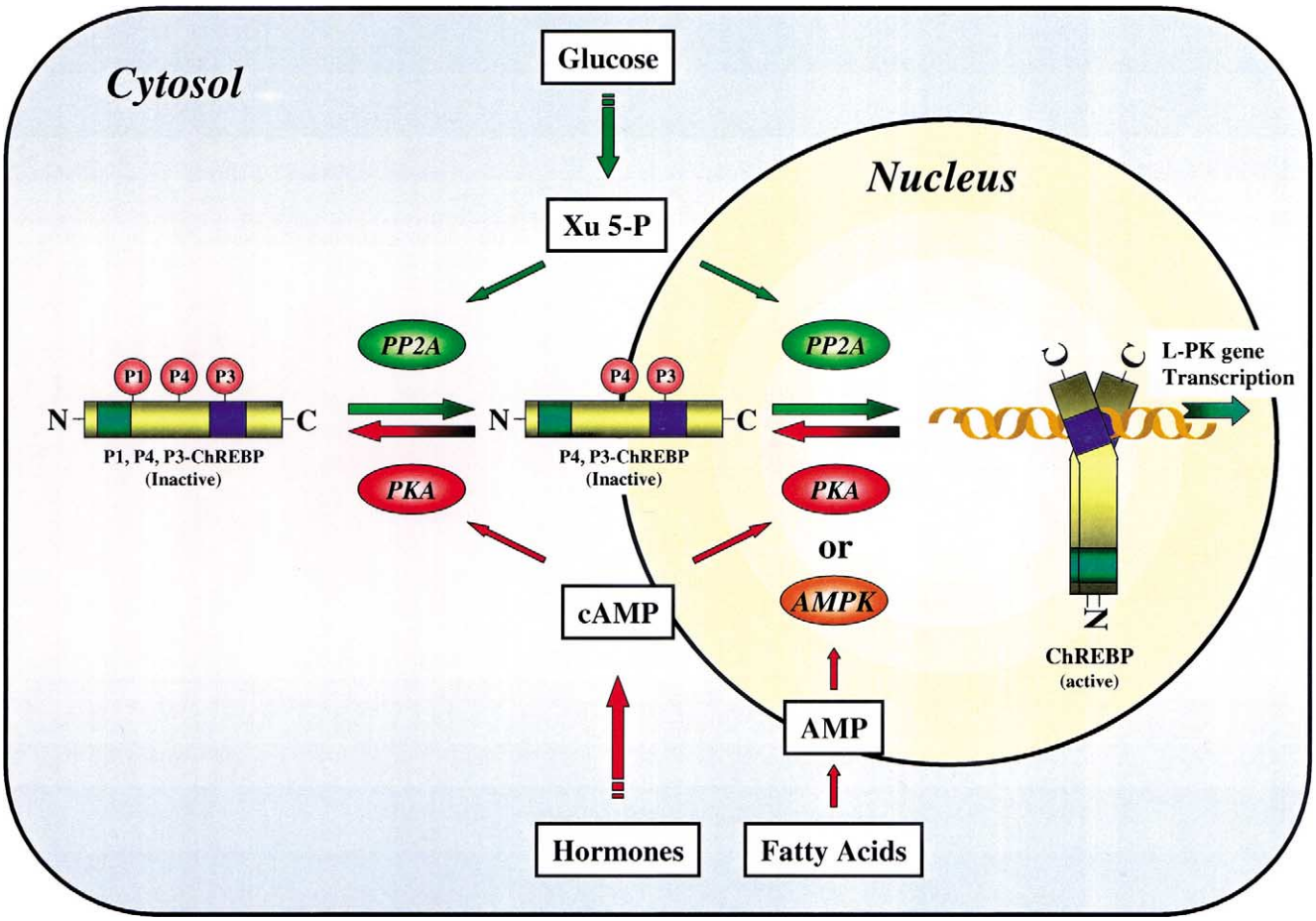

Fig. 2. Schematic representation of a possible regulatory mechanism of ChREBP by glucose and cAMP in hepatocytes. Three PKA phosphorylation sites of ChREBP, Ser196 (P1), Thr666 (P3), and Ser568 (P4), play crucial roles in high glucose-induced activation of ChREBP and inhibition by cAMP and AMP. The P2 site at Ser626 is not shown because its role is not known at present. Glucose signaling may activate PP2A via xylulose-5-phosphate (Xu 5-P). In the cytoplasm, Xu 5-P-activated PP2A dephosphorylates the P1 site of ChREBP, which results in activation of the import of ChREBP into the nucleus. Once ChREBP is localized in the nucleus, glucose activates the inactive form of ChREBP (P4, P3-ChREBP) by dephosphorylation of the P3 site catalyzed by nuclear PP2A. The P2 probably serves as an accessory factor. ChREBP, which is dephosphorylated at P1 and P3, binds to the ChRE of the LPK gene and activates transcription of the LPK gene. Fatty acids increase the AMP/ATP level in hepatocytes, which activates AMP-activated protein kinase (AMPK). AMPK phosphorylates Ser568 and inactivates the DNA-binding activity of ChREBP.

phosphorylation site, Thr666, is phosphorylated with PKA *in vitro*, the DNA-binding activity is inhibited completely [24]. The rates of ^{32}P incorporation into Thr666 of the truncated ChREBP and the loss of the DNA-binding activity are the same, indicating that inhibition is the direct result of phosphorylation. When the phosphorylated/inactive ChREBP is treated with the PP2A catalytic subunit, the DNA-binding activity is reversibly activated, and both rates are similar, again supporting the direct regulation by dephosphorylation. Double mutants of ChREBP consisting of Ala or Asp mutants of Ser626 with a transport-active Ser196Ala were tested for the effect of phosphorylation of Ser626. A Ser626Asp (with Ser196Ala) mutant loses transcriptional activity, probably as a result of the loss of DNA-binding activity. However, the similar double mutant of Thr666Ala (Ser196Ala) shows LPK promoter activity even in the presence of cAMP, suggesting that Thr666 is a more important phosphorylation site than Ser626, and that Ser626 may play a passive role in the overall transcriptional activity. Thus, there appear to be two levels of regulation of ChREBP by PKA-mediated phosphorylation as a result of a rise in cAMP. One is the phosphorylation of Ser196, which inhibits nuclear import, and the other is Thr666, which inhibits DNA-binding activity (Fig. 2). These studies delineate the pathway for the cAMP-mediated action of glucagon.

4. Fatty acid inhibition of ChREBP

A high fat diet inhibits glucose metabolism, and sometimes is referred to as the "fatty acid sparing effect of glucose". We showed that ChREBP was inhibited significantly in the liver of rats fed a high fat diet compared with livers from those fed the normal laboratory chow or a high carbohydrate diet [23]. Short-chain fatty acids such as acetate and long-chain fatty acids such as octanoate and palmitate inhibit the glucose-induced activation of LPK

transcription in hepatocytes [26]. In these hepatocytes, the cytosolic AMP concentration increases 30-fold, and, as a result, AMPK is activated. 5-Amino-4-imidazolecarboxamide ribotide (AICAR), a specific activator of AMPK, also inhibits LPK transcription, further supporting the idea that AMPK is responsible for the inhibition by phosphorylation. A Ser568Ala mutant of a truncated ChREBP consisting of the C-terminal region can be phosphorylated by the AMPK catalytic subunit, which results in the loss of inhibition by fatty acids and retention of DNA-binding activity. On the other hand, a Ser568Asp mutant shows weak DNA-binding and LPK transcriptional activity even in the absence of fatty acids. Thus, the fatty acid inhibition of glucose-induced LPK transcription appears to be the result of phosphorylation of ChREBP at Ser568 by AMPK, which inactivates DNA-binding activity. AMPK is activated by the increased AMP, which likely results from fatty acid activation by fatty acyl CoA synthetase.

5. Mechanism of activation by glucose

Glucose is able to reverse these inhibitory effects at both levels. The simplest mechanism for this reversal is by dephosphorylation of these phosphorylation sites by a protein phosphatase(s) (PPase). Since there are two sites of activation involved, namely dephosphorylation of Ser196 in the cytosol and of Thr666 in the nucleus, the protein phosphatases catalyzing these reactions must reside in these two separate compartments. The question is, are the PPases different and, if so, are the glucose-signaling mechanisms also different. The type of PPase (cytosolic) specific for Ser196 appears to be PP2A because it is inhibited by canthradic acid, which is known to be specific for this type of PPase. Another question is whether the same PP2A, which we discovered several years ago [27], can catalyze the dephosphorylation of both sites. The previously described PP2A is also activated in liver by high glucose and catalyzes the dephosphorylation of an important enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (Fig. 3). This bifunctional enzyme synthesizes and degrades Fru 2,6-P_2, the most potent activator of a key regulatory enzyme, phosphofructokinase, and thus controls glycolysis in the liver. The phosphorylation by PKA results in inactivation of the kinase and activation of the phosphatase, resulting in a decrease in Fru 2,6-P_2 levels. Under increased glucagon such as in starved conditions, hepatic glycolysis is then inhibited, because of low Fru 2,6-P_2 resulting from the phosphorylation of the bifunctional enzyme (Fig. 3). However, administration of excess glucose activates a specific PP2A that dephosphorylates the inactive/phosphorylated bifunctional enzyme, resulting in activation of the kinase and inactivation of the phosphatase, which, in turn, results in a rise in Fru 2,6-P_2 levels. The search for a glucose-signaling compound revealed that xylulose-5-P (Xu 5-P) is specifically able to activate the PP2A [28]. Xu 5-P is an intermediate of the nonoxidative part of the pentose shunt pathway and is formed from two glycolytic intermediates, glyceraldehyde 3-P (GAP) and fructose-6-P (Fru 6-P) catalyzed by transketolase (GAP + Fru 6-P ↔ Xu 5-P + erythrose 4-P). Thus, these findings demonstrate for the

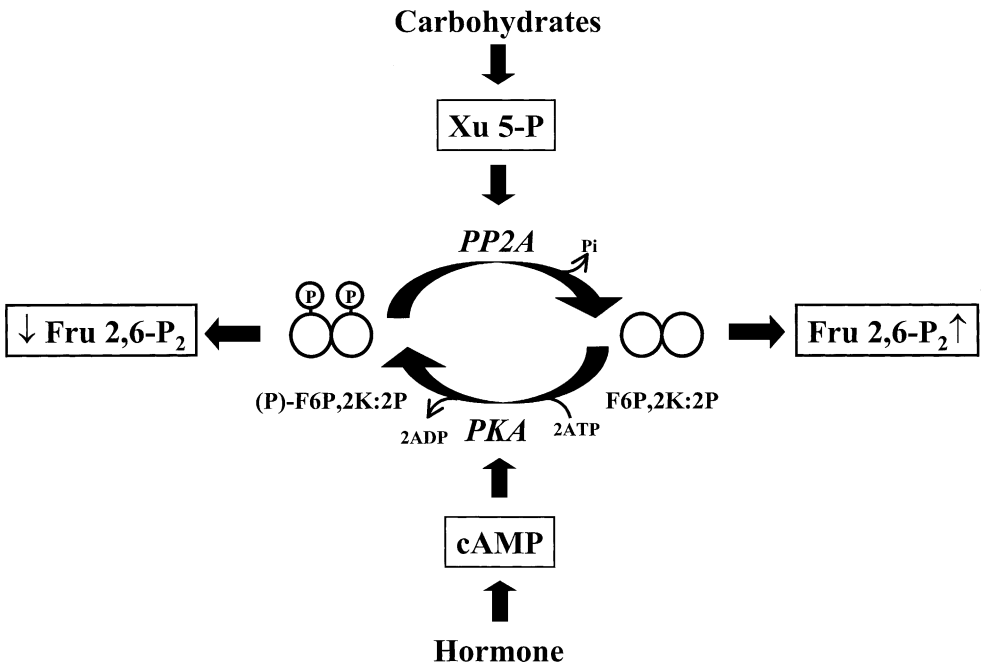

Fig. 3. Scheme for the regulation of the concentration of Fru 2,6-P_2 in the liver by Fru 6-P,2-kinase:Fru 2,6-Pase mediated by two adversaries, cAMP and excess glucose. (P)-Fru 6-P,2K:2P, phosphorylated Fru 6-P,2-kinase:Fru 2,6-Pase.

first time a pentose shunt intermediate involved directly in the control of the most important activator of glycolysis, Fru 2,6-P_2, and emphasizes coordinated control of these two pathways of glucose metabolism. Furthermore, Xu 5-P appears to be the signaling metabolite for the glucose activation of glycolysis. We are currently investigating the possibility that the same Xu 5-P-activated PP2A is involved in the activation of both nuclear transport and the DNA-binding activities of ChREBP.

6. SREBP activation of lipogenesis and sterol synthesis

Ingestion of a diet high in carbohydrate results in increased insulin, and a pathway by which lipogenesis is stimulated appears to involve a transcription factor called SREBP. SREBP was discovered by Brown and Goldstein (reviewed in [29]) as a transcription factor that controls genes involved in cholesterol uptake and biosynthesis. In addition to cholesterol gene regulation, the SREBP1c isoform has been shown to regulate the transcription of genes of the lipogenic pathway [30]. SREBP has a unique dual DNA-binding specificity and binds to a consensus sequence: CAC(C/G)CAC, a so-called sterol regulatory element-1 or SRE-1, or to palindromic sequences termed E-boxes within the promoters of lipogenesis genes [30,31].

When cells are cultured in medium containing high cholesterol, SREBPs are bound in the endoplasmic reticulum (ER) by two membrane spanning domains of the protein [32]. Upon sterol deprivation, the amino terminal region of the SREBPs is cleaved by two proteolysis steps catalyzed by two separate proteases [33], and released from the ER. The released SREBPs are then imported into the nucleus where they activate the transcription of various genes. Fatty acids show similar activation processing of the SREBPs; thus, the SREBPs, especially SREBP1c, become an important regulator of both cholesterol and fatty acid synthesis.

ChREBP and SREBP are a pair of important transcription factors involved in the nutrient and hormonal regulation of genes encoding enzymes of glucose metabolism and lipogenesis pathways. The regulation of ChREBP is mediated by phosphorylation/dephosphorylation, especially in response to cAMP and glucose, while SREBP is regulated by an insulin-mediated proteolytic cleavage to generate an active factor. The discovery of ChREBP should aid in the future investigation into the mechanisms of conversion of excess glucose to storage fat. The important problems remaining include the glucose-signaling compound and its mechanism of activation, which may involve a protein phosphatase such as Xu 5-P-activated PP2A. ChREBP may play an essential role(s) in the regulation of a number of other enzyme genes, besides the LPK gene, involved in glucose and lipid metabolism. To gain insight into these possibilities, we are in the process of preparing ChREBP gene knockout mice. They may provide important information regarding the potential roles of ChREBP *in vivo*.

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