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Invited Mini Review

CREB and FoxO1: two transcription factors for the regulation of hepatic gluconeogenesis

*Kyoung-Jin Oh , Hye-Sook Han, Min-Jung Kim & Seung-Hoi Koo**
Division of Life Sciences, Korea University, Seoul 136-713, Korea

Liver plays a major role in maintaining glucose homeostasis in mammals. Under fasting conditions, hepatic glucose production is critical as a source of fuel to maintain the basic functions in other tissues, including skeletal muscle, red blood cells, and the brain. Fasting hormones glucagon and cortisol play major roles during the process, in part by activating the transcription of key enzyme genes in the gluconeogenesis such as phosphoenol pyruvate carboxykinase (PEPCK) and glucose 6 phosphatase catalytic subunit (G6Pase). Conversely, gluconeogenic transcription is repressed by pancreatic insulin under feeding conditions, which effectively inhibits transcriptional activator complexes by either promoting post-translational modifications or activating transcriptional inhibitors in the liver, resulting in the reduction of hepatic glucose output. The transcriptional regulatory machineries have been highlighted as targets for type 2 diabetes drugs to control glycemia, so understanding of the complex regulatory mechanisms for transcription circuits for hepatic gluconeogenesis is critical in the potential development of therapeutic tools for the treatment of this disease. In this review, the current understanding regarding the roles of two key transcriptional activators, CREB and FoxO1, in the regulation of hepatic gluconeogenic program is discussed. [BMB Reports 2013; 46(12): 567-574]

INTRODUCTION

Glucose functions as a primary fuel for mammals. Thus, the maintenance of the glucose homeostasis is one of the primary mechanisms for the survival of the organisms. Intake of dietary carbohydrates and their absorption in the intestine provide one of the primary events to control the rate of glucose metabolism. In addition, utilization of glucose by skeletal muscles or adipocytes, as well as the glucose output by the liver, are also key components for controlling glucose homeostasis (1).

*Corresponding author. Tel: +82-2-3290-3403; Fax: +82-2-3290-4144; E-mail: koohoi@korea.ac.kr

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The liver is critical in maintaining glucose homeostasis, as an organ that controls the equilibrium of plasma and hepatic glucose levels by the regulated uptake and storage process and the output of glucose (1-3). Under feeding conditions, the intake of carbohydrates is generally supplied to the bloodstream as a form of glucose via the intestinal system, which can be used by peripheral tissues such as skeletal muscle, red blood cells, and the brain. Excessive glucose in the liver is initially stored as glycogen via glycogenesis, and eventually processed as a triglyceride by a combination of metabolic pathways including glycolysis, fatty acid biosynthesis, triglyceride synthesis, and maturation. The latter process is termed as lipogenesis. The resultant triglyceride is converted into very low density lipoprotein (VLDL), and is transported into the white adipose tissues (4). Pancreatic insulin plays a major role in the process by activating key regulatory enzymes in the process by acute post-translational modifications. Furthermore, expression of genes involved in the lipogenesis is increased by transcription factors such as SREBP-1c and ChREBP. The roles of these transcription factors are described elsewhere (5-7). Under fasting conditions, the increase in glucagon levels that are associated with the decrease in insulin levels in the plasma is critical in the glucose output from the liver by initially enhancing glycogen breakdown. Prolonged fasting induces *de novo* glucose synthesis from the liver, termed gluconeogenesis, by acute activation of key regulatory enzymes that include glucose 6 phosphatase (G6Pase), fructose 1,6-bisphosphatase (Fbpase), pyruvate carboxylase (PC), and phosphoenolpyruvate carboxykinase (PEPCK). Furthermore, chronic activation of gluconeogenesis is ultimately achieved by the transcriptional activation of the aforementioned gluconeogenic genes (2, 3, 8, 9).

As major catabolic hormones, glucagon and stress hormone cortisol instigate the intracellular signaling pathways to activate key transcription factors such as cAMP response element binding protein (CREB) and forkhead box class Os (FoxOs) (10-13). Transcriptional co-activators are necessary to modulate the full activity of these transcription factors. The transcriptional co-activators that have been linked to these factors include CREB binding protein (CBP)/p300, CREB regulated transcription co-activator 2 (CRTC2), peroxisome proliferator-activated receptor gamma co-activator 1 alpha (PGC-1α), and protein arginine methyltransferases (PRMTs) (14-19). On the other hand,

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the anabolic hormone insulin inhibits the gluconeogenic program either via inactivation of transcriptional activators/co-activators or activation of transcriptional repressors of gluconeogenic genes. Examples of the transcriptional repressors include orphan nuclear receptor SHP and Transcription factor 7-like2 (TCF7L2) (20-22).

In this review, we would like to delineate the molecular mechanisms by which CREB and FoxO1 control the hepatic gluconeogenic program. We will also provide the involvement of transcriptional co-activators and repressors to modulate the activity of transcriptional circuits for hepatic gluconeogenesis.

CREB

General facts

CREB is one the first transcription factors purified by DNA affinity column using the cyclic AMP binding element (CRE) of the somatostatin gene (23). G-protein coupled receptor (GPCR)-mediated activation of adenylyl cyclase increased levels of cyclic AMP in the cell, which activates protein kinase A (PKA) and the subsequent phosphorylation of Ser 133 of CREB. These phosphorylation events lead to the recruitment of histone acetyltransferasese CBP/p300 onto the promoter, and resultant acetylation of histones H3 and H4 (24-26). In addition, CREB-dependent transcription is further enhanced by association with its CRTC transcriptional co-activators (27, 28). Attenuation of CREB activity can be achieved by dephosphorylation of this residue by Ser/Thr phosphatases such as protein phosphatase 1 (PP1) and PP2A (29, 30).

Regulation of hepatic gluconeogenesis

Under fasting conditions, increased secretion of pancreatic glucagon leads to the activation of cAMP-CREB-dependent gluconeogenesis in the liver. The presence of CRE on the promoters of key gluconeogenic genes including PEPCK, G6Pase, Fbpase, and PC were identified, and it was subsequently confirmed that CREB could directly activate the transcription of these genes (31). The significance of CREB in the regulation of hepatic gluconeogenesis was shown by a study utilizing alb-ACREB TG mice that express ACREB, a dominant negative inhibitor of CREB, in the liver (10). Alb-ACREB TG mice result in the reduction in blood glucose levels with reduced mRNA levels for hepatic gluconeogenic genes, showing that CREB is a physiological transcriptional regulator of gluconeogenesis *in vivo*. Furthermore, acute inhibition of CREB activity by the delivery of adenoviral ACREB, siRNA for CREB, or inhibitory chemicals also reduces blood glucose levels *in vivo*, suggesting that CREB could be a potential target for the development of anti-diabetes drugs (10, 32).

Transcriptional co-activators for CREB

CBP/p300: CBP and its orthologue p300 are members of histone acetyltransferases (HATs) acetylate lysine residues of both histone and non-histone proteins (33, 34). Homozygotic null mice for either CBP or p300 are embryonically lethal, showing that they are critical in the process of embryonic development (35, 36).

As a transcriptional co-activator for CREB, the role of CBP/p300 has been implicated in glucose metabolism. The role for CBP in gluconeogenesis is still unresolved. While mice heterozygous for a CBP mutant allele showed insulin sensitive phenotypes in an earlier report, recent studies utilizing CBP mutant mice revealed that the disruption of CREB-CBP interaction did not result in reduced hepatic gluconeogenesis (16, 37). Furthermore, mice containing CBP with mutations in CH1 domain are resistant to insulin-mediated regulation of hepatic gluconeogenesis, and mutant mice producing CH1 null products (ΔCH1) display normal fasting gluconeogenesis (14, 38, 39). These data suggest that CBP alone is not sufficient to regulate CREB activity in mediating hepatic gluconeogenesis. Further studies are necessary to delineate the potential involvement of p300 and other HATs in the transcriptional control in this setting.

CRTC2: The CRTC family of transcriptional co-activators (also known as TORC, transducers of regulated CREB activity) consists of CRTC1, CRTC2, and CRTC3, which possess an N-terminal CREB binding domain, a central regulatory domain that is Ser/Pro-rich, and a C-terminal transactivation domain (40). The activity of the CRTC family mainly regulates phosphorylation-dependent changes in cellular localization. Under basal conditions, CRTCs are highly phosphorylated and reside in the cytosol via interaction with 14-3-3 proteins. Most notably, phosphorylation of Ser 171 (for CRTC2) is mediated by AMP-activated protein kinase (AMPK) and its related kinases (AMPKRK) such as salt-inducible kinase 1(SIK1) and SIK2. Exposure to cAMP agonist or the treatment of calcium ionophore directs a rapid dephosphorylation of CRTCs by inactivation of kinases and activation of Ser/Thr phosphatases (e.g. as PP2B or SMEK/PP4C) (41-43).

CRTC2 is the most prominent isoform in the mammalian liver. Fasting-dependent dephosphorylation of CRTC2 results in its nuclear translocation and interaction with CREB to activate hepatic gluconeogenesis (16). Recent studies also revealed the physiological significance of CRTC2 in the regulation of hepatic gluconeogenesis. Acute knockdown of CRTC2 in mice by RNA interference reduces fasting plasma glucose with reduction in gluconeogenic gene expression (16, 44). Similarly, CRTC2 knockout mice exhibit low blood glucose levels and increased glucose tolerance, confirming that CRTC2 is critical in the control of hepatic gluconeogenesis and energy homeostasis (45). CRTC2 was also shown to co-activate other basic leucine zipper transcription factors such as CREBH and ATF6, each of which could influence glucose homeostasis in mammals (12, 46). Further study is required for the potential competition between transcription factors to recruit the CRTC2 as a transcriptional co-activator.

Downstream targets for CREB in the energy metabolism

CREB is not only important in the direct transcriptional activa-

tion of gluconeogenic genes, but is also critical in modulating the fasting-mediated transcriptional activation of PGC-1α and estrogen-related receptor gamma (ERRγ), which serve as crucial transcriptional regulators for the activation of gluconeogenic genes during the prolonged fasting or starvation (10, 11, 19). We have also identified that mammalian phosphatidic acid phosphatase Lipin1 is a transcriptional target of CREB, and is responsible for the progression of hepatic insulin resistance in the diet-induced or the genetic mouse models of obesity (47). Thus, further study is necessary to delineate the diverse roles of CREB in the transcriptional control of energy homeostasis.

FoxO1

General facts

FoxOs belong to a subclass within the forkhead family of transcription factors that possess a forkhead box-type DNA binding domain, which recognize a specific regulatory element termed insulin response element (IRE) on the promoter. Four major isoforms, FoxO1, FoxO3, FoxO4, and FoxO6 are identified in mammals, and they function as critical transcription factors for various cellular signaling pathways in the energy metabolism, stress resistance, and longevity (48, 49).

As in the case of CRTCs, subcellular localization of FoxOs is determined by the phosphorylation status of Ser/Thr residues. Insulin/PI3K signaling pathway activates Akt/serum and glucocorticoid-induced kinase (SGK)-dependent phosphorylation of key Ser/Thr residues of FoxOs (Thr24, Ser253, and Ser316 for murine FoxO1), which promotes association with 14-3-3, the cytoplasmic localization, and subsequent degradation by a ubiquitin/proteasome-mediated pathway (50-52). Additionally, acetylation of FoxOs (at Lys242, 245, and 262 for murine FoxO1) could also modulate their transcriptional activity, though the exact outcome of such modification is still in debate (53-55).

Regulation of hepatic gluconeogenesis

Among FoxO family members, FoxO1 has been tightly linked with hepatic gluconeogenesis (56). FoxO1 binding sites (IRE) were mapped on the promoters of G6Pase and PEPCK, and were shown to be critical in mediating the insulin/Akt-dependent inhibition of these genes (57). Further study revealed that in conjunction with PGC-1α, FoxO1 confers insulin-dependent regulation of hepatic gluconeogenesis in mouse models (18). Chronic deletion of FoxO1 in the liver also results in reduced hepatic glucose production in mice, showing that FoxO1 is indeed a major transcription factor for modulating hepatic gluconeogenesis (56, 58). Recently, a role of FoxO6, another member of the FoxO family, was demonstrated in hepatic gluconeogenesis, suggesting that some redundancy might be present among FoxO transcription factors in the control of hepatic glucose metabolism *in vivo* (59).

Transcriptional co-activators for FoxO1

PGC-1α: First identified as a co-activator of PPARγ, PGC-1α plays a crucial role in the control of adaptive thermogenesis in response to cold shock in brown adipocytes (60). PGC-1α is also shown to interact with other transcriptional co-activators such as CBP/p300 and the mediator complex to fully activate transcriptional initiation (61, 62). Initially, PGC-1α can only function as a co-activator for nuclear receptors such as PPARα, PPARδ, liver X receptor (LXR)α/β, GR, retinoid-related orphan nuclear receptor (ROR) subfamily, and farnesoid X receptor (FXR) using its LXXLL motifs. PGC-1α was also shown to co-activate various types of transcription factors, such as SREBP, Sox9, and FoxO1 (63, 64).

In the liver, the expression of PGC-1α is markedly induced upon fasting via a CREB-dependent transcriptional mechanism, and is critical in maintaining prolonged gluconeogenesis under starvation (10, 19). Indeed, PGC-1α promotes the expression of gluconeogenic genes by enhancing the transactivating potential of FoxO1 (10, 18, 19). Conversely, the depletion of PGC-1α in the liver of mice results in the reduction of fasting glucose levels with the reduced expression of gluconeogenic genes (65, 66). These data underscore the physiological role of PGC-1α as a transcriptional co-activator for FoxO1 in the regulation of hepatic gluconeogenesis.

PRMTs: Protein arginine methyltransferases (PRMTs) catalyze the transfer of (a) methyl group to the Arg residues of histones and non-histone proteins in eukaryotes. PRMT1 is among the eleven mammalian PRMTs that have been identified in mammals to date, and belongs to the type I enzymes that include PRMT1, 3, 4, 6, and 8. It catalyzes the formation of asymmetrically dimethylated Arg on its substrates. FoxO1 was recently identified as a substrate for PRMT1, and Arg methylation of FoxO1 at Arg248 and 250 by PRMT1 enhances nuclear localization of FoxOs, thereby promoting downstream signaling such as oxidative stress response (15, 67-69).

The role of PRMT1 as a co-activator for FoxO1 in the hepatic gluconeogenesis was recently established (15, 67). In mouse liver, PRMT1-dependent Arg-dimethylation increases nuclear localization and chromatin occupancy of FoxO1 on the gluconeogenic promoter. Furthermore, either acute knockdown or chronic haploinsufficiency of PRMT1 in mice reduces FoxO1-mediated gluconeogenesis, showing that PRMT1 is critical in enhancing FoxO1 activity in the physiological context. Further study is required to explore the potential involvement of other PRMT families in the regulation of gluconeogenesis or FoxO1 activity.

TRANSCRIPTIONAL REPRESSORS FOR CREB AND FoxO1 IN THE GLUCONEOGENESIS

SHP

SHP, also known as NR0B2, is a member of atypical nuclear receptors (NRs) that lack a DNA-binding motif, and mostly functions as a transcriptional repressor of NRs via its LXXLL

motifs (70, 71). In addition, SHP can inhibit the activity of other classes of transcription factors, suggesting that atypical NRs might function as a more general transcriptional repressor for various cellular signaling pathways.

SHP is ubiquitously expressed in most tissues but shows a higher expression in the mammalian liver, suggesting a role in the regulation of hepatic energy metabolism (72, 73). Indeed, metformin-dependent activation of AMPK leads to the transcriptional induction of SHP in the liver, which in turn reduces hepatic glucose output in mice, showing a novel mechanism for this glucose-lowering reagent (74). Subsequently, it was shown that SHP directly inhibits cAMP-dependent hepatic gluconeogenesis by binding to CREB to block the association with CRTC2 (20). These results illustrate an alternative mechanism by which metformin regulates hepatic glucose production via SHP-dependent inhibition of gluconeogenic gene transcription. It is worth noting that DAX-1, another member of atypical NRs, reduces hepatic glucose production by inhibiting the recruitment of another transcription co-activator for gluconeogenesis, PGC-1α, onto the promoters of PEPCK and G6Pase. Further study is necessary to delineate the physiological and pathological relevance of these atypical NRs in the regulation of glucose homeostasis.

TCF7L2

TCF7L2 is a member of the LEF/TCF family of transcription factors with an HMG-box-type DNA-binding domain, and was shown as a nuclear transcription factor for the well-known

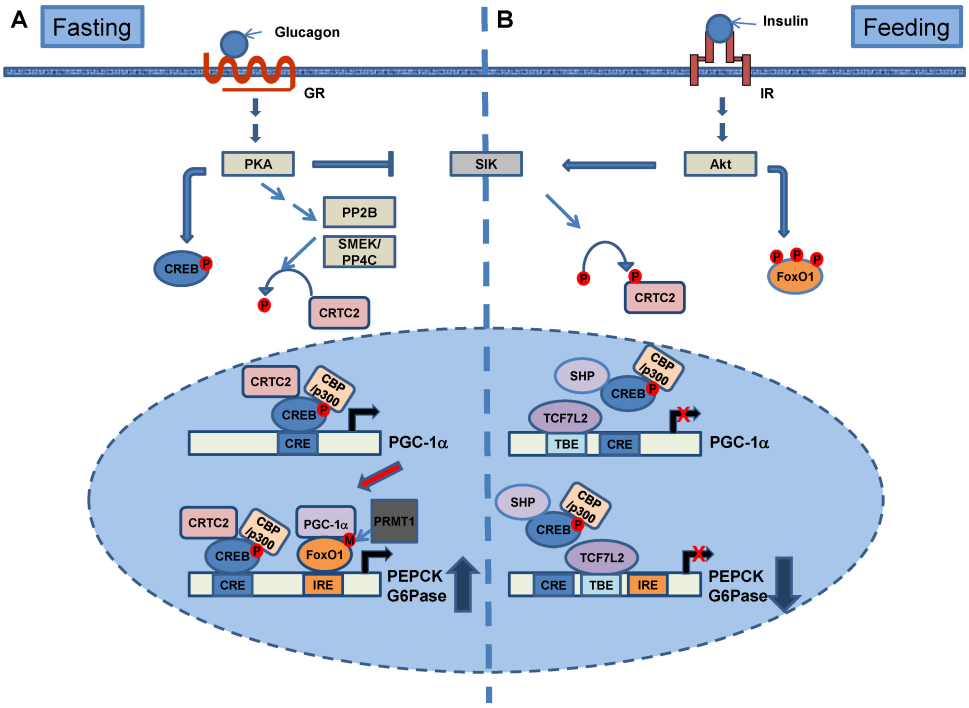

Fig. 1. Models for transcriptional regulation of hepatic gluconeogenesis under fasting and feeding conditions. (A) Transcriptional activation of hepatic gluconeogenesis under fasting conditions. Under fasting conditions, increased secretion of pancreatic glucagon triggers activation of PKA, which phosphorylates CREB at the serine 133 residue, leading to the increased association of this factor with co-activator CBP/p300 onto the chromatin. In addition, PKA promotes dephosphorylation of CRTC2 at the serine 171 residue by inactivating SIK kinases and activating serine/threonine phosphatases SMEK/PP4C and PP2B, leading to the nuclear localization and increased association of CRTC2 with chromatin-bound CREB. These events lead to the increased expression of gluconeogenic genes such as PEPCK and G6Pase, leading to the increases in hepatic gluconeogenesis during the early phase of fasting. At the same time, CREB/CRTC2 can enhance the expression of PGC-1α and ERRγ, which are the key transcriptional regulators of hepatic gluconeogenesis during the later phase of fasting. PRMP1 is also involved in the regulation of gluconeogenesis by modifying the arginine residues (Arg 248 and 250) of FoxO1 during this process. (B) Transcriptional repression of hepatic gluconeogenesis under feeding conditions. By contrast, feeding leads to the reduced plasma concentration of glucagon and enhanced secretion of pancreatic insulin, which leads to the activation of insulin signaling pathways in the liver. Activation of Akt leads to the activation of SIK kinases, thus promoting increased phosphorylation of CRTC2 and association with 14-3-3 (not shown). At the same time, Akt also directly phosphorylates critical residues of FoxO1 (see the text for details), turning off the transcription of hepatic gluconeogenesis. Transcriptional repressors for hepatic gluconeogenesis such as SHP, DAX-1, and TCF7L2 are induced under this condition, which helps to ensure the inactivation of transcription for gluconeogenic genes as well as PGC-1α.

Wnt/β-catenin signaling pathway (75-78). Wnt-mediated cellular signaling promotes the nuclear entry of β-catenin, a transcriptional co-activator of TCF7L2, leading to the activation of TCF7L2 target gene transcription. The TCF7L2 signaling pathway was linked to the developmental processes and proliferation events of cancer cells or stem cells (79).

Recently, TCF7L2 has been genetically linked to diabetes, and several human single nucleotide polymorphisms (SNPs) have been identified within the allele of TCF7L2 and correlate with increases in the incidence of this disease (80-84). Recent studies also revealed that TCF7L2 can promote insulin secretion *in vivo*, either by enhancing the secretion of GLP-1 in the intestinal endocrine L cells or mediating GLP-1-dependent insulin secretion in the pancreatic β cells (85).

The functional role of TCF7L2 as a transcriptional repressor for hepatic glucose metabolism was recently described (22, 86). Under feeding conditions, increased TCF7L2 binds to the regulatory elements near CRE and IRE, thereby inhibiting the occupancies of CREB and FoxO1 over the gluconeogenic promoters. This leads to the inhibition of hepatic gluconeogenesis under feeding conditions, which can be mimicked by the overexpression of TCF7L2 in the mouse liver. Under insulin-resistant conditions, however, the expression of medium and short isoforms of TCF7L2, which are exclusively found in the nucleus, are selectively decreased, suggesting that failure to inhibit CREB/FoxO1-dependent gluconeogenesis could occur. Corroborating this hypothesis, we also found that the depletion of TCF7L2 by RNA interference or genetic knockout results in hyperglycemia due to the reduction in hepatic gluconeogenesis. These data suggest that TCF7L2 is critical in the regulation of CREB- and FoxO1-dependent gluconeogenesis in both physiological and pathological conditions. Further study is necessary to determine whether a similar inhibitory loop is present in other TCF7L2-expressing tissues.

CLOSING REMARKS

In this review, we attempted to summarize the role of two major transcription factors for hepatic gluconeogenesis, CREB and FoxO1 (Fig. 1). Under fasting conditions, the increase in the secretion of glucagon results in the activation of cAMP signaling pathway in the liver, leading to the activation of CREB-dependent as well as CBP/p300- and CRTC2-dependent (its co-activators) transcriptional activation of gluconeogenic genes. Interestingly, some of the important transcriptional regulators themselves are also transcriptionally induced (e.g. PGC-1α, ERRγ), amplifying the magnitude of the gluconeogenic response. FoxO1 is also induced under fasting conditions, in part due to the lack of insulin/Akt pathway in the liver. The transcriptional activity of FoxO1 is greatly enhanced with its co-activator PGC-1α, suggesting that this pathway could be more critical in the later phase of the adaptive response to fasting. Under feeding conditions, the termination of hepatic gluconeogenesis is achieved by inhibition of these transcriptional machineries. The direct role of anabolic hormone insulin by Akt-dependent modification of FoxO1 is well described. Enhanced expression of transcriptional repressors under feeding conditions such as SHP or TCF7L2 also contributes greatly to effectively turning off the hepatic gluconeogenesis. The generation of liver-specific knockout mice for each transcriptional regulators is necessary to delineate the complex transcriptional regulatory mechanisms for hepatic gluconeogenesis in the future.

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