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# Role of CRTC2 in the control of hepatic gluconeogenesis

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Fasting glucose metabolism in the liver is critical in maintaining energy homeostasis in mammals. To provide sufficient amounts of glucose in the bloodstream, a preferred fuel for most tissues, glucose production from the liver is activated under starvation conditions. Short-term fasting elicits the production of glucose from glycogen, a storage form of glucose in the liver, by activating glycogenolysis. Longer-term fasting or starvation also triggers the activation of de novo glucose synthesis, or gluconeogenesis, by utilizing various precursors such as lactate, amino acids, or glycerol. Activation of the latter pathway is achieved mainly via transcriptional mechanism, and fasting hormone glucagon or stress hormone epinephrine induces cAMP-dependent pathway in the liver, thus activating transcription factor cAMP response element binding protein (CREB) and the resultant transcription of downstream target genes in the gluconeogenesis including phosphoenolpyruvate carboxykinase (PEPCK) and glucose 6-phosphatase catalytic subunit (G6Pase). While CREB-dependent transcriptional pathway is activated under fasting conditions, feeding effectively reduces glucose production from the liver in part by reducing CREB-dependent transcriptional activation of gluconeogenic gene expression. Increased hepatic glucose production under insulin resistance or type 2 diabetes is an important underlying mechanism for hyperglycemia, and it was shown that hyperactivation of CREB-dependent transcription can be attributable for such phenomenon. In this review, we would like to delineate the mechanistic insight into the role of CREB regulated transcription coactivator 2 (CRTC2), a new transcriptional coactivator of CREB, in the control of hepatic gluconeogenesis.

## INTRODUCTION

The liver is a major organ to regulate energy homeostasis in mammals. It plays a central role in the control of glucose metabolism, lipid metabolism, as well as detoxification processes that is essential for the everyday life. In particular, it plays a key role in the metabolism of glucose, a key nutrient that is mainly utilized by most species from bacteria to men. Under feeding, dietary carbohydrate is processed in the intestine and the resultant monosaccharide, which is mostly glucose, is transported into the various tissues via bloodstream (Pilkis and Claus, 1991; Pilkis et al., 1988). The glucose in excess is stored in the tissues as a storage form of glucose, glycogen. In particular, the liver stores large amount of glycogen that can be utilized by tissues that are mainly dependent on the glucose as a preferred fuel such as brain and red blood cells. Under fasting conditions, the stored glycogen is mobilized as glucose via a process of glycogen breakdown or glycogenolysis to provide glucose to the various tissues. Under longer term fasting or in time of starvation, the liver provides more glucose by activation of de novo glucose synthesis or gluconeogenesis. The precursor of gluconeogenesis includes lactate, amino acids and glycerol. Lactate is derived from the anaerobic glycolysis mainly from the skeletal muscle and is transport to the liver via Cori cycle. Starvation process also triggers protein breakdown and provides its building block

amino acids as precursors for the gluconeogenesis. Fasting also triggers lipolysis in the adipose tissues, resulting in the increased serum free fatty acids as well as glycerol. While free fatty acids can be taken up by various tissues and are utilized as alternative fuels, glycerol taken up by the liver is converted into glycerol 3-phosphate and is introduced into the gluconeogenesis.

Glucagon is a major hormone that is responsible for the mobilization of glucose under fasting conditions. Low blood glucose triggers increased secretion of glucagon from the pancreatic alpha cells, which in turn activates intracellular cAMP signaling pathway by activation of G protein signaling and adenylate cyclase activity mostly in the liver. cAMP directly activates protein kinase A (PKA), which is responsible for the increased glycogenolysis as well as hepatic gluconeogenesis by increased phosphorylation of enzymes in the pathway. In addition, PKA is also involved in the chronic activation of hepatic gluconeogenesis by enhancing transcription of key gluconeogenic genes including PEPCK, G6Pase, and pyruvate carboxylase (PC). cAMP response element binding protein (CREB) is responsible for such transcriptional regulation in response to the activation of cAMP pathway (Herzig et al., 2001; Moore et al., 1998). Briefly, cAMP binds to the regulatory subunits of PKA, releasing its catalytic subunits. Activated PKA enters the nucleus and phosphorylates serine 133 residue of CREB

that is bound to the cAMP response element (CRE) on the target promoter. Phosphorylated CREB undergoes a conformational change that enables it to recruit CREB coactivators CREB binding protein (CBP)/p300, thus activating target gene transcription. Indeed, CRE sequences have been identified in the 5'-upstream sequences of various gluconeogenic genes such as PEPCK, G6Pase, PC, and fructose 1,6-bisphosphase (Fbpase1). In addition, peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1 $\alpha$ ) also contains CRE on its promoter sequence and is transcriptionally induced by CREB. Since PGC-1 $\alpha$  is an important coactivator for fasting glucose and lipid metabolism in the liver, its transcriptional regulation indicates the importance of cAMP-dependent transcriptional control in energy homeostasis in the liver. The importance of CREB in the control of hepatic gluconeogenesis has been verified in *in vivo* settings using animal models. Transgenic mice expressing A-CREB, a CREB inhibitor, in the liver reduces gluconeogenic gene expression in the liver (Herzig et al., 2001). Adenovirus mediated expression of A-CREB in the liver reduces gluconeogenic gene expression including PEPCK, G6Pase, PC, and PGC-1 $\alpha$ , with a concomitant reduction in blood glucose levels. In addition, acute delivery of shRNA against CREB in the liver leads to the reduced hepatic gluconeogenesis, confirming the importance of CREB in the control of hepatic gluconeogenesis *in vivo* (Erion et al., 2009). Recently, a new class of CREB coactivator, CREB regulated transcriptional coactivators (CRTC), has been characterized (Conkright et al., 2003). Among the family members, CRTC2 is most abundant in the liver, and is linked to the CREB-dependent control of hepatic glucose metabolism (Koo et al., 2005). In this review, we would like to delineate the recent findings regarding the role of CRTC2 in the glucose metabolism in the liver as well as molecular mechanisms in controlling its activity in detail.

## IDENTIFICATION OF CRTC PROTEINS

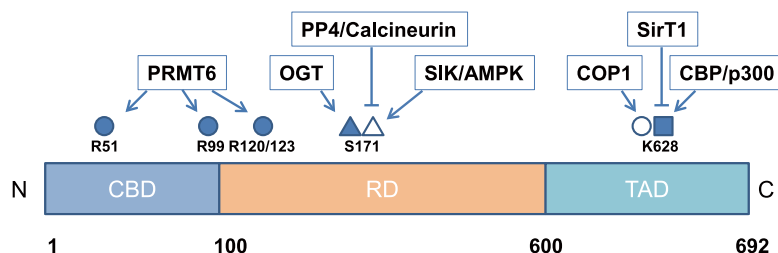
CRTC proteins were first identified as a new regulator of cAMP-dependent transcriptional events by expression library screening using CRE luciferase (Conkright et al., 2003). CRTC1, the founding member of CRTC family of proteins, was shown to increase CRE luciferase activity in response to cAMP treatment in the HEK 293 cells. The CRTC1 was first reported as a fusion gene in the name of MECT1-MAMML2. Via a recombination event, DNA sequences encoding the CREB binding domain of CRTC1 (termed Mucoepidermoid carcinoma translocated-1 (MECT1)) was fused to the C-terminal transcriptional activation domain of the Notch coactivator Mastermind-like 2 (MAML2) leading to the generation of a fusion gene encoding strong transcriptional activator found in the Mucoepidermoid carcinoma. As a transcriptional coactivator CRTC1 does not possess an intrinsic DNA binding motif.

Subsequently, two other mammalian paralogues, CRTC2 and CRTC3 were identified in mammals. Indeed, CRTC orthologues were also identified in lower eukaryotes including *Caenorhabditis elegans* and *Drosophila Melanogaster*, showing a conservation of this CREB coactivator family during evolution (Altarejos and Montminy, 2011).

## THE STRUCTURE OF CRTC2 AND ITS POST-TRANSLATIONAL MODIFICATIONS

CRTC proteins consist of an amino terminal domain termed CREB binding domain that is responsible for the binding of CRTC proteins with CREB and other basic leucine zipper (bZIP) transcription factors, a carboxy terminal domain termed transactivation domain, and a central regulatory serine/proline rich domain that is mainly targeted by various post-translational modifying enzymes for regulating activity of this protein family (Figure 1) (Altarejos and Montminy, 2011). Recently, the function of amino terminal CREB binding domain was delineated in detail (Luo et al., 2012). According to the study, 28 residues of the amino terminus of CRTC2 consist of alpha helix structure, and directly bind to leucine zipper region of CREB. Since alpha helix of CRTC2 lacks basic amino acids, CRTC2 is not directly involved in the DNA binding, but promotes stronger interaction among CREB, CRTC2 and its cognate DNA (containing CRE sequences) via non-specific interaction with phosphate group of DNA.

Several reports also attempted to characterize the nature of transactivation domain of CRTC2. It appeared that Lys628 residue of CRTC2 is targeted by a couple of post-translational modification marks. Acetylation of Lys628 of CRTC2 by CBP/p300 led to the activation of this protein via tighter association of CREB-containing transcriptional complex (Liu et al., 2008). On the other hand, histone deacetylase Sirt1 promotes deacetylation of Lys628, leading to the dissociation of CRTC2-containing transcription machinery. Lys628 is also target of COP1



**FIGURE 1 | Post-translational modification on CRTC2.** Post-translational modifications on CRTC2 are shown. PRMT6 catalyzes asymmetric dimethylation on arginine residues (R51, 99, 120, and 123) in the nucleus. AMPK/SIK phosphorylates, while PP4/Calcineurin dephosphorylates serine 171 residue. The same serine 171 residue is targeted by OGT for O-GlcNAcylation. Acetylation of lysine 628 by CBP/p300 activates CRTC2, while deacetylation of this residue by SirT1 inhibits it. Lys628 is also targeted by COP1 ubiquitin ligase for polyubiquitination. A filled circle indicates asymmetric dimethylation, while an open circle indicates ubiquitination. A filled triangle is for O-GlcNAcylation, and an open triangle denotes phosphorylation. Finally, a filled square indicates acetylation. CBD: CREB binding domain; RD: Regulatory domain; TAD: transactivation domain

ubiquitin ligase. Polyubiquitination of CRTC2 is then targeted by proteasome and is subsequently degraded (Dentin et al., 2007). Thus, acetylation of Lys628 protects CRTC2 from ubiquitin proteasome mediated degradation and termination of cAMP-dependent transcriptional signal.

Recruitment of other transcriptional regulator by transactivation domain of CRTC2 was recently suggested. In the report, they showed that the transactivation domain of CRTC2 associates with a class of histone acetyltransferase KAT2B, leading to the increased acetylation of histone 3 lysine 9 (H3K9) of gluconeogenic promoters, resulting in the increased association of CREB-CRTC2 and transcriptional activation of target genes (Ravnskjaer et al., 2013).

Serine 171 residue in the central regulatory domain of CRTC2 is a key site for the regulation of this protein. In the absence of cAMP signaling, AMPK and its related kinases are activated, thus resulting in the phosphorylation of serine 171 of CRTC2 and its tight association with 14-3-3 proteins in the cytosol. AMP activated protein kinase (AMPK) and its related kinases (AMPKRKs), including salt inducible kinase (SIK)1 and SIK2, are associated with the phosphorylation of this residue (Screaton et al., 2004). Upon activation of cAMP signaling pathway under fasting conditions, PKA phosphorylates and inactivates these kinases, leading to dephosphorylation and activation of CRTC2. Recent reports also suggested the role of serine/threonine phosphatases in the control of dephosphorylation event of CRTC2. Serine/threonine protein phosphatase 4 (PP4) is activated under fasting or in insulin resistant states, in part via transcriptional activation of Suppressor of MEK null (SMEK)1 and SMEK2, a pair of PP4 regulatory subunit 3 proteins, leading to the increased dephosphorylation and activation of CRTC2 (Yoon et al., 2010). CREB also induces expression of inositol 3 phosphate receptor, resulting in the release of calcium from the ER stores under fasting conditions. Increase calcium concentration in turn activates calcineurin, a PP3 catalytic subunit, which also promotes dephosphorylation and activation of CRTC2 in the liver (Wang et al., 2012). Dephosphorylation of CRTC2 on serine 171 residue leads to the dissociation of CRTC2 from 14-3-3 and subsequent nuclear translocation. Thus, concomitant inactivation of kinases and activation of phosphatases result in the activation and the nuclear entry of CRTC2 under fasting conditions, leading to the activation of gluconeogenic gene transcription.

In addition to the phosphorylation/dephosphorylation, serine 171 is also shown to be O-GlcNAcylated by O-linked N-acetylglucosamine (O-GlcNAc) transferase (OGT) under hyperglycemic conditions such as type 2 diabetes (Dentin et al., 2008). O-GlcNAcylated serine residue is refractory to the phosphorylation by AMPK family of kinases, providing a potential mechanism for the hyperactivation of CRTC2 during hyperglycemia in conditions such as type 2 diabetes.

Protein arginine methyltransferases (PRMTs) have been also shown to be involved in the control of CRTC2 activity. PRMT4 interacts with CREB and activates CREB-dependent transcription

by promoting histone acetylation (Krones-Herzig et al., 2006). However, the direct interaction between this protein and CRTC2 was not tested. Recent report suggests that PRMT5 can directly bind to CRTC2 and also promotes histone acetylation of gluconeogenic promoters to activate CREB-CRTC2 dependent transcription in the liver (Tsai et al., 2013). In either case, direct modification of CRTC2 by PRMTs was not shown. On the other hand, PRMT6 promotes CRTC2 activity via a direct asymmetric dimethylation of arginine residues of CRTC2 (Han et al., 2014). PRMT6 resides in the nucleus, and directly methylates arginine residues of CRTC2 in its amino terminus, thus enhancing the interaction between CREB and CRTC2 onto the gluconeogenic promoters. Interestingly, the identified arginine residues (arginine 51, 99, 120, and 123) are conserved among various species as well as other paralogues (CRTC1 and CRTC3), showing the importance of this modification in the control of CRTC2 activity. Future study will clarify the relative contribution among these PRMTs in the control of CRTC2-dependent transcriptional pathway in the liver.

## DIRECT CONTROL OF HEPATIC GLUCONEOGENESIS BY CRTC2

CRTC2 is a predominant isoform among CRTC proteins in the liver. Under fasting conditions, pancreatic alpha cell secretes glucagon, leading to the activation of glucagon receptor mediated signal transduction mainly in the liver. Activation of PKA results in the activation of serine/threonine phosphatases and the inactivation of serine/threonine kinases, promoting dephosphorylation and nuclear translocation of CRTC2. In the nucleus, CRTC2 binds to CREB on the gluconeogenic promoters such as PEPCK, G6Pase, and PGC-1 $\alpha$ , and turns on gluconeogenesis (Koo et al., 2005).

The role of CRTC2 in the hepatic gluconeogenesis has been also confirmed in *in vivo* animal models. Depletion of hepatic CRTC2 by RNA interference lowered blood glucose levels with reduced gluconeogenic gene expression (Koo et al., 2005; Saberi et al., 2009). A couple of CRTC2 knockout mice models were suggested. In one model, deletion of exon 4 through 11 of CRTC2 did not elicit changes in the blood glucose levels in mice, but showed a reduced response to cAMP treatment in hepatocytes, suggesting that CRTC2 may not function as a regulator of glucose metabolism *in vivo* (Le Lay et al., 2009). However, careful investigation reveals that the deletion of exons 4 through 11 led to the potential generation of in-frame mutant form of CRTC2. Indeed, deletion of exons 4 through 11 removes most of the regulatory domain of CRTC2 including serine 171 residue, resulting in the generation of constitutively active form of CRTC2, at least in the cultured cells (Altarejos and Montminy, 2011). Thus, further characterization is necessary before fully accepting the result of the *in vivo* study utilizing these knockout mice. Another systemic knockout mouse of CRTC2 was generated and reported recently. In this line of mice, exon 1 of CRTC2 was targeted for the generation of knockout mice,

**TABLE 1** | Metabolic phenotypes of CRTC knockout animals

Isoform	Site of Major Expression	Major Target Genes	Knockout phenotype
CRTC1	Brain (Hypothalamus)	CART1, IRS2	Hyperphagia, Obesity, Infertility
CRTC2	Liver	PEPCK, G6Pase, PGC-1 $\alpha$ , ERR $\gamma$ , Lipin1	Reduced hepatic gluconeogenesis/ Improved insulin sensitivity
CRTC3	Adipocytes	RGS2	Resistant to diet-induced obesity, Improved insulin sensitivity
dTORC ( <i>D. Melanogaster</i> )	Brain	Cox, catalase	Reduced resistance against oxidative stress, Reduced lifespan
CRTC-1 ( <i>C. Elagans</i> )	Ubiquitous	Abu (ER stress related gene)	Increase lifespan

precluding the potential generation of chimeric form of CRTC2 that could hinder the interpretation of the result that is associated with CRTC2 KO mice (Wang et al., 2010). Indeed, CRTC2 KO mice displayed lower blood glucose levels, and improved glucose tolerance with reduced gluconeogenic gene expression in the liver, confirming the critical role of CRTC2 in the control of hepatic gluconeogenesis *in vivo*.

### MODULATION OF TARGET GENES OF CRTC2: INDIRECT REGULATION OF GLUCOSE METABOLISM BY CRTC2

As a transcriptional coactivator of CREB, CRTC2 regulates hepatic glucose metabolism not only via a direct transcriptional control of gluconeogenic genes, but also via an indirect mechanism by controlling transcription of genes that encode various proteins affecting glucose homeostasis (Table 1).

As described previously, CREB and CRTC2 are involved in the transcriptional activation of PGC-1 $\alpha$ . First as identified as a coactivator for peroxisome proliferator activated protein (PPAR)  $\gamma$  in brown adipocytes, PGC-1 $\alpha$  functions as a coactivator for various nuclear hormone receptors including PPAR $\alpha$ , PPAR $\delta$ , liver X receptors (LXRs) and glucocorticoid receptor (GR) via a direct interaction by using LXXLL motifs (Finck and Kelly, 2006; Puigserver et al., 1998). In the liver, PGC-1 $\alpha$  is shown to promote gluconeogenic gene expression by coactivating hepatic nuclear factor 4 (HNF4)  $\alpha$  and FoxO1 (Puigserver and Spiegelman, 2003). While depletion of CRTC2 by RNA interference reduced gluconeogenic gene expression, the effect was effectively rescued by ectopic expression of PGC-1 $\alpha$ , showing indeed that CREB and CRTC2 are involved in the transcriptional control of hepatic gluconeogenesis both via a direct activation of gluconeogenic genes and an indirect mechanism through activation of PGC-1 $\alpha$  (Koo et al., 2005).

Estrogen receptor related (ERR)  $\gamma$  has also been shown to a direct transcriptional target of CREB and CRTC2 in the liver (Kim et al., 2012). As in the case of PGC-1 $\alpha$ , expression of ERR  $\gamma$  is transcriptionally activated by CREB and CRTC2-dependent

manner under fasting conditions. Inhibition of ERR  $\gamma$  activity by either RNA interference or inverse agonist GSK5182 restores euglycemia in a mouse model of type 2 diabetes. Furthermore, ERR  $\gamma$  also controls gluconeogenic gene expression in conjunction with PGC-1 $\alpha$ , further showing the case for the control of hepatic gluconeogenesis by CREB and CRTC2 via an indirect pathway.

Lipin1 is another transcriptional target of CREB and CRTC2 whose expression is increased under fasting or by insulin resistance in the liver (Ryu et al., 2009). Lipin1 encodes a protein termed LPIN1, a phosphatidic acid phosphatase that catalyzes the conversion of phosphatidic acids to diacylglycerol (DAG) on the surface of smooth endoplasmic reticulum (ER). Increased expression of lipin1 under obesity results in the accumulation of DAG. DAG-mediated signaling pathway aggravates insulin resistance and enhances hepatic glucose production, which contributes to the hyperglycemic phenotype. Thus, CRTC2-dependent transcription mechanism is shown to be a novel target of controlling insulin resistance and hyperglycemia. Based on this result, further study will be necessary to delineate other potential CREB-CRTC2 target genes that could impact on the control of hepatic glucose metabolism. Utilization of CRTC2 knockout mice will be a valuable asset to conduct such a study in the future.

### CRTC2 AS A TRANSCRIPTIONAL COACTIVATOR FOR OTHER BZIP FAMILY OF PROTEINS

Since CRTC2 functions as a transcriptional coactivator of CREB by its interaction with bZIP domain, it is plausible to expect that CRTC2 could interact with other members of bZIP factors. Indeed, activating transcription factor (ATF) 6, a class of ER-bound bZIP transcription factor, was shown to interact with CRTC2 in the liver (Wang et al., 2009). ATF6 is a critical transcriptional activator in response to ER stress, and enhances expression of key gene products in the unfolded protein response (UPR) pathway. Normally resides in the ER, ATF6 is activated under ER stress by proteolytic cleavage that would

release an active transcription factor moiety into the nucleus. According to the recent report, ER stress also triggers the ER-stored calcium release in the liver, leading to the activation of calcineurin and resultant dephosphorylation/activation of CRTC2. Thus, concomitant activation of ATF6 and CRTC2 enable them to function as an active transcriptional machinery to activate genes involved in the UPR pathway.

Another ER-bound bZIP factor CREBH was also shown to interact with CRTC2, using it as a transcriptional coactivator in the liver (Lee et al., 2010b). As a member of CREB3 subfamily of bZIP proteins, CREBH is mainly expressed in the liver, and its expression and activity is enhanced under fasting or by insulin resistance. Thus, CREBH and CRTC2 can be also activated at the same time to enhance a new set of target genes. Interestingly, binding sites of CREBH were identified among 5'-upstream sequences of gluconeogenic genes PEPCK and G6Pase, which are distinct from the well-characterized CRE sequences for CREB binding. Depletion of CRTC2 in the liver almost completely abrogated CREBH-dependent activation of gluconeogenesis in mice, showing the importance of CREBH in the control of CRTC2-dependent glucose metabolism in the liver. Based on these results, CRTC2 can control hepatic glucose metabolism by interaction with various bZIP transcription factors such as CREB, ATF6, and CREBH. Further study is required to delineate potential interaction of other bZIP factors with CRTC2 in the control of glucose metabolism or other metabolic pathways in the liver.

### INHIBITORY PROTEINS THAT ARE INVOLVED IN THE CONTROL OF CRTC2 ACTIVITY

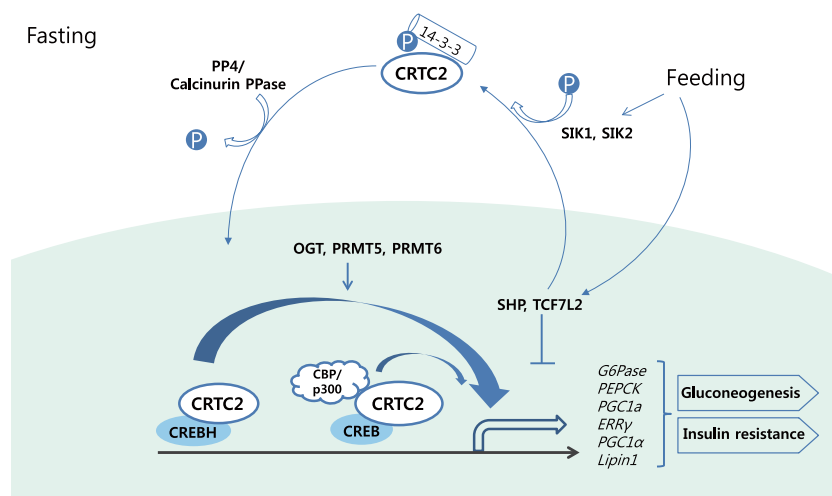
A couple of reports identified inhibitory proteins that interfere with CRTC2 action in the liver. Small heterodimer partner

(SHP), also known as NR0B2, is a member of nuclear hormone receptors that lacks DNA binding domain, and inhibits activity of other transcriptional factors by protein-protein interaction. SHP is highly expressed in the liver, and its expression is induced by metformin, an anti-diabetic drug that also enhances activity of AMPK (Kim et al., 2008). By interaction with CREB, SHP disrupts CREB/CRTC2 interaction, thus reducing hepatic gluconeogenesis in mice (Lee et al., 2010a). Recently, transcription factor 7-like 2 (TCF7L2), a transcriptional regulator of Wnt/beta catenin signaling pathway, was shown to control hepatic glucose metabolism. Expression of TCF7L2 is increased under feeding conditions in the liver, resulting in the increased occupancy of this protein onto the cognate binding sites that are adjacent to the CREB binding sites present in the gluconeogenic promoters (Oh et al., 2012). Thus, CREB/CRTC2 binding to the promoter is effectively inhibited under feeding conditions, providing another layer of regulation in controlling glucose homeostasis in the liver. Interestingly, TCF7L2 expression is reduced by insulin resistance, showing reduced TCF7L2 occupancy over gluconeogenic promoters that could also contribute to the increased expression of gluconeogenic genes by CREB-CRTC2 transcriptional machinery in this setting. These results suggest the involvement of various signaling pathways in the control of CRTC2 activity.

### FUNCTIONS OF OTHER CRTC PROTEINS AND ROLES OF CRTC ORTHOLOGUES IN OTHER ORGANISMS

Expression of CRTC1 is highly restricted in the central nervous system. In particular, CRTC1 protein is found to be most highly expressed in the hypothalamic arcuate cells. Upon feeding, dephosphorylation and activation of CRTC1 is promoted in the hypothalamus. Activated CRTC1 then functions as a CREB

coactivator and controls transcription of cocaine- and amphetamine-regulated transcript (CART), an anorexigenic protein at the level of transcription, thus involved in the control of feeding behavior. Indeed, systemic knockout of CRTC1 in mice resulted in the obesity phenotype that is associated with leptin resistance, showing the importance of this protein in the control of CNS-directed control of feeding behavior and obesity (Altarejos et al., 2008). On the other hand, CRTC3 is shown to be highly expressed in white and brown adipocytes. Normally, catecholamine induces lipolysis in white adipocytes and fatty acid oxidation and uncoupling protein-mediated heat dissipation in brown adipocytes, via a binding of beta adrenergic receptors and subsequent activation of G protein-coupled adenylate cyclase activity and cAMP signaling pathway.



**FIGURE 2 | Regulation of hepatic glucose metabolism by CRTC2.** A model showing transcriptional regulation of hepatic glucose metabolism by CRTC2. See the main text for a detailed explanation.

Interestingly, CRTC3 knockout mice were protected against the diet-induced obesity, in part by enhanced energy expenditure, and also displayed improved insulin sensitivity. It turned out that one of the major transcriptional targets of CRTC3 is regulator of G protein signaling 2 (RGS2), which encodes GTPase-activating protein that limits G protein activity (Song et al., 2010). Thus, CRTC3 knockout mice displayed prolonged activation of cAMP-dependent pathway in adipocytes that would increase lipolysis (white adipocytes) and energy expenditure (brown adipocytes), protecting the mice from diet-induced obesity and insulin resistance.

CRTC was also shown to be involved in the control of energy metabolism in the lower eukaryotes. In *Drosophila Melanogaster*, only a single orthologue of CRTC, termed dTORC, is present. Deletion of dTORC in the fly resulted in the reduced lipid accumulation and enhanced sensitivity to fasting. dTORC is mainly expressed in the brain, and controls energy balance and resistance to the oxidative stress, resulting in the prolonged longevity (Wang et al., 2008). As in the case of mammalian orthologue, dTORC activity is regulated by phosphorylation of key serine residue. In the fly, SIK, a member of AMPK related kinase, is involved in the phosphorylation of dTORC, and its activity is induced under feeding conditions. Ultimately, phosphorylated dTORC undergoes proteasomal degradation. In *Caenorhabditis elegans*, also only a single orthologue of CRTC, termed CRTC-1, is identified to date. As in the case of the mammalian orthologue, its activity is controlled by AMPK- and calcineurin-dependent phosphorylation events. In this organism, CRTC-1 functions as a coactivator of CRH-1, an orthologue of CREB. While dTORC promotes resistance to oxidative stress and increased longevity in the fly, CRTC-1 appears to impair longevity in *C. elegans*, since the deletion of either CRTC-1 or CRH-1 increases the longevity of this animal (Mair et al., 2011). Further study will be necessary whether dTORC and CRTC-1 regulate expression of different subset of genes to control the oxidative stress or aging pathways in the respective organism (See table 1 for comparison).

## CONCLUDING REMARKS

In this review, we delineate the structure, function, and the regulatory mechanisms of CRTC2 as a master regulator of hepatic glucose metabolism (Figure 2). CRTC2 is activated under fasting conditions, via inhibition of AMPK and its related kinases, activation of PP4/calcineurin phosphatases, PRMTs, and CBP/p300. Activated CRTC2 binds to CREB or CREBH onto the gluconeogenic promoters in the nucleus, enhancing gluconeogenesis at the level of transcription. Conversely, feeding can effectively turn off transcription of gluconeogenic genes by inhibition of CRTC2 via activation of kinases, inactivation of phosphatases, and activation of inhibitory proteins SHP and TCF7L2. In addition, CRTC2 is shown to transcriptionally regulate various target genes including PGC-1 $\alpha$ , ERR $\gamma$ , and Lipin1 to control energy metabolism and insulin signaling pathway in

the liver. Further study involving the identification of additional transcription factors that function as partners for CRTC2 could elicit additional CRTC2-controlled signaling pathway in the future.

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## AUTHOR INFORMATION

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