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REVIEW

Metabolic regulation by salt inducible kinases

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Abstract **In fasting mammals, the liver is the primary source of glucose production for maintenance of normoglycemia. In this setting, circulating peptide hormones and catecholamines cause hepatic glucose output by stimulating glycogen breakdown as well as *de novo* glucose production through gluconeogenesis. Fasting gluconeogenesis is regulated by a complex transcriptional cascade culminating in elevated expression of hepatic enzymes that promote gluconeogenesis and glucose export to the blood. The cAMP response element binding protein CREB and its co-activator CRTC2 play crucial roles in signal-dependent transcriptional regulation of gluconeogenesis. Recent work has identified a family of serine/threonine kinases, the salt inducible kinases (SIKs), which are subject to hormonal control and constrain gluconeogenic and lipogenic gene expression in liver. As normal regulation of gluconeogenesis and lipogenesis is disrupted in diabetic states, SIK kinases are poised to serve as therapeutic targets to modulate metabolic disturbances in diabetic patients. The purpose of this review is to 1) describe the identification of CRTCs CREB co-activators and their regulation by SIKs, 2) discuss recent progress toward understanding regulation and function of SIKs in metabolism and 3) examine the potential clinical impact of therapeutics that target SIK kinase function.**

Keywords salt inducible kinases (SIKs), cAMP response element binding protein (CREB), CRTC, gluconeogenesis, lipogenesis, type 2 diabetes, transcription

Introduction

Maintenance of glucose homeostasis in mammals is a complex process involving multiple organ systems including liver, skeletal muscle, adipose tissue and brain. Blood glucose concentration is normally maintained within a small physiologic range by the actions of the counter-regulatory hormones glucagon and insulin, which coordinately control hepatic gluconeogenesis and peripheral glucose uptake (Moller, 2001). Hyperglycemia in diabetic and pre-diabetic patients arises from excess hepatic glucose output during fasting as well as hepatic and peripheral insulin resistance following feeding. As such, tremendous experimental effort has been devoted to elucidating molecular mechanisms that control the balance between glucose output and uptake in the liver.

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During fasting, pancreatic glucagon stimulates cAMP-dependent signaling pathways through cAMP-dependent protein kinase (PKA) in the liver. cAMP-PKA signaling not only acutely stimulates glycogen breakdown but also induces gluconeogenesis through transcriptional induction of rate-limiting gluconeogenic enzymes (Salway, 2004). PKA phosphorylates serine 133 of the cAMP response element binding protein CREB (reviewed in Mayr and Montminy, 2001) on promoter regions of the genes encoding phosphoenolpyruvate carboxykinase (Pepck) (Liu et al., 1991) and glucose 6-phosphatase (G6Pase) (Argaud et al., 1996) as well as the transcriptional co-activator PGC1, which further induces G6Pase expression (Herzig et al., 2001; Yoon et al., 2001) (Fig. 1). The finding that mice lacking CREB activity in liver have dramatically impaired gluconeogenesis established CREB as a key regulator of the transcriptional response in the liver during fasting (Herzig et al., 2001). Subsequent work identified additional players in the regulation of CREB-dependent transcription: a new family of CREB co-activators (CRTC1-3) and the salt inducible kinases (SIK1 and SIK2), which modulate CRTC function.

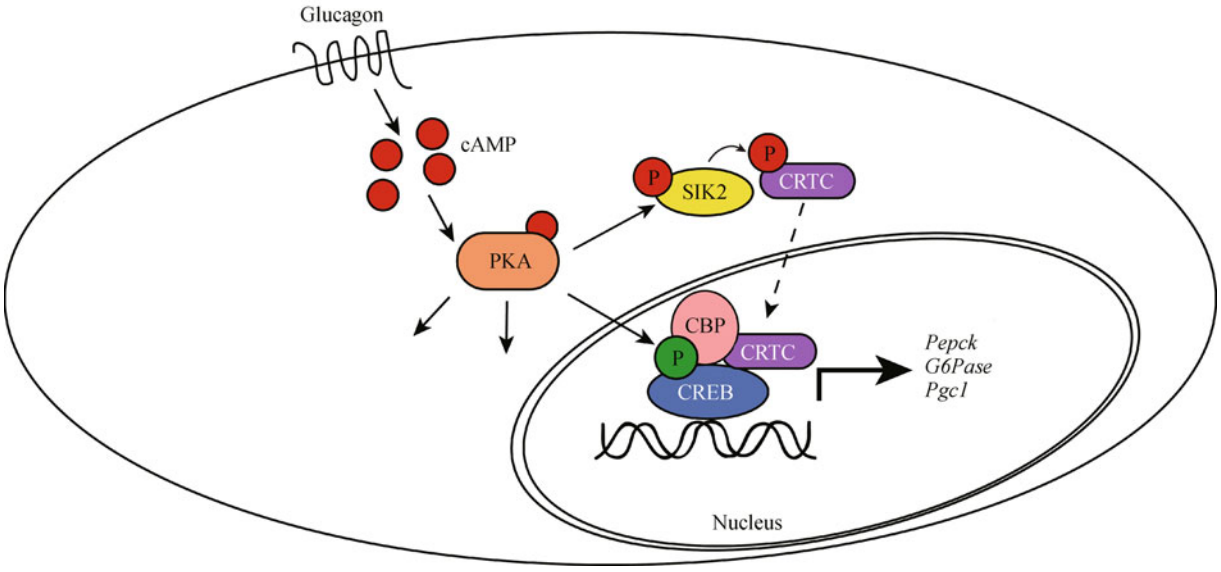

Figure 1 Diagram of CREB/CRTC activation in liver during fasting. During fasting, glucagon induces cAMP accumulation, which activates PKA. PKA catalyzes phosphorylation of CREB and SIK kinases (shown as SIK2). Phosphorylated CREB on target gene promoters recruits CBP/p300 histone acetyltransferases. Phosphorylation of SIK by PKA inhibits SIK activity on CRTC2. Dephosphorylated CRTC2 translocates to the nucleus and forms a ternary complex with CREB/CBP. The CREB complex directs transcription of *Pepck*, *G6Pase* and *Pgc1* to promote gluconeogenesis. Inhibitory phosphorylation is shown in red; activating phosphorylation shown in green.

Identification of CREB co-activators

Mechanistic studies investigating CREB regulation revealed that although phosphorylation on Ser133 is necessary for CREB-dependent transcription, it is not sufficient (Fisch et al., 1987; Bonni et al., 1995; Brindle et al., 1995; Mayr et al., 2001). These observations led Montminy and colleagues to search for the so-called "second event" by which PKA activates CREB-dependent transcription. Using a high-throughput screening approach to identify modulators of CREB, Dr. Montminy's group identified a previously unknown transcriptional co-activator family they named TORCs (transducer of regulated CREB activity), which are sufficient to activate CREB-dependent luciferase reporter genes when overexpressed (Conkright et al., 2003). Labow and colleagues identified TORCs independently by a similar approach (Iourgenko et al., 2003). TORCs were subsequently re-named CRTCs (cAMP-regulated transcriptional co-activators) to prevent confusion with mTOR signaling complexes. There are three CRTC family members (CRTC1–3) that share similar domain structures. CRTCs do not bind to DNA, nor are they phosphorylated by PKA; rather they bind to CREB on promoters and form a ternary complex with the histone acetyltransferase paralogs CBP/p300 (Ravnskjaer et al., 2007; He et al., 2009) and enhance recruitment of basal transcription machinery to target gene promoters (Conkright et al., 2003) (Fig. 1). A recent review provides a comprehensive description of regulation and functional roles of CRTCs (Altarejos and Montminy, 2011).

Linking SIKs to CREB

Seminal studies in pancreatic islet cells elucidated the molecular mechanism by which PKA signaling regulates CRTC2. Screaton and colleagues (2004) found that CRTC2 accumulates in the cytoplasm of resting islet cells, but rapidly translocates to the nucleus upon simultaneous treatment with cAMP and calcium stimuli. In non-excitable cells, cAMP is sufficient to promote nuclear entry of CRTC2. Mass spectrometry and two-dimensional tryptic peptide mapping allowed identification of amino acids within CRTC2 that are phosphorylated in resting cells, but become dephosphorylated after cAMP stimulation. Serine 171 is the primary phosphorylation site that mediates CRTC2 association with 14-3-3 proteins, which sequester CRTC2 in the cytoplasm under resting conditions (Screaton et al., 2004). In response to cAMP and calcium signals, CRTC2 (Ser171) becomes dephosphorylated, and CRTC2 accumulates in the nucleus where it cooperates with CREB to drive transcription. Mass spectrometry on immunoprecipitates of cytoplasmic CRTC2 allowed the authors to identify salt inducible kinase 2 (SIK2) as the inhibitory kinase that maintains CRTC2 in latent cytoplasmic complexes (Fig. 1). Salt inducible kinase 1 (SIK1) had been previously identified as a modulator CREB-dependent transcription in adrenocortical carcinoma cells (Doi et al., 2002), but the molecular mechanism remained elusive. The discovery of the link between CRTCs and SIKs opened a new area of investigation that uncovered complex regulation of CRTC2 activity by multiple post-translational

modifications under normal and pathophysiologic states and prompted deeper investigation into the metabolic roles of SIKs.

Characterization and regulation of SIKs

SIK1 was first isolated from the developing myocardium of mice (Ruiz et al., 1994) and subsequently cloned as a transcript enriched in the adrenal glands of rats fed a high-salt diet, from which its name was derived (Wang et al., 1999). SIK2 and SIK3 were identified in mammalian genomes by similarity (Katoh et al., 2004a). These kinases are evolutionarily conserved; *D. melanogaster* and *C. elegans* each have SIK homologs, called *dSIK* and *Kin-29*, respectively (Lanjuin and Sengupta, 2002; Okamoto et al., 2004; Wang et al., 2008; Choi et al., 2011). In mammals, *Sik1* mRNA is most abundant in the adrenal gland, brain, testes and skeletal muscle, with less expression in adipose, liver, and heart (Horike et al., 2003). SIK1 is unique in that it is transcriptionally induced by cAMP stimuli in multiple cell types (Katoh et al., 2004b; Koo et al., 2005; Berdeaux et al., 2007). *Sik2* is enriched in metabolic tissues (liver, adipose, brain), while *Sik3* is expressed ubiquitously (Okamoto et al., 2004). The SIKs are members of the AMP-dependent protein kinase (AMPK)-related family of 14 serine/threonine kinases (Bright et al., 2009). However, they function as single subunit enzymes and therefore lack the AMP and glycogen sensitivity conferred on AMPK by its associated βγ subunits (reviewed in Hardie, 2004).

Like other AMPK-related kinases, SIKs contain a highly homologous N-terminal kinase domain and adjacent ubiquitin-associated (UBA) domain (Fig. 2) and are not catalytically active until requisite T-loop phosphorylation by LKB1 (Lizcano et al., 2004; Shaw et al., 2004; Bright et al., 2009). Although additional upstream kinases are also capable of phosphorylating the conserved residue on AMPK, including CaM kinase kinase-α and-β (CaMKK) isoforms (Hawley et al., 1995; Hawley et al., 2005; Witczak et al., 2007) and TGFβ-activated kinase 1 (TAK1) (Momcilovic et al., 2006; Xie et al., 2006), *Lkb1*-deficient cells and tissues lack detectable SIK kinase activity (Lizcano et al., 2004; Sakamoto et al., 2005; Shaw et al., 2005). Thus it is still unclear to what extent CaMKK enzymes contribute to SIK regulation *in vivo*. T-loop phosphorylation on SIKs is thought to be constitutive, although this is yet to be thoroughly investigated. The UBA domains of AMPK-related kinases neither bind ubiquitin *in vitro* nor are they modified by ubiquitylation; rather, the UBA domain is important for phosphorylation by LKB1 (Jaleel et al., 2006).

All three SIK family members share a conserved C-terminal PKA phosphorylation site that lies near an arginine-lysine rich region (Fig. 2), which functions as a non-canonical nuclear localization signal (NLS) (Katoh et al., 2004b). Phosphorylation of this site (SIK1 Ser577; SIK2 Ser587) by PKA causes nuclear export by a CRM1-dependent mechanism (Katoh et al., 2002; Takemori et al., 2002; Katoh et al., 2004b). Although cytoplasmic and nuclear SIK1 mutants both repress CREB-dependent transcription in adrenocortical carcinoma cells (Katoh et al., 2004b), constitutively nuclear SIK1(S577A) and SIK2(S587A) exhibit stronger repressive activity than their wild type counterparts on CREB-dependent promoters in adrenocortical cells, hepatocytes and brown adipocytes (Takemori et al., 2002; Dentin et al., 2007; Muraoka et al., 2009). Surprisingly, PKA phosphorylation of SIK has little effect on SIK kinase activity in cell-free assays

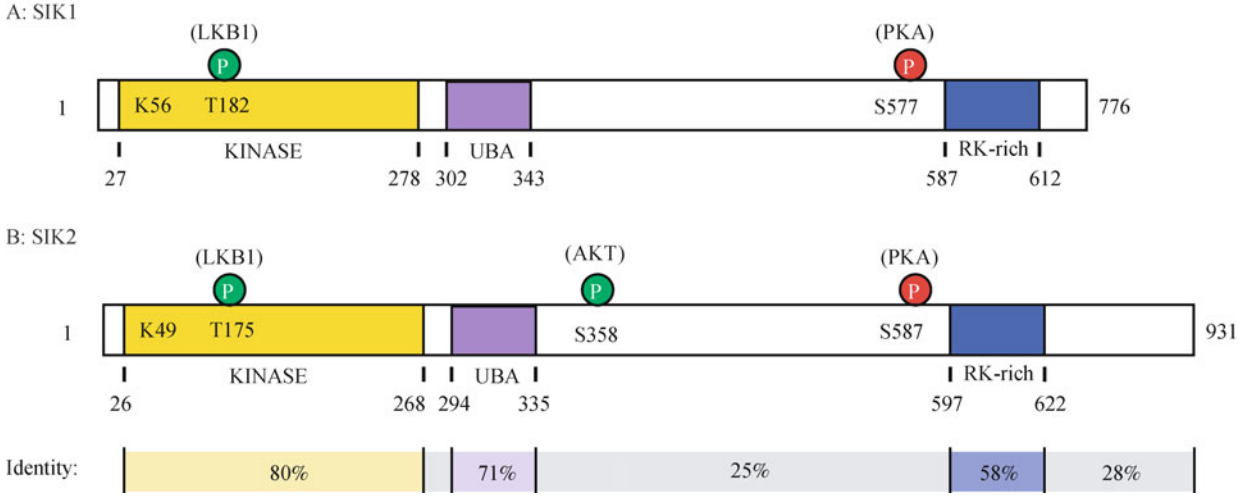

Figure 2 Domain structure and percent identity of SIK1 (A) and SIK2 (B). Domain structures of A) SIK1 and B) SIK2 showing the N-terminal kinase domain (yellow, with catalytic lysine indicated), the T-loop LKB1 phosphorylation site, UBA domain (purple), AKT phosphorylation site (Ser358 in SIK2), PKA phosphorylation site (Ser577), and RK-rich nuclear localization signal (blue). Mouse numbering shown. Phosphorylation sites are colored green for activating and red for inhibitory phosphorylation. Percent identity between domains was determined by the CLUSTALW alignment tool and is shown beneath the SIK2 diagram.

(Katoh et al., 2006; Berdeaux et al., 2007), suggesting that subcellular sequestration from substrates plays a major regulatory role.

SIKs catalyze phosphorylation on serine and threonine residues in the consensus motif LXR[S/T]X**S**XXXL (Horike et al., 2003; Screaton et al., 2004), forming a binding site for 14-3-3 chaperone proteins. Substrates including CRTCs (Screaton et al., 2004) and class II HDACs (Berdeaux et al., 2007) are regulated by 14-3-3 dependent cytoplasmic sequestration. IRS1 (Horike et al., 2003), SREBP-1c (Yoon et al., 2009) and the histone acetyltransferase p300 (Bricambert et al., 2010) have recently been found to be SIK substrates, though the mechanism by which SIK phosphorylation inhibits their activities was not shown to involve 14-3-3 binding. Several of these substrates (CRTC, class II HDACs, SREBP1-c, IRS1) are also phosphorylated by AMPK (Jakobsen et al., 2001; Koo et al., 2005; Takemori et al., 2009; Yoon et al., 2009). It will be interesting to determine if other AMPK substrates are also phosphorylated by SIK kinases and uncover the molecular determinants of substrate selection among these related kinases.

Regulation of gluconeogenesis by SIKs

CREB transcriptional activity is required for fasting gluconeogenesis (Herzig et al., 2001). The finding that CRTC2 is expressed in hepatocytes prompted the Montminy group to evaluate the role of CRTC2 and SIK kinases in this setting. Through an elegant set of *in vitro* and *in vivo* studies, Koo and colleagues (2005) demonstrated that CRTC2 plays a key role in fasting-induced gluconeogenic gene expression and glucose output. CRTC2 accumulates in the nuclei of hepatocytes during fasting or after exposure to cAMP agonists, and acute expression of CRTC2 in liver promotes gluconeogenesis. Adenoviral delivery of a CRTC2-specific shRNA has the opposite effect (Koo et al., 2005). As expected, *Crtc2* knockout mice also show reduced gluconeogenic gene expression during fasting, though the ultimate effect on fasting glucose varied among the two independent knockout strains created (Le Lay et al., 2009; Wang et al., 2010). Effects of *Crtc2* deletion were more pronounced when the animals were fed high fat diet. Although they gained similar amounts of bodyweight as controls, mice lacking CRTC2 retained better insulin sensitivity, judged by glucose and insulin tolerance tests (Wang et al., 2010). These data are in keeping with effects of acute CRTC2 knockdown in diabetic rodents (Saberi et al., 2009).

The observation that CRTC2 subcellular localization was regulated in hepatocytes suggested CRTC2 regulation by salt inducible kinases is a conserved mechanism among hepatocytes and islet cells (Koo et al., 2005). Two SIK isoforms, SIK1 and SIK2 are expressed in liver tissue, and forced expression of either inhibits fasting gluconeogenesis. Intriguingly, *Sik1* mRNA is induced by CREB-CRTC2 binding to the *Sik1* promoter as part of the fasting program and serves as a feedback inhibitor on CRTC2 at the end of the fasting period (Koo et al., 2005). This manner of cAMP-dependent feedback regulation on CREB-CRTC2 transcription by SIK1 also occurs in adrenocortical carcinoma cells treated with adrenocorticotropic hormone (ACTH) (Lin et al., 2001). As in islet cells, SIK1 phosphorylation of CRTC2 Ser171 causes nuclear exclusion in hepatocytes; mutation of this site to alanine renders CRTC2 constitutively nuclear and strongly active on CREB-responsive promoters. The finding that additional AMPK-related kinases, including AMPK itself and SIK2 (Koo et al., 2005; Shaw et al., 2005), also phosphorylate CRTC2 in hepatocytes and intact liver suggests that CRTC2 is a nodal point in transcriptional regulation of glucose output.

Indeed, further studies on hepatic CRTC2 regulation revealed additional signaling mechanisms that serve to inhibit CREB/CRTC2 stimulated transcription at the fasting-to-feeding transition. During re-feeding, blood glucose rises rapidly and stimulates secretion of insulin from β-cells of the pancreas. Insulin, in turn, signals to skeletal muscle, adipose tissue and liver to take up the excess glucose and cease gluconeogenesis. One of the molecular mechanisms by which insulin inhibits gluconeogenesis is via SIK2-dependent inhibition and proteasomal degradation of CRTC2 (Dentin et al., 2007). Within 2 h of re-feeding of mice, hepatic CRTC2 becomes re-phosphorylated on serine 171, transported out of the nucleus and degraded by a ubiquitin-dependent mechanism (Fig. 3). Remarkably, insulin-stimulated AKT was shown to directly phosphorylate serine 358 on SIK2 and promote SIK2-dependent phosphorylation and nuclear export of CRTC2. This phosphorylation event is critical for cessation of gluconeogenesis after re-feeding, as knockdown of SIK2 in mouse liver results in elevated blood glucose during both fasting and re-feeding. SIK2 primes CRTC2 for recognition and ubiquitylation by the E3 ubiquitin ligase COP1 in the cytoplasm. This pathway is thought to restore the CREB transcriptional program to basal levels under *ad libitum* conditions (Dentin et al., 2007). As both SIK1 and SIK2 have been implicated in regulation of CRTC2 and hepatic glucose output, it is unclear to what extent their functions overlap. It is surprising that SIK2 knockout mice do not display overt metabolic abnormalities (Sasaki et al., 2011); this finding suggests that there is either compensation among the SIK family members or that SIK2 downregulation accompanies and contributes to diet-induced diabetes, but is not sufficient to play a causal role.

The clinical importance of the SIK-CRTC2 pathway in hepatic gluconeogenesis is underscored by several key observations. First, overexpression of SIK1 or SIK2 is sufficient to normalize fasting blood glucose in genetically diabetic *(db/db)* mice (Koo et al., 2005). Second, in livers from *db/db* mice, CRTC2 phosphorylation is strongly blunted and the protein accumulates to high levels, consistent with elevated activity of CREB-luciferase reporter genes (Dentin et al., 2007). CRTC2 becomes additionally hyperactive in

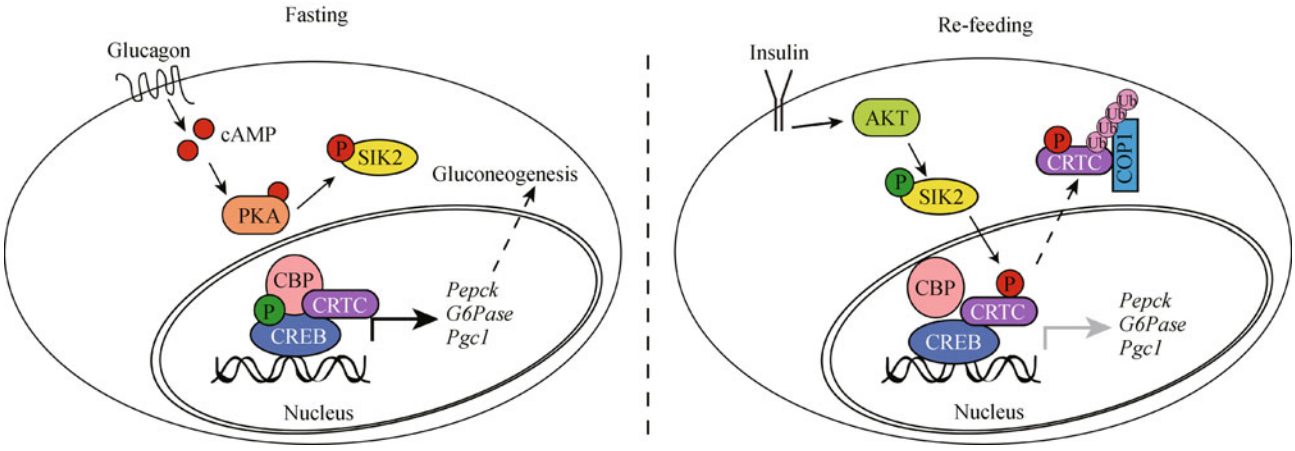

Figure 3 Diagram of SIK2-dependent CRTC2 regulation in hepatocytes. Left) During fasting, SIK2 is inhibited by PKA phosphorylation on Ser587. CRTC2 localizes to the nucleus and cooperates with CREB/CBP to activate transcription of gluconeogenic genes. Right) After re-feeding, insulin activates AKT, which phosphorylates SIK2 on Ser358, promoting SIK2 kinase activity. SIK2 in turn phosphorylates CRTC2 on Ser171, causing CRTC2 to exit the nucleus. In the cytoplasm, phospho-CRTC2 is polyubiquitylated by the COP1 E3 ubiquitin ligase complex and degraded by the proteasome. In livers of diabetic animals, CRTC2 is de-phosphorylated and O-glycosylated, and the protein accumulates inappropriately.

obese mice through O-glycosylation on serine 171, which blocks the inhibitory effects of SIK kinases (Dentin et al., 2008). Additionally, mice lacking CRTC2 retain better insulin sensitivity when fed high fat diet (Koo et al., 2005). Moreover, the widely prescribed glucose-lowering drug metformin was shown to act on CRTC2 through an LKB1-dependent signaling pathway (Shaw et al., 2005) and by inhibition of the CREB co-activator CBP and disruption of the CREB-CRTC2-CBP complex (He et al., 2009). Finally, acute suppression of CRTC2 or CREB expression in liver restores normoglycemia in diabetic rodents (Erion et al., 2009; Saberi et al., 2009). It is therefore clear that CREB and its co-activators are central regulators of hepatic glucose output that become aberrantly active in diabetic states by multiple mechanisms. As SIKs potently inhibit CREB/CRTC2 activity, therapeutic strategies to activate SIK kinases in liver would likely have beneficial effects in patients with type 2 diabetes.

Control of hepatic lipogenesis by SIKs

Another primary function of the liver is lipogenesis: synthesis and esterification of fatty acids from glucose to triacylglycerols for storage of excess glucose taken in during feeding (Salway, 2004). Insulin and glucagon regulate not only the balance between glucose uptake and production, but also lipid storage and liberation. The insulin surge after feeding promotes lipogenesis through a gene expression program directed in large part by the sterol regulatory element binding proteins (SREBPs) (reviewed in Raghow et al., 2008). During gene profiling studies of hepatocytes overexpressing or lacking SIK1, Koo and colleagues noticed that not only were gluconeogenic genes altered but an entire program of genes involved in lipogenesis was also regulated, including the genes encoding fatty acid synthase (*Fasn*) and acetyl CoA carboxylase (*Acaca*) (Yoon et al., 2009). They determined that SIK1 blocks lipogenesis by direct phosphorylation of Srebp1-c on multiple serine residues. Ectopic expression of SIK1 in mouse livers reduces lipogenic gene expression and hepatic triglyceride accumulation. This effect was reversed by co-expression of a phosphorylation-deficient Srebp1-c mutant (Fig. 4A) (Yoon et al., 2009). These findings suggest that strategies to promote SIK1 activity or expression in livers of diabetic patients may revert the excessive lipogenesis often observed in diabetic mammals. Indeed, prior studies showed reduced SIK2 kinase activity in livers of diabetic rodents despite elevated amounts of *Sik1* and *Sik2* mRNAs (Horike et al., 2003). Although SIK1 kinase activity was not measured in that study, it is tempting to speculate that SIK1 kinase activity is reduced in livers of diabetic mice, given the dramatic reduction of CRTC2 phosphorylation (Dentin et al., 2007).

Perhaps not surprisingly, SIK2 was also recently implicated in lipogenesis, but through a different signaling pathway. Glucose stimulates activity of the carbohydrate responsive element binding protein (ChREBP) in the liver (Yamashita et al., 2001). One function of ChREBP is to promote glucose storage by transcriptional induction of lipogenic genes in cooperation with SREBP1-c (Dentin et al., 2004). In an effort to understand how excess glucose stimulates pathologic lipogenesis, Dentin and colleagues searched for mechanisms by which ChREBP is aberrantly activated in diabetic states (Bricambert et al., 2010). They found that knockdown of SIK2 in mouse liver causes not only fasting hyperglycemia, but also dramatic hepatic steatosis (pathologic accumulation of triglycerides in the liver) and concomitant upregulation of genes involved in the lipogenic

program, similar to the observations with SIK1 knockdown (Yoon et al., 2009). However, the molecular mechanism is distinct. The histone acetyltransferase p300 contains a SIK consensus phosphorylation site (Ser89) (Screaton et al., 2004), which was shown by Dentin's group to be functional *in vivo* (Bricambert et al., 2010). In response to glucose, p300 acetylates and activates ChREBP; SIK2 blunts this response by catalyzing an inhibitory phosphorylation on p300(S89) (Bricambert et al., 2010). SIK2 thereby reduces lipogenic gene expression downstream of ChREBP (Fig. 4B). SIK2-specific shRNA or p300 overexpression in mouse liver induces hepatic steatosis, which can be rescued by co-expression of SIK2. Importantly, reduced SIK2 activity in livers of diabetic mice was found to correlate with enhanced p300 activity, ChREBP hyperacetylation and activation, and steatosis. These findings strongly suggest that small molecules that activate SIK kinases may have therapeutic benefit in humans with hepatic lipid storage diseases like non-alcoholic fatty liver disease.

SIK2 function in adipocytes

In mammals, *Sik2* mRNA is most abundant in white and brown adipose tissue (WAT and BAT) (Horike et al., 2003). Expressed at low levels in pre-adipocytes, SIK2 mRNA and protein are strongly upregulated during adipogenesis in cultured cells and in primary adipocytes derived from epididymal fat. SIK1 is poorly expressed in this cell type. Database searches for adipocyte-enriched proteins containing the consensus SIK2 substrate motif suggested an additional link to the insulin signaling pathway via direct phosphorylation of IRS1 on serine 789 (mouse; Ser794 in human). Indeed, SIK2 phosphorylates IRS1(Ser789), and the authors suggest that SIK2 inhibits the insulin signaling pathway through this interaction (Horike et al., 2003). However, the effects of SIK2 on insulin signaling in adipocytes have not been tested directly. Interestingly, AMPK phosphorylation of the same site on IRS1 in differentiated C2C12 myotubes promotes insulin signaling, as more PI(3)-kinase activity is associated with phospho(S789)-IRS1 in that cell type (Jakobsen et al., 2001). Similar to the case in hepatocytes, SIK2 over-expression in white adipocytes repressed lipogenic gene expression, possibly through regulation of SREBP transcription factors (Du et al., 2008). Together these observations indicate that SIK kinases may modulate insulin signaling and lipid storage in white adipose tissue, and further study is warranted to identify the molecular mechanisms involved.

Notably, the CREB co-activator CRTC3 is also enriched in adipose tissue (Song et al., 2010). *Crtc3* knockout mice fed a high-fat diet are more insulin sensitive and store less lipids in WAT than wild type littermate controls. This unexpected phenotype was found to result from enhanced cAMP signaling in *Crtc3*-deficient WAT due to reduced expression of the CREB/CRTC3 target gene *Rgs2* in the knockout cells (Song et al., 2010). RGS2 (regulator of G-protein signaling 2) is a feedback inhibitor on heterotrimeric G-proteins. Reduced *Rgs2* expression in cells lacking CRTC3 resulted in increased cAMP production. cAMP promotes lipolysis in adipose tissue (Salway, 2004); thus enhanced cAMP signaling tone in $Crtc3^{-/-}$ fat cells results in elevated lipolysis and a lean phenotype. As SIK kinases normally inhibit CRTC3 (Song et al., 2010), one would expect that SIK expression in WAT would promote lipolysis; conversely, SIK-deficient animals would be predicted to store more triglycerides in WAT after high-fat diet feeding.

Brown adipose tissue (BAT) is a mitochondria-rich tissue specialized for thermogenesis in response to catecholamines and can affect energy balance by increasing energy expenditure (Kajimura et al., 2010). SIK2 is enriched in BAT, where it becomes phosphorylated on the inhibitory serine 587 in response to insulin signaling, though the kinase

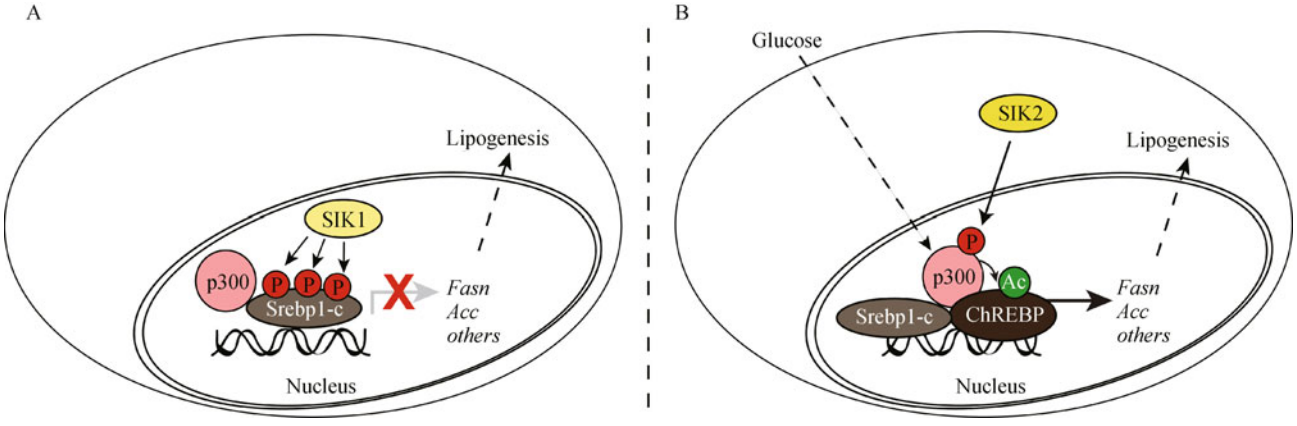

Figure 4 Mechanisms by which SIKs inhibit hepatic lipogenesis. A) SIK1 directly phosphorylates Srebp1-c on three serine residues (Ser265/266/329). This blocks transcription of lipogenic genes including *Fasn* and *Acaca* and inhibits hepatic triglyceride synthesis. SIK1 RNAi promotes lipogenic gene expression. B) Glucose stimulates hepatic lipogenesis by activating p300-dependent acetylation of ChREBP, which cooperates with Srebp1-c to induce lipogenic genes. SIK2 opposes this pathway by phosphorylating p300(Ser89), inhibiting p300 acetylation of ChREBP.

responsible has not been identified (Muraoka et al., 2009). Concomitant with SIK2 inhibition, CRTC co-activators become dephosphorylated and associated with promoters of thermogenic genes *Pgc1α* and *Ucp1* after insulin stimulation of brown adipocytes (Muraoka et al., 2009). As in other tissues, forced expression of SIK2 in brown adipocytes blocks CREB-dependent gene expression. BAT in obese animals contains elevated SIK2 activity and CRTC2 phosphorylation; this correlates with reduced *Pgc1α* and *Ucp1* expression, suggesting that SIK kinases inhibit thermogenesis in BAT. Consistent with this notion, transgenic mice expressing constitutively nuclear SIK2(S587A) in BAT gain more weight on a high-fat diet, have defective thermogenesis, exhibit worse glucose tolerance after high-fat feeding and accumulate more triglyceride in brown adipocytes than wild-type control animals (Muraoka et al., 2009). Physiologic effects of constitutively nuclear SIK2 in promoting lipid accumulation in BAT are in apparent contrast to effects of CRTC3 deletion in promoting lipolysis in WAT (Song et al., 2010). Thus, careful examination of SIK2 action in BAT and WAT is required to fully predict how therapeutics that target SIK2 will affect lipid metabolism in adipose tissue.

Lessons from model organisms: indirect metabolic control by SIKs and CRTCs

SIK kinases also affect whole-body metabolism in fruit flies. Flies have a single SIK orthlog, termed *dSIK2* or *Drosophila SIK* (Wang et al., 2008; Choi et al., 2011) and a single CRTC ortholog *dTORC* (Wang et al., 2008). *dTORC* mutant flies have reduced storage of glycogen and lipids, which may underlie their profound sensitivity to starvation and stress (Wang et al., 2008). As in mammalian tissues, dTORC is activated by starvation or stress in flies, and insulin signaling activates dSIK2, which phosphorylates and inhibits dTORC. Moreover, epistasis analysis confirmed that *dSIK2* and *dTORC* lie in a common genetic pathway (Wang et al., 2008; Choi et al., 2011). As expected, deletion of *Drosophila SIK* resulted in the opposite phenotype of *dTORC* deletion: flies were less sensitive to metabolic stress and stored more lipids (Choi et al., 2011). However, metabolic control by SIK and TORC in *Drosophila* occurs via different mechanisms than those discussed above in mammalian systems. In flies, *dTORC* and *dSIK2* are primarily expressed in the head, and neuronal expression of dTORC or SIK2 shRNA can rescue the phenotypes observed in TORC-deficient flies (Wang et al., 2008). Although the identity of neuronal dTORC target genes that regulate whole-body metabolism is unknown, it is clear that the SIK-TORC pathway can affect metabolism by mechanisms in addition to direct effects on metabolic pathways in liver and adipose.

Indeed, recent studies of CRTC proteins in mammals revealed prominent roles in the response to feeding in the brain. Mice lacking the brain-restricted CREB co-activator *Crtc1* exhibit dramatic hyperphagia, weight gain, leptin resistance and reductions in energy expenditure (Altarejos et al., 2008). CRTC1 is enriched in the ventromedial hypothalamus, which is a center of the brain that senses nutritional state and regulates food intake. Neuronal CRTC1 becomes activated in response to leptin and glucose, whereupon it associates with the proximal promoter region of the *Cartpt* gene, which may mediate the effects of CRTC1 on satiety (Altarejos et al., 2008). CRTC2 is also enriched in the hypothalamus (Lerner et al., 2009), where it accumulates in the nucleus after feeding, suggesting that CRTC2 also participates in the transcriptional response to feeding. CRTC2 activity is antagonized by AMPK, as the AMPK activator AICAR promotes CRTC2 phosphorylation and prevents CRTC2 from associating with the *Irs2* promoter in response to glucose. While the roles of SIK kinases are yet to be evaluated in this context, it is important to note that the primary metabolic effectors of SIKs have varying actions in different tissues.

Non-metabolic functions of SIK kinases

Although best studied in the context of metabolism, it is notable that additional roles have been identified for SIK kinases using targeted genetic approaches as well as high-throughput screens. Both SIK1 and SIK2 have been implicated in neuronal responses to injury, with opposing effects. *Sik2* knockout mice have been reported; while these mice evidently do not show overt metabolic phenotypes, they do have enhanced melanogenesis in skin (Horike et al., 2010) and enhanced neuronal survival after ischemia, perhaps by virtue of enhanced CRTC1/CREB activity on pro-survival genes such as *Bdnf* (Sasaki et al., 2011). On the other hand, SIK1 has been implicated as a pro-survival factor in neurons subjected to cerebral ischemia (Cheng et al., 2011). SIK1 was previously shown to be upregulated in the hippocampus of rats treated with the seizure-inducing agent kainic acid (Feldman et al., 2000). Hurn and colleagues investigated the functional consequences of SIK1 induction following cerebral ischemic injury in mice; they injected lentiviral vectors encoding SIK1-specific shRNAs into the brains of mice and found that, by contrast with genetic deletion of *Sik2*, *Sik1* knockdown exacerbated neuronal death after ischemic injury, possibly through de-repression of class II HDACs (Cheng et al., 2011). These intriguing findings show that any therapeutics targeting SIK kinases should be tested for effects on the central nervous system.

Sik1 knockout mice have not been reported, but shRNA-based knockdown strategies and genetic models have revealed many important roles in different cell types. In transformed fibroblasts, SIK1 acts as a tumor suppressor by promoting anchorage-dependent cell death called anoikis (Cheng et al., 2009). This effect may result from direct phosphorylation of the p53 tumor suppressor protein, but

additional work is required to prove this definitively. Mouse embryonic stem cells lacking *Sik1* have delayed upregulation of the cardiogenic program in vitro, which was ascribed to deficiency in upregulation of the cell cycle inhibitor $p57^{Kip2}$ (Romito et al., 2010). However, it remains to be determined how SIK1 regulates expression of $p57^{Kip2}$ and whether this effect will be observed in whole animals. It is notable in this regard that *Sik1* transcripts are enriched in the early developing mouse myocardium during embryogenesis (Ruiz et al., 1994). We previously demonstrated that *Sik1* is the critical CREB target gene responsible for maintenance of skeletal muscle fiber survival (Berdeaux et al., 2007). Mice lacking CREB function in skeletal muscle have ongoing myofiber degeneration and regeneration. We found this was due to reduced *Sik1* expression, as restoration of SIK1 by adeno-associated virus was sufficient to rescue the degenerative phenotype. Through this study we defined a new signaling pathway whereby CREB transcriptionally induces SIK1, which in turn phosphorylates and inhibits class II histone deacetylases, ultimately allowing full transcriptional activity of MEF2 proteins (Berdeaux et al., 2007). It will be interesting to determine how this pathway affects muscle development. The same SIK-HDAC-MEF2 pathway was shown to function in *C. elegans* sensory neurons (van der Linden et al., 2007) as well as in chondrocytes (Kozhemyakina et al., 2009). These varied roles for SIK kinases suggest that multiple regulatory mechanisms exist in different tissues that will allow targeted therapy depending on the disease.

Misregulation of SIK kinases in diabetic states: prospects for diabetes therapy

The hallmark feature of type 2 diabetes is elevated fasting blood glucose. Dysregulated gluconeogenesis contributes to hyperglycemia in diabetic rodents and humans (Magnusson et al., 1992; Yoon et al., 2001), though the contribution of elevated gluconeogenic gene expression to this phenomenon is controversial (Herzig et al., 2001; Yoon et al., 2001; Samuel et al., 2009). Nonetheless, it is clear that CREB-dependent transcription is required for appropriate control of glucose output during fasting and that CREB and its regulators become mis-regulated in diabetic livers (Herzig et al., 2001; Koo et al., 2005; Dentin et al., 2007; Bricambert et al., 2010). The involvement of these pathways in pathologic hyperglycemia was recently further substantiated by the findings that delivery of CRTC2- or CREB-specific siRNAs reduced hyperglycemia in diabetic rodents (Erion et al., 2009; Saberi et al., 2009) and that *Crtc2* deletion promoted insulin sensitivity in high fat diet fed mice (Wang et al., 2010). Moreover, the anti-diabetic drug metformin acts through pathways culminating in reduced CREB transcriptional activity (Shaw et al., 2005; He et al., 2009). As described in this article, appropriate regulation of CRTC/CREB activity by SIK kinases is crucial for inhibiting excessive hepatic glucose output. In livers of diabetic rodents, SIK1 and SIK2 activities are reduced (Horike et al., 2003; Bricambert et al., 2010), and CRTC2 becomes aberrantly activated due to O-glycosylation on the SIK phosphorylation site, rendering CRTC2 constitutively active (Dentin et al., 2008). Finally, SIK1 and SIK2 inhibit both gluconeogenesis (Koo et al., 2005; Dentin et al., 2007) and lipogenesis (Yoon et al., 2009; Bricambert et al., 2010), both of which contribute to metabolic abnormalities in diabetic patients.

It is unknown whether human mutations in SIK kinases are linked with diabetes or metabolic syndrome. Nonetheless, these are attractive molecular targets for metabolic therapy; enhancing SIK activity with a specific pharmacologic agent would be predicted to reduce blood glucose and hepatic triglyceride storage in diabetic patients. It is noteworthy that SIKs have distinct regulatory roles in other tissues and cell types like skeletal muscle, brain, bone chondrocytes and the adrenal gland. Thus any therapeutic agent targeted to SIK kinases or SIK activators should be evaluated in multiple organ systems. Perhaps the most promising avenue will be identification of tissue-specific mechanisms by which SIK kinase activities are regulated. Such mechanisms would allow identification of even more selective molecular targets by which hyperglycemia and fatty liver can be blunted. Given the potent effects of SIK kinases on key metabolic organs, uncovering these mechanisms will certainly yield exciting and clinically relevant results in the years to come.

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