# Novel role for the CRTC2 in lipid homeostasis



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COMMENTARY

# Novel role for the CRTC2 in lipid homeostasis

Insulin resistance, a state of reduced responsiveness to insulin, is frequently associated with obesity, which subsequently leads to type 2 diabetes. Although major advances have been made in unraveling the underlying mechanisms that cause insulin resistance, many of the pathways and regulators that link insulin to its downstream metabolic effects are not completely understood.

Studies in rodent models showed a peculiar feature of hepatic insulin resistance, namely its selectivity. Insulin exerts two predominant actions in the liver: (i) it suppresses glucose production (gluconeogenesis); and (ii) it activates the synthesis of fatty acids and triglycerides (lipogenesis). However, in the insulin-resistance state, insulin fails to suppress gluconeogenesis, and lipogenesis is paradoxically overactivated. As suggested by Brown and Goldstein[1], the signaling pathways utilized by insulin to stimulate sterol regulatory element-binding protein (SREBP)-1c could remain intact in obesity and type 2 diabetes, even as the pathways that regulate glucose metabolism become resistant. These dual actions contribute to the lethal combination of hyperglycemia and hypertriglyceridemia that characterizes the diabetic state.

Increased lipogenesis observed in the insulin-resistant state is partially because of dysregulation of the master transcriptional regulator of lipogenesis, the SREBP-1c. The SREBP family consists of three isoforms: SREBP-1a, SREBP-1c and SREBP-2[2]. SREBP-1c is an isoform that controls the biosynthesis of fatty acids and triglycerides by increasing transcription of genes encoding acetyl-CoA carboxylase, fatty acid synthase, elongation of very long chain fatty acids family member 6, stearoyl-CoA desaturase and others. Insulin activates SREBP-1c through at least two mechanisms: (i) it increases SREBP-1c transcription; and (ii) it increases the proteolytic cleavage of SREBP-1c from an inactive endoplasmic reticulum membrane-bound precursor to release their N-terminal domain capable of translocating to the nucleus to activate transcription (Figure 1).

In hepatocytes, insulin signaling through phosphatidylinositol 3-kinase and Akt results in the activation of SREBP-1c expression and accumulation of nuclear SREBP-1c protein. The mammalian target of rapamycin complex 1 (mTORC1) is the major phosphatidylinositol 3-kinase/Akt downstream effector, because insulin-stimulated SREBP activation and lipogenesis are both blocked by the mTORC1 inhibitor rapamycin. In addition, lipin-1, a phosphatidic acid

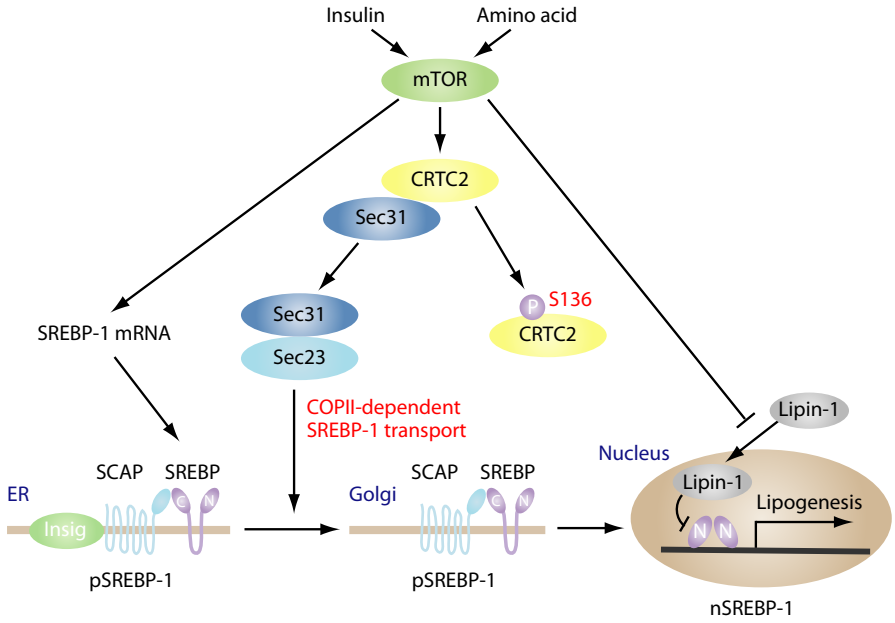


**Figure 1** | The mammalian target of rapamycin (mTOR) pathways involved in the regulation of sterol regulatory element-binding protein (SREBP)-1. There are multiple signals that regulate expression, endoplasmic reticulum (ER)-to-Golgi transport and the proteolytic cleavage of SREBP-1c. The mTOR pathways involved in the regulation of SREBP-1. During feeding, Sec31A, a subunit of the coat protein complex II (COPII) complex, dissociates from cyclic adenosine monophosphate response element-binding protein (CREB) regulated transcription coactivators (CRTC)2, which becomes phosphorylated at Ser136 by mTOR, and interacts instead with Sec23A, thus promoting COPII-dependent transport and processing of SREBP-1 in the Golgi. In obesity, enhanced phosphorylation of CRTC2 by mTOR partially contributes to increase SREBP-1 activation and hepatic lipogenesis. The mTOR pathways are also able to increase SREBP-1c messenger ribonucleic acid (mRNA), and activate the processing of SREBP-1c and maximal lipogenic gene expression. C, sterol regulatory element-binding protein C-terminal fragment; N, sterol regulatory element-binding protein N-terminal fragment; SCAP, sterol regulatory element-binding protein cleavage-activating protein.

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phosphatase and transcriptional coactivator, is a direct substrate of mTORC1 and regulator of nuclear SREBP activity. Lipin-1 phosphorylation by mTORC1 blocks lipin's nuclear localization and activates SREBP-1c activity. In the insulin-resistant state, insulin might continue to activate mTORC1 while losing its ability to inhibit forkhead box O1.

The cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB)-regulated transcription coactivators (CRTCs) physically interact with CREB through its N-terminal portion, and coactivate the transcriptional activity of CREB through its C-terminally located transactivation domain. cAMP-dependent activation of this transcriptional machinery promotes hepatic gluconeogenic programs by upregulation of key enzyme genes, such as phosphoenolpyruvate carboxykinase and glucose-6-phosphatase catalytic subunit[3,4]. Although the function of CRTCs in the liver has been extensively studied in the context of gluconeogenesis, Han *et al.*[5] recently showed that cytosolic CRTC2, as a critical mediator of mTOR, modulates coat protein complex II (COPII) activity, which subsequently leads to SREBP-1 processing and enhancement of de novo lipogenesis.

These authors measured hepatic lipid levels in *Crtc2*$^{+/+}$ and *Crtc2*$^{-/-}$ mice. They found that *Crtc2*$^{-/-}$ mice showed increased hepatic triglyceride levels, but not cholesterol, when compared with *Crtc2*$^{+/+}$ mice fed with both regular chow and a high-fat diet. Transcript analysis showed specific upregulation of SREBP1-target genes involved in triglyceride synthesis in the livers of *Crtc2*$^{-/-}$ mice. In *Crtc2*$^{-/-}$ mice fed with both chow and a high-fat diet, the active, nuclear SREBP-1 was significantly increased while the precursor, full-length SREBP-1 was slightly decreased, although total *Srebp-1c* mRNA levels remained unchanged. These results suggest that CRTC2 mediates SREBP-1 activity at the post-transcriptional level. In addition, the abnormal nuclear SREBP-1 accumulation and the enhanced lipogenic gene expression in *Crtc2*$^{-/-}$ mice were normalized to the level found in fed *Crtc2*$^{+/+}$ mice by adenovirus-mediated CRTC2 overexpression or knockdown of *Srebp-1*. Taken together, these results show that CRTC2 modulates triglyceride synthesis through regulation of SREBP-1 maturation.

In the liver, cAMP signaling activates CRTC2 activity through Ser171 dephosphorylation resulting in its nuclear localization and increased association with CREB on the promoters of gluconeogenic genes. Han *et al.*[5] examined the effects of the cellular localization of CRTC2 on SREBP1 maturation using two CRTC2 mutants: (i) ΔTAD (amino acids 1–630), which lacks the transactivation domain, but still shuttles between the cytoplasm and nucleus; and (ii) ΔTAD/AA, which is confined to the nucleus because of serine-to-alanine mutations at positions 171 and 275. Similar to wild-type CRTC2, the ΔTAD mutant also blocks SREBP-1 maturation in *Crtc2*$^{-/-}$ mice, whereas ΔTAD/AA mutant was not able to suppress the processing of SREBP-1, suggesting that SREBP-1 processing depends on cytosolic but not nuclear CRTC2.

Using mass spectrometry and co-immunoprecipitation analysis, they found that CRTC2 binds to proteins involved in SREBP-1 transport regulation. Insulin regulates SREBP activity through stimulation of endoplasmic reticulum-to-Golgi SREBP-1c transport by promoting its phosphorylation and association with COPII vesicles. Indeed, CRTC2 binds to Sec31A, a subunit of the COPII complex, and negatively regulates SREBP-1 processing by competing with Sec23A for binding to Sec31A.

Finally, Han *et al.*[5] found that the CRTC2–Sec31 interactions modulate hormonal and nutritional activation of SREBP-1. Indeed, both insulin and amino acid stimulation attenuated the CRTC2–Sec31A interaction with concomitant enhancement of the Sec23A–Sec31A association. The regulatory effects of CRTC2 on the Sec23–Sec31A interaction were inhibited in the presence of torin1, an inhibitor of mTOR, which controls lipid metabolism and SREBP-1 activation. The conserved serine site at position 136 of CRTC2, which occurs in the context of a classic mTOR substrate motif (S/T-P), was phosphorylated without and dephosphorylated with rapamycin treatment. In addition, a co-immunoprecipitation assay and *in vitro* kinase assay showed that mTOR interacts with CRTC2, and mTOR directly phosphorylates CRTC2 at Ser136. Both insulin and amino acids stimulate CRTC2 Ser136 phosphorylation in an mTOR-dependent manner, suggesting that CRTC2 is a bona fide substrate of mTOR. Taken together, these results show that mTOR modulates COPII-dependent SREBP1 processing through Ser136 phosphorylation of CRTC2 (Figure 1).

The elegant study by Han *et al.*[5] greatly advances our understanding of the specific signaling pathways by which insulin stimulates lipogenesis, and provides new insight into how SREBP-1 activity is enhanced in obesity and type 2 diabetes. This study thus identifies mTOR-CRTC-SREBP-1 signaling as a potential therapeutic target for ameliorating hyperglycemia, hypertriglyceridemia and hepatic steatosis in type 2 diabetes. Further unveiling of the molecular mechanism(s) that regulate this signaling axis in obesity and diabetes will hopefully result in effective ways to overcome insulin resistance and provide a basis for beneficial treatment of type 2 diabetes.

## DISCLOSURE

The authors declare no conflict of interest.

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