

Short Conceptual Overview

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New insights into the activation of sterol regulatory element-binding proteins by proteolytic processing

Abstract: Sterol regulatory element-binding proteins (SREBPs) are transcription factors that regulate a wide variety of genes involved in cholesterol and fatty acid synthesis. After transcription, SREBPs are controlled at multiple post-transcriptional levels, including proteolytic processing and post-translational modification. Among these, proteolytic processing is a crucial regulatory step that activates SREBPs, which are synthesized as inactive endoplasmic reticulum membrane proteins. In this review, we focus on recent progress with regard to signaling pathways and small molecules that affect activation of SREBPs by proteolytic processing.

Keywords: AMP-activated protein kinase (AMPK); insulin; proteolytic processing; SREBP.

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Introduction

In humans, cholesterol is derived from two main sources: diet and *de novo* synthesis in the liver. Cholesterol homeostasis is tightly regulated at transcriptional, translational, and post-transcriptional levels, and its disruption increases the risk of atherosclerotic disease. Initially, sterol regulatory element-binding proteins (SREBPs) were thought to be transcriptional regulators of cholesterol homeostasis (1), comprising the transcription factors SREBP-1a, SREBP-1c, and SREBP-2 (2, 3). SREBP-1a and SREBP-1c are produced from the same gene but are transcribed from different promoter and transcription start sites, and the resulting proteins have different target genes. SREBP-2 preferentially regulates the expression of genes involved in cholesterol synthesis, whereas SREBP-1c

basically regulates genes of fatty acid biosynthetic pathways. SREBP-1a has been shown to regulate genes of cholesterol and fatty acid synthesis (4, 5). All three SREBPs are synthesized as membrane proteins of the endoplasmic reticulum (ER) and contain N-terminal transcription factor domains and C-terminal regulatory domains (Figure 1). These domains are located in the cytosol and are separated by two transmembrane domains. Therefore, SREBPs require liberation from N-terminal halves to function as transcription factors in the nucleus. This activation by proteolytic processing is tightly regulated by the interaction with ER membrane proteins, SREBP cleavage-activating protein (SCAP), and insulin-induced gene (Insig). SREBPs form a complex with SCAP, which functions as a cholesterol sensor through an interaction between each C-terminal domain. When the cells are depleted of sterols, SCAP binds to the COPII proteins Sar1 and Sec23/24, resulting in the incorporation of SCAP/SREBPs complexes into COPII-coated vesicles. Consequently, SREBPs enter the Golgi and are processed by site-1 and site-2 proteases (S1P and S2P, respectively). With increases in the ER cholesterol content, SCAP binds to cholesterol, which induces conformational changes, and gets attached to Insig. The interaction between Insig and SCAP results in inhibition of SCAP binding to COPII proteins, and SREBPs remain on the ER membrane as inactive precursors (Figure 1). Regulation of proteolytic processing of SREBPs by cholesterol has been described in more detail in another review (1).

Under conditions of low cholesterol, SREBPs are activated by proteolytic processing and their isoforms are then stimulated to activate respective target genes. Although nuclear SREBP-1 is increased under these conditions in cultured cells, treatment with a bile acid-binding resin (colestipol) and a cholesterol synthesis inhibitor (mevinolin), which lowers total plasma cholesterol levels and decreases nuclear SREBP-1 despite increasing nuclear SREBP-2 in hamster liver cells (6). Therefore, cholesterol levels are not a prominent factor in the activation of SREBP-1 in the living animal liver. Interestingly, the

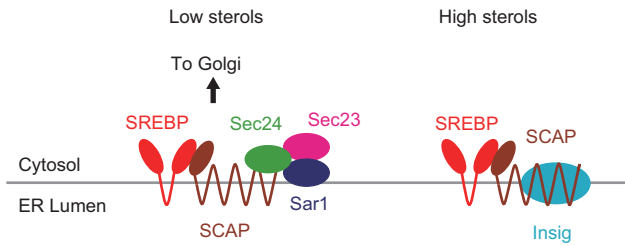


Figure 1 Regulation of SREBP proteolytic processing by intracellular cholesterol levels.

Under conditions of low cholesterol (Low sterols), SCAP/SREBPs complexes are incorporated into COPII-coated vesicles and are transported to the Golgi. Subsequently, SREBPs are cleaved by S1P and S2P within the Golgi. With increased ER cholesterol content (High sterols), Insig binds to SCAP and retains SCAP/SREBPs on the ER membrane as an inactive precursor.

expression of SREBP-1c is increased in the livers of diabetic animals, and its overactivation is associated with hepatic steatosis (7). In this review, we discuss the regulation of SREBP-1c activity, with a focus on proteolytic processing.

In addition to proteolytic processing, SREBP activities are controlled by multiple transcription, translation, and protein degradation factors (Figure 2). Of note, SREBP-1c and SREBP-2 promoters are autoregulated by SREBPs (8, 9). Furthermore, rapid degradation of active nuclear SREBPs by the ubiquitin-proteasome system is triggered by GSK-3-mediated phosphorylation (10). The activities of nuclear SREBPs are also controlled by post-translational modifications, such as phosphorylation, acetylation, and SUMOylation (11–15). Although many reports identify signaling pathways and small molecules that regulate SREBP activities, these multiple modes of regulation are often indistinguishable from each other. Several well-designed experiments have recently revealed signaling pathways and small molecules other than cholesterol that regulate proteolytic processing of SREBPs.

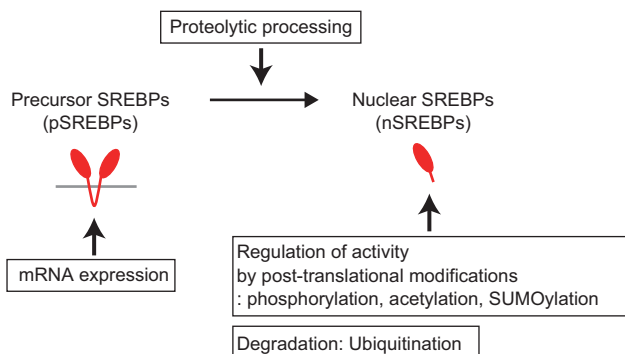


Figure 2 Multiple modes of SREBP regulation.

Regulation of SREBP-1c by insulin signaling pathway

In rodent livers, SREBP-1c mRNA and active nuclear SREBP-1 protein levels are upregulated following re-feeding (16), and lipogenic gene expression and *de novo* lipogenesis are subsequently increased. SREBP-1c expression in the rat liver reduces after treatment with streptozotocin, which destroys pancreatic β -cells and leads to acute insulin deficiency. However, SREBP-1c expression returns to normal levels when diabetic rats are treated with insulin, indicating that insulin mediates the increase in SREBP-1c expression after re-feeding (17). In agreement, wortmannin, a potent inhibitor of phosphoinositide 3-kinase (PI3K), blocks induction of SREBP-1c by insulin (18) and activation of Akt then induces SREBP-1c expression (19), confirming the involvement of insulin/PI3K/Akt signaling in the induction of SREBP-1c. Akt signaling also activates SREBP-2 (20, 21), and this issue has been described in detail in another review (22).

The SREBP-1c-activating actions of insulin are numerous and complex (Figure 3). Akt directly phosphorylates precursor SREBP-1c, which stimulates its proteolytic processing by enhancing the affinity of the SCAP/SREBP-1c complex for Sec23/24 proteins of COPII vesicles (23). Other studies show that ERK1/2-mediated phosphorylation of nuclear SREBPs stimulates their transcriptional activities (11, 24, 25). Moreover, the ubiquitin ligase Fbw7 has been shown to ubiquitinate and degrade nuclear SREBPs following phosphorylation by GSK-3 (10).

A recent study revealed that PI3K/Akt-mediated induction of nuclear SREBP-1 protein is abolished by treatment with the mTORC1 inhibitor rapamycin in human retinal pigment epithelial cells (26). Furthermore, insulin-induced SREBP-1c expression is abolished by rapamycin

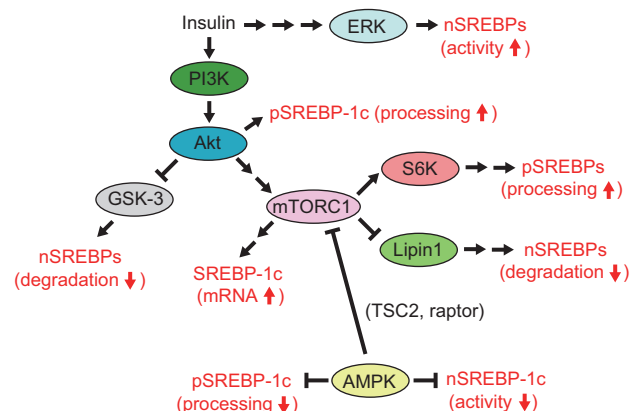


Figure 3 Multiple effects of insulin signaling on SREBP activation.

in primary cultured rat hepatocytes (27), indicating that the Akt/mTORC1 pathway is involved in insulin activation of SREBP-1c. Indeed, constitutive activation of mTORC1 by TSC1/2 complex disruption leads to increased expression of SREBP-1c in mouse embryonic fibroblasts (28). However, contrary to expectations, constitutive activation of mTORC1 reduces SREBP-1c expression in TSC1-deficient mouse hepatocytes (29). These reductions in SREBP-1c expression follow mTORC1-driven inhibitory feedback mechanisms that attenuate Akt signaling (29, 30). Insulin inhibits the expression of Insig2a, which is a predominant liver-specific isoform of Insig2, and results in proteolytic processing of SREBP-1c (31). Impairment of Akt signaling by constitutive mTORC1 activation results in maintenance of high levels of Insig2a gene expression, thereby inhibiting SREBP-1c activation. Indeed, si-RNA-mediated suppression of Insig2a in TSC1-deficient hepatocytes restores insulin-stimulated induction of SREBP-1c (29), indicating tissue-specific activation of SREBP-1c.

Peterson et al. reported the involvement of lipin1, which functions as a phosphatidic acid phosphatase and transcriptional co-activator, in the mTORC1-mediated activation of SREBP-1c (32). In particular, mTORC1 directly phosphorylates lipin1 and promotes its cytoplasmic localization, which results in increased nuclear SREBP proteins through an unknown mechanism. Therefore, lipin1 localization may contribute to mTORC1-mediated activation of SREBP-1c.

Because insulin triggers an increase in nuclear SREBP-1 protein levels in the livers of living animals and in freshly isolated hepatocytes, it was hypothesized that insulin may stimulate proteolytic processing of SREBP-1. Using an elegant assay system that eliminated transcriptional effects of insulin, Owen et al. expressed an insulin-insensitive epitope-tagged human apoE promoter/enhancer-driven SREBP-1c expression cassette in rats (33). In these animals, exogenous nuclear SREBP-1c was decreased during fasting and increased on re-feeding, without a change in exogenous SREBP-1c mRNA, suggesting that proteolytic processing of SREBP-1c is regulated by feeding. This mode of regulation was recapitulated by insulin treatments of primary hepatocytes from these transgenic rats. Treatment with the proteasome inhibitor MG132 caused an increase in nuclear SREBP-1c, which increased further with insulin, indicating that insulin increases the production of the nuclear form rather than blocking degradation. Importantly, this insulin-mediated increase in SREBP-1c proteolytic processing was blocked by the kinase inhibitors wortmannin (PI3K inhibitor), rapamycin (mTORC1 inhibitor), and LYS6K2 (S6K inhibitor). Interestingly, wortmannin and rapamycin inhibited the effects of insulin on

endogenous SREBP-1c mRNA whereas LYS6K2 did not, indicating the varying involvement of these kinase signaling pathways in insulin-mediated SREBP-1c gene expression and proteolytic processing. At present, it is unclear how this S6K inhibitor blocks insulin-mediated increases in SREBP-1c proteolytic processing. A putative target is phosphorylated by S6K and then stimulates SREBP-1c proteolytic processing by enhancing transport of SCAP/SREBPs from ER to the Golgi. Insulin may stimulate transport of SCAP/SREBPs from ER to the Golgi through direct phosphorylation of SREBPs by Akt, whereas inhibition of S6K blocks the activities of proteases such as S1P and S2P. It will be necessary to determine whether inhibition of S6K affects insulin-induced transport of SCAP/SREBPs.

SREBP-1c regulation by AMP-activated protein kinase (AMPK)

AMPK, a major cellular energy sensor, plays a key role in maintaining energy homeostasis. When the intracellular AMP/ATP ratio is increased, AMPK is activated following phosphorylation by the upstream kinase LKB1. Activated AMPK regulates several metabolic processes, including gluconeogenesis, fatty acid and cholesterol synthesis, and fatty acid oxidation, by phosphorylating various downstream substrates (34). Previous studies suggest that AMPK activation leads to decreased SREBP-1c expression in mouse livers and rat hepatoma cells (35–37). Recent studies have demonstrated that the induction of SREBP-1c gene expression and nuclear localization of SREBP-1 by high-fat and high-sucrose diets is completely reversed by treatment with the synthetic polyphenol S17834, which activates AMPK in the livers of low-density lipoprotein (LDL) receptor-deficient mice (38). Similar observations were made in HepG2 hepatoma cells. In particular, nuclear SREBP-1 increases after treatments with glucose and insulin, and this effect is abolished by S17834. Moreover, dominant-negative AMPK abrogates the ability of S17834 to repress glucose and insulin-mediated accumulation of nuclear SREBP-1 in HepG2 cells, suggesting that S17834 inhibits activation of AMPK. Furthermore, AMPK directly phosphorylates nuclear SREBP-1c at Ser372, leading to decreased activity (38). The effects of AMPK activation on proteolytic processing of SREBP-1c were examined in HEK293T cells expressing exogenous epitope-tagged SREBP-1c. In these experiments, exogenous nuclear SREBP-1c decreased with AMPK activation in the presence of the proteasome inhibitor ALLN, suggesting that AMPK acts by decreasing production of the nuclear form rather

than by stimulating its degradation. Although it has been demonstrated that active AMPK decreases nuclear SREBP-1, the mechanisms by which AMPK suppresses proteolytic processing of SREBP-1c remain unclear. AMPK physically associates with SREBP and directly phosphorylates precursor and nuclear SREBP-1c at Ser372. Potentially, phosphorylation of SREBP-1c at Ser372 inhibits the transport of SCAP/SREBP-1c complexes from ER to the Golgi. Indeed, active AMPK may suppress proteolytic processing of SREBP-1c by inhibiting mTORC1 activity through direct phosphorylation of both TSC2 and raptor [(39); Figure 3]. This interpretation is supported by suppression of Akt-induced nuclear SREBP-1 by activated AMPK (26).

SREBP regulation by small molecules

Several small molecules other than cholesterol have been reported to regulate proteolytic processing of SREBPs. Among these, unsaturated fatty acids – such as oleate, linoleate, and arachidonate – downregulate nuclear SREBP-1 by suppressing transcription and proteolytic processing of SREBPs (40). Unsaturated fatty acids inhibit proteasomal degradation of ubiquitinated Insig-1 (41). A recent report revealed that the primary mechanism for polyunsaturated fatty acid-mediated suppression of SREBP-1 involves the inhibition of proteolytic processing and that this in turn leads to decreased transcription of SREBP-1c through an autoregulatory mechanism (42).

Grand-Perret et al. showed that the steroid-like analog GW707 and the non-steroidal compound GW300 stimulate transcription of LDL receptors (43). These molecules increased nuclear localization of exogenous SREBPs in CHO cells transfected with plasmids encoding precursor SREBP-1a or SREBP-2. Because these compounds bind SCAP, they are referred to as SCAP ligands that stimulate the proteolytic processing of SREBPs. However, a subsequent report showed that GW707 stimulates SREBP target genes independent of SCAP-binding activity. Treatment with GW707 leads to the accumulation of free lysosomal cholesterol even in the absence of SCAP, which in turn disturbs appropriate cholesterol-mediated suppression of SREBP proteolytic processing (44).

Betulin, which is abundant in birch bark, suppresses SREBP activity by directly binding to SCAP and then stimulating an interaction between SCAP and Insig-1 that suppresses proteolytic processing of SREBPs (45).

Choi et al. showed that the small molecule diarylthiazole derivative fatostatin, formerly known as 125B11, inhibits insulin-induced adipogenesis in 3T3-L1 cells (46). Fatostatin inhibits proteolytic processing of SREBPs and binds SCAP. However, the mechanism by which fatostatin inhibits proteolytic processing of SREBPs remains unclear (47).

We performed luciferase reporter assays with promoter regions of SREBP target genes and found several small molecules that regulate SREBP activities. Among these, glutamine and resveratrol stimulated, and 4'-hydroxyflavanone suppressed SREBP activities (48–50). Glutamine is the most abundant free amino acid in the body that plays an important role in cell proliferation (51). Glutamine accelerates proteolytic processing of SREBPs by increasing PI3K/Akt-mediated transport of the SCAP/SREBPs complex from ER to the Golgi. Interestingly, a PI3K inhibitor, but not an mTORC1 inhibitor, blocked glutamine-stimulated proteolytic processing of SREBP-1 (48). As it is well known that activation of SREBP stimulates the synthesis of lipids, glutamine-mediated induction of lipid synthesis may be, at least in part, responsible for the effects of glutamine on cell proliferation. The polyphenol resveratrol is present in a variety of plant species and exhibits a wide range of health benefits. Although resveratrol increases the activity of AMPK and inhibits proteolytic processing of SREBP-1 (38), our data showed that resveratrol stimulates proteolytic processing of SREBPs (49). At present, this conflict of observations is unexplained. However, we also observed increased activity of AMPK in the presence of resveratrol, indicating stimulation of proteolytic processing of SREBPs under AMPK-activated conditions.

In vivo effects of small molecules that inhibit proteolytic processing of SREBPs

Fatostatin treatment produces several metabolic benefits in obese *ob/ob* mice (47). Among these, expression of lipogenic genes in the liver, activities of enzymes such as ACC and FAS, and liver triglycerides and cholesterol are significantly lowered by fatostatin. Interestingly, despite suppression of liver LDL receptor mRNA, which is an SREBP-2 target gene that mediates endocytosis of lipoproteins containing apolipoproteins B and E, particularly LDL, serum LDL cholesterol levels are significantly lowered in the presence of fatostatin. This discrepancy may be explained by reduced *de novo* synthesis of cholesterol and VLDL

secretion. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is another SREBP-2 target gene that stimulates LDL receptor protein degradation (52). Impairment of SREBP-2 activity by fatostatin may cause suppression of PCSK9 and LDL receptor gene expression, in turn stabilizing LDL receptor protein and reducing serum LDL cholesterol. Clinically, fatostatin prevents increases in body weight and blood glucose in obese *ob/ob* mice. Accordingly, fatostatin-treated *ob/ob* mice have increased serum ketone bodies, indicating a significant increase in fatty acid oxidation.

The effects of betulin on atherosclerosis and type II diabetes were investigated in Western diet-fed mice (45). In this study, dietary betulin reduced nuclear SREBP-1 and lipogenic gene expression in the livers of the mice, indicating that proteolytic processing of SREBPs is inhibited by betulin *in vivo*. Treatment with betulin led to decreased triglycerides and cholesterol in the liver, decreased serum LDL cholesterol, and increased serum high-density lipoprotein cholesterol. While LDL receptor gene expression was not altered, other SREBP-2 target genes such as HMG-CoA synthase, HMG-CoA reductase, and PCSK9 were suppressed by betulin in the liver. Hence, betulin improved diet-induced insulin resistance and ameliorated atherosclerosis in LDL receptor-deficient mice.

Small molecules that block proteolytic processing of SREBPs, such as fatostatin and betulin, exhibit excellent efficacy against diet-induced obesity, insulin resistance, and atherosclerosis (Figure 4). However, both compounds produced additional effects that were not related to suppression of SREBPs. In particular, treatment with fatostatin caused increases in fatty acid oxidation and dietary betulin stimulates adiponectin mRNA expression in white adipose tissue through unknown mechanisms. In addition to improvements in lipid metabolism, suppression of SREBPs activities may indirectly lead to further beneficial effects. In any case, it is clear that small molecules that suppress SREBPs are promising therapeutic agents for metabolic diseases.

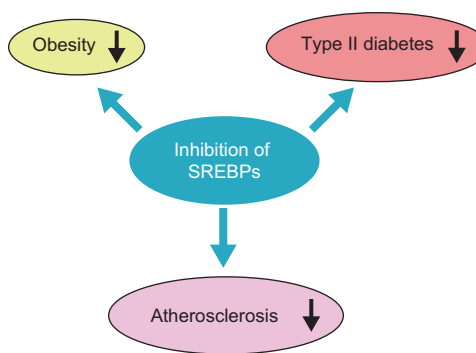


Figure 4 Inhibition of SREBP activity is a promising therapeutic target for metabolic diseases.

Conclusion

SREBPs are transcription factors that regulate the expression of genes involved in lipid metabolism after proteolytic processing. However, further studies are urgently required to precisely determine the mechanisms of proteolytic processing of SREBPs. In particular, little is known of regulatory differences between SREBP-1 and SREBP-2, despite knowledge of their target gene specificity. With the exception of unsaturated fatty acids, the small molecules described above equally regulate proteolytic processing of SREBP-1 and SREBP-2. However, further studies will be required to determine whether SREBPs isoforms are differentially regulated at the proteolytic processing level and to define the mechanisms involved.

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