

Transcriptional Control of Hepatic Lipid Metabolism by SREBP and ChREBP

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Abstract

The liver is a central organ that controls systemic energy homeostasis and nutrient metabolism. Dietary carbohydrates and lipids, and fatty acids derived from adipose tissue are delivered to the liver, and utilized for gluconeogenesis, lipogenesis, and ketogenesis, which are tightly regulated by hormonal and neural signals. Hepatic lipogenesis is activated primarily by insulin that is secreted from the pancreas after a high-carbohydrate meal. Sterol regulatory element binding protein-1c (SREBP-1c) and carbohydrate-responsive element-binding protein (ChREBP) are major transcriptional regulators that induce key lipogenic enzymes to promote lipogenesis in the liver. Sterol regulatory element binding protein-1c is activated by insulin through complex signaling cascades that control SREBP-1c at both transcriptional and posttranslational levels. Carbohydrate-responsive element-binding protein is activated by glucose independent of insulin. Here, the authors attempt to summarize the current understanding of the molecular mechanism for the transcriptional regulation of hepatic lipogenesis, focusing on recent studies that explore the signaling pathways controlling SREBPs and ChREBP.

Keywords

- ▶ SREBP
- ▶ ChREBP
- ▶ gene expression
- ▶ lipid metabolism
- ▶ steatosis

Mammals adapt to the fluctuation of nutrient availability by storing surplus nutrient mainly in adipose tissue in the form of triglyceride (TG). Ingestion of carbohydrates stimulates the conversion of carbohydrate into TG in the liver, which is followed by the mobilization of TG from the liver to adipose tissue for long-term storage. Increased glucose levels in the circulation after a high-carbohydrate meal activate hepatic lipogenesis through multiple mechanisms. Pancreatic hormones, glucagon, and insulin play central roles in the regulation of both glucose and lipid metabolism. Glucose triggers insulin secretion from pancreatic β cells, which stimulates glucose uptake and utilization, and promotes glycogen synthesis and lipogenesis in the liver. Insulin also suppresses hepatic glucose production, fat oxidation, and ketogenesis, shifting the balance to fat storage. Glucose itself also acts as a signaling molecule to regulate the genes encoding important enzymes in glycolysis and lipogenesis.¹

Metabolic and hormonal cues such as glucose, insulin, and glucagon regulate the gene-expression program of glycolysis and lipogenesis via transcription factors. Sterol regulatory

element binding protein-1c (SREBP-1c) is considered the master transcriptional regulator of fatty acid and TG synthesis in response to insulin stimulation. SREBP-1c is expressed at a low level in the liver of fasted animals, but is dramatically induced upon feeding, which is mediated by insulin.^{2,3} The function of SREBP-1c is also activated by insulin at the posttranslational level. Activated SREBP-1c binds to SRE (sterol regulatory element) sequences found on the promoters of its target genes as a homodimer. Sterol regulatory element binding protein-1c induces mRNAs encoding enzymes catalyzing various steps in the fatty acid and TG synthesis pathway, such as ATP-citrate lyase (ACL), acetyl-CoA synthetase (ACS), acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), stearoyl-CoA desaturase-1 (SCD1), and glycerol-3-phosphate acyltransferase (GPAT).^{2,4,5}

Carbohydrate-responsive element-binding protein (ChREBP) has been recognized as a transcription factor that is activated by high glucose independent of insulin, and plays a key role in glycolysis and lipogenesis.¹ It induces L-type pyruvate kinase (L-PK), ACC, and FAS genes by directly

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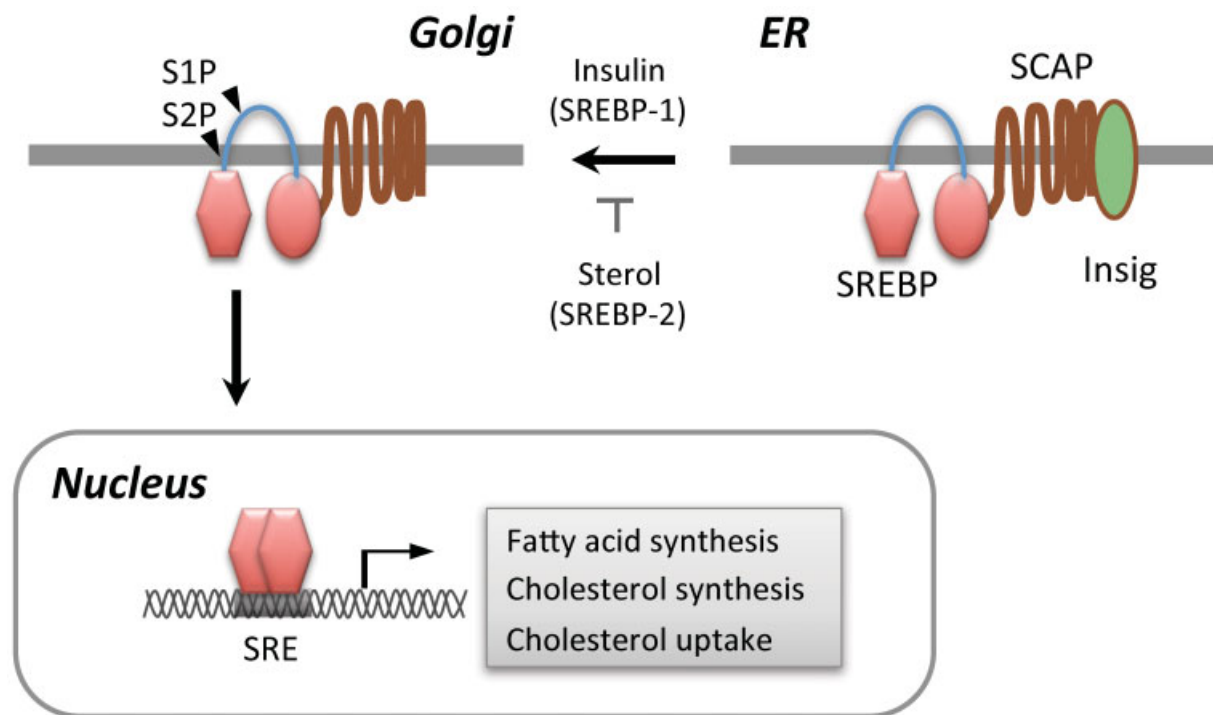


Fig. 1 Schematic illustration of the proteolytic activation of sterol regulatory element binding proteins (SREBPs). Sterol regulatory element binding proteins are synthesized as endoplasmic reticulum-(ER-) anchored precursor forms. Low cellular sterol concentrations trigger the release of the SREBP cleavage activating protein- (SCAP- SREBP-2 complex from Insig. Insulin stimulates the transport of SREBP-1c to the Golgi. Sterol regulatory element binding protein is sequentially cleaved by S1P and S2P proteases in the Golgi apparatus. The processed SREBP enters the nucleus to activate the transcription of genes regulating fatty acid and cholesterol metabolism.

binding to carbohydrate response elements (ChoRE) found in their promoters.⁶⁻⁸ Carbohydrate-responsive element-binding protein is a bZIP transcription factor that forms a heterodimeric complex with another bZIP protein, Max-like protein X (MLX).⁹

During recent years, significant advancement has been made in our understanding of the mechanisms by which SREBP and ChREBP are activated in the liver and regulate lipid metabolism. In this review, we will focus on recent studies that provide new insights into the transcriptional regulation of hepatic lipid metabolism.

SREBP Transcription Factors

Sterol regulatory element binding proteins are major transcription factors that regulate the expression of genes involved in fatty acid, TG, and cholesterol metabolism in the liver.¹⁰⁻¹² The SREBP family consists of SREBP-1a, SREBP-1c, and SREBP-2.^{13,14} Both SREBP-1a and SREBP-1c are encoded by a single gene, but are transcribed by different promoters, producing similar proteins that differ only in the N-terminal region.¹⁴ Whereas SREBP-1c is the predominant isoform expressed in the liver, SREBP-1a is produced in certain cell types in the immune system as well as in cultured cell lines.^{14,15} Although there is some functional overlap between different isoforms, SREBP-1c is mostly responsible for the expression of genes involved in fatty acid biosynthesis, whereas SREBP-2 activates cholesterol metabolism genes.¹⁰

Sterol regulatory element binding proteins are synthesized as precursor forms containing two transmembrane helices that anchor the protein in the endoplasmic reticulum (ER) membrane (►Fig. 1).¹⁶ They are associated with the SREBP cleavage activating protein (SCAP) and ER retention protein called Insig.¹⁷ To be activated, the SREBP-SCAP complex should be dissociated from Insig, associate with COPII-coated vesicles, and then migrate to the Golgi apparatus.^{18,19} Sterol regulatory element binding proteins are sequentially cleaved by site 1 (S1P) and site 2 (S2P) proteases in the Golgi, which releases the N-terminal cytosolic portion of the protein that enters the nucleus to act as the active transcription factor.¹⁰

Regulation of SREBP Activation by Proteolytic Cleavage

SREBP cleavage activating protein is a polytopic protein containing eight transmembrane helices and seven loops.²⁰ Transmembrane helices 2 to 6 are required for the binding of SCAP to Insig.^{18,21,22} Cholesterol binds to SCAP in loop 1 located in the ER lumen, which triggers a conformational change in loop 6 facing the cytoplasm.^{18,21,22} This conformational change of SCAP precludes its interaction with COPII proteins, and hence suppresses the mobilization of the SCAP-SREBP-2 complex to the Golgi. When the ER cholesterol level is decreased, the SCAP-SREBP-2 complex binds to COPII vesicles to be transported to the Golgi for proteolytic

activation. SCAP responds to the changes in ER cholesterol concentration with high precision, such that small changes in the ER cholesterol levels from the threshold level (5%) abruptly turn on/off SCAP–SREBP-2 association with COPII and the consequent SPREP-2 activation, enabling the precise regulation of SREBP-2 by cholesterol abundance.^{21,22}

It is well known that insulin transcriptionally activates SREBP-1c in the liver.^{3,23} But it has been less clear whether insulin also stimulates proteolytic processing of SREBP-1c because it is technically difficult to distinguish the contribution by the transcriptional activation and the proteolytic processing to the increased nuclear SREBP-1c level in response to insulin stimulation. To distinguish the effect of insulin on SREBP-1c processing from the transcriptional activation of SREBP1c mRNA, Hegarty et al pretreated rat hepatocytes with the LXR α agonist TO-901317 before adding insulin.²⁴ TO-901317 induced SREBP-1c mRNA, which was not further increased by insulin. Under such conditions, insulin significantly increased the processed nuclear SREBP-1c protein, indicating that insulin stimulated SREBP-1c processing. Similarly, insulin increased the nuclear processed SREBP-1c level in hepatocytes infected with SREBP-1c adenovirus or in transgenic rat liver that expressed human SREBP-1c under control of the apoE promoter.^{25,26} In contrast, insulin did not increase SREBP-2 processing, highlighting the specific role of insulin in SREBP-1c processing.²⁵ These independent studies clearly demonstrate that insulin not only activates SREBP-1c transcription, but also stimulates SREBP-1c processing.

How does insulin stimulate SREBP-1c processing? Stimulation of SREBP-1c processing by insulin was inhibited by small molecule inhibitors of phosphoinositide 3-kinase (PI3K), Akt, and mammalian target of rapamycin (mTOR) complex 1 (mTORC1), and p70 ribosomal S6 kinase (p70S6K), indicating that the PI3K/mTOR signaling pathway plays a critical role in SREBP-1c processing.^{24–27} It has been proposed that Akt directly phosphorylates SREBP-1c, which increases the affinity of SCAP–SREBP-1c complex for Sar1/Sec23/24 proteins of COPII-coated vesicles and facilitates the Golgi transportation of SREBP-1c.²⁵ Interestingly, insulin strongly suppresses the expression of Insig-2a, which is the major Insig isoform expressed in the liver.²⁸ The Insig2 gene has two different promoters, from which Insig-2a and Insig-2b mRNAs are transcribed. These two transcripts differ in noncoding exon 1, and hence produce same protein. Suppression of Insig-2a requires Akt activation, and involves mRNA destabilization.^{27,29} It is notable that Insig-2a preferentially interacts with the SCAP–SREBP-1c complex, while Insig-1 binds to SCAP–SREBP-2.²⁹ Hence, it is conceivable that insulin selectively activates SREBP-1c processing through two distinct mechanisms that involve the suppression of Insig-2a and the induction of SREBP-1c phosphorylation, which facilitates the association of SCAP–SREBP-1c complex with COPII-coated vesicles (–Fig. 2). However, the precise mechanism by which insulin stimulates SREBP-1c processing remains to be further investigated. For example, although SREBP-1c activation correlates well inversely with the Insig-2a level, it is not known if the disappearance of Insig-2a protein precedes SREBP-1c

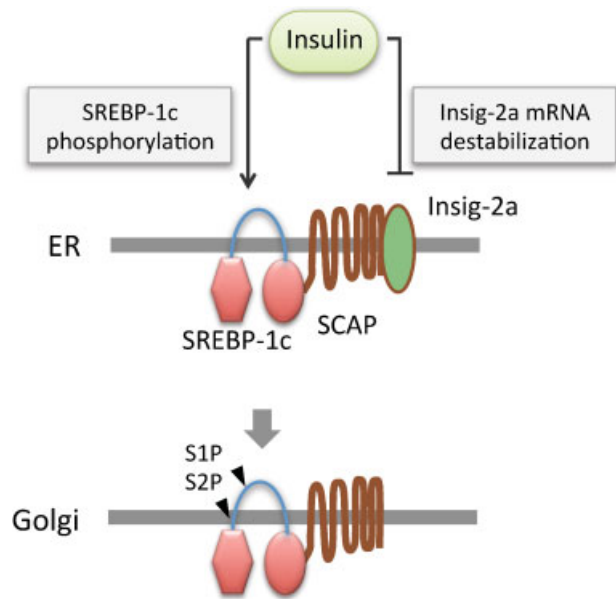


Fig. 2 Insulin promotes sterol regulatory element binding protein 1c (SREBP-1c) processing. Insulin induces AKT-mediated SREBP-1c phosphorylation, which stimulates the transport of SREBP-1c–SCAP complex to the Golgi apparatus. Insulin also induces the degradation of Insig-2a mRNA to promote the Golgi transport and proteolytic processing of SREBP-1c.

activation. Furthermore, Insig-2a is strongly induced by the LXR α agonist TO-901317, but does not suppress insulin-mediated SREBP-1c processing, suggesting that Insig-2a downregulation is not a prerequisite for SREBP-1c processing.²⁴ It is possible that the decreased expression of Insig-2a contributes to SREBP-1c processing upon chronic insulin stimulation, while acute insulin stimulation activates SREBP-1c processing through a distinct mechanism that does not involve Insig-2a downregulation.

Regulation of SREBP Activation by Nuclear Translocation

Although the processed SREBPs contain a nuclear localization signal in the HLH-Zip domain that mediates spontaneous import of the protein to the nucleus,³⁰ a recent study suggests that the nuclear entry of processed SREBP-1 and SREBP-2 could be regulated by mTORC1.³¹ Lipin 1, a phosphatidic acid phosphatase, is an mTORC1 substrate. Dephosphorylation of lipin 1 by mTOR inhibitor treatment triggers the entry of lipin 1 into the nucleus. Interestingly, dephosphorylated nuclear lipin 1 inhibits nuclear localization of SPREBPs in NIH-3T3 cells. In the presence of lipin 1 in the nucleus, SREBPs appear to localize to the perinuclear area in proximity to the nuclear matrix component lamin A. A lipin 1 construct carrying a mutation in the mTORC1 phosphorylation site suppressed the nuclear entry of processed SREBP-1c and the expression of lipogenic target genes, indicating that lipin-1 suppresses the transcriptional function of SREBPs by suppressing their nuclear localization. Lipin 1 appears to be critically involved in SREBP regulation by mTORC1 in mouse liver as well because

lipin 1 silencing restored lipogenic gene expression in the liver of Raptor knockout mice, where mTORC1 was inactivated. Lipin 1 also regulates fatty acid metabolism through other mechanisms. Lipin 1 dephosphorylates phosphatidic acid to produce diacylglycerol.^{32,33} It also stimulates fatty acid oxidation in concert with peroxisome proliferator-activated receptor α (PPAR α) and its coactivators.³⁴ Further studies will reveal the significance of lipin 1 regulation of SREBP in hepatic lipid metabolism under various pathophysiological conditions.

Transcriptional Regulation of SREBP-1c

Hepatic SREBP-1c mRNA level is dynamically regulated by nutritional status. SREBP-1c mRNA expression in liver is suppressed in fasted animals, and highly induced by ingestion of a high carbohydrate diet.² Expression of SREBP-1c was suppressed in diabetic rats induced by streptozotocin treatment, but normalized by insulin injection, indicating that insulin mediates the induction of SREBP-1c mRNA by carbohydrate diet ingestion.²³ Insulin strongly induces SREBP-1c mRNA in cultured hepatocytes.^{14,35} In contrast, glucagon suppresses SREBP-1c mRNA expression via the cyclic adenosine 3',5'-monophosphate/protein kinase A signaling pathway.^{35,36}

The engagement of insulin with its cell surface receptor induces the phosphorylation of the scaffolding protein family

insulin-receptor substrates (IRS), which then initiates a signaling cascade that culminates with the transcriptional suppression of gluconeogenesis and the activation of lipogenesis (**► Fig. 3**).^{37,38} Tyrosine phosphorylation of IRS by the insulin receptor recruits phosphoinositide-3-kinase (PI3K), which then phosphorylates phosphatidylinositol (4,5) bisphosphate (PtdIns(4,5)P₂) to produce Ptd(3,4,5)P₃ (PIP₃). As a phospholipid second messenger, PIP₃ recruits the Ser/Thr kinase AKT to the plasma membrane, where it is phosphorylated by 3-phosphoinositide-dependent protein kinase-1 (PDK1) to be activated. Consequently, active AKT phosphorylates a wide range of downstream targets involved in cell metabolism, such as forkhead box protein O1 (Foxo1), glycogen synthase kinase 3 (GSK3), and tuberous sclerosis 2 (TSC2) within the TSC1-TSC2 complex.^{39,40} Tuberous sclerosis 2 is a critical regulator of the mammalian TOR (mTOR) complex 1 (mTORC1), which plays a central role in cell growth and metabolism.^{41,42} Phosphorylation of TSC2 by AKT results in the activation of mTORC1, as the phosphorylated TSC2 no longer inhibits the Ras homolog enriched in brain (Rheb) protein that is critically required for mTORC1 functions.⁴⁰

Recently, mTORC1 has emerged as an important regulator of SREBP-1c that activates both SREBP-1c transcription^{26,43,44} and the proteolytic processing in response to insulin stimulation.^{24–27} Suppression of mTORC1 activity by rapamycin inhibited the expression of SREBP-1c and lipogenic genes regulated by SREBP-1c in the livers of rodents subjected to

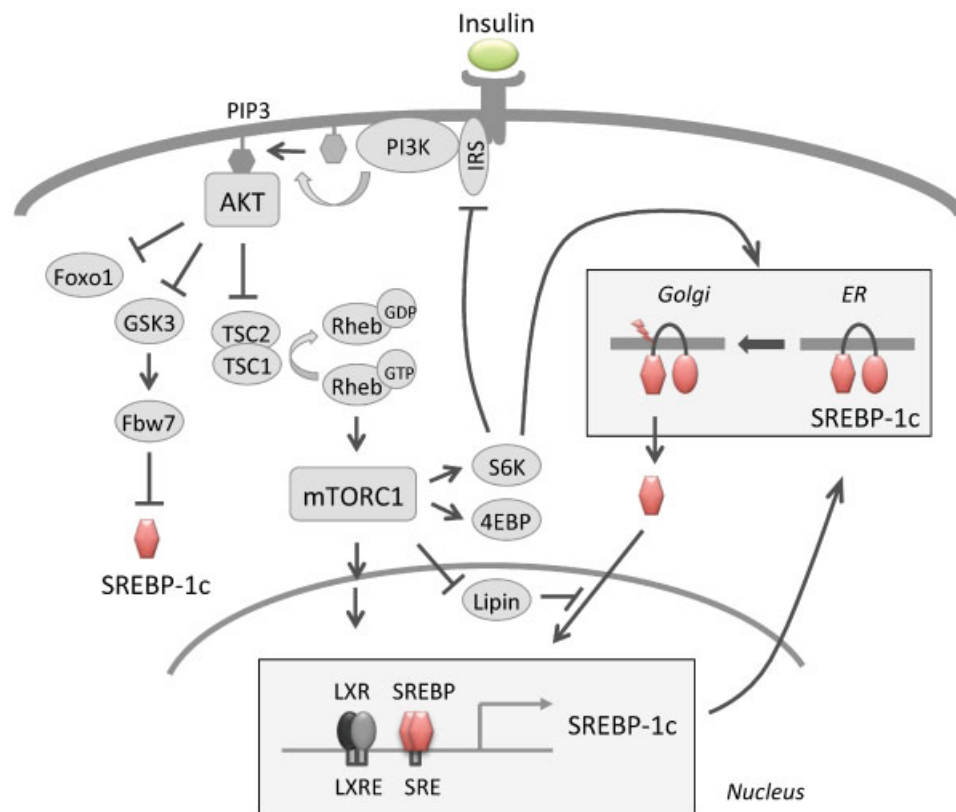


Fig. 3 Regulation of sterol regulatory element binding protein (SREBP) by the insulin signaling pathway. Insulin activates SREBP-1 through multiple mechanisms. Insulin stimulates SREBP-1c transcription, promotes proteolytic processing, facilitates the nuclear import of the processed protein, and suppresses the proteasomal degradation of SREBP-1.

fasting followed by refeeding with a high-carbohydrate diet, uncovering a critical role of the mTORC1 pathway in the insulin-induced lipogenesis program.⁴³ Similarly, insulin-induced SREBP-1c mRNA expression was abolished by rapamycin or small molecule inhibitors of PI3K or AKT that block mTORC1 activation in cultured rat hepatocytes.⁴³ mTORC1 protein kinase directly phosphorylates two major downstream targets, initiation factor 4E-binding protein (4E-BP) and p70 ribosomal S6 kinase (p70S6K), increasing mRNA translation.^{41,45,46} p70S6K has a growing number of downstream targets in addition to the ribosomal protein S6.^{47,48} Notably, a p70S6K inhibitor had no effect on insulin-induced SREBP-1c mRNA expression,⁴³ but inhibited the proteolytic processing of SREBP-1c protein,²⁶ indicating that mTORC1 regulates SREBP-1c mRNA expression and protein processing through distinct mechanisms.

Although the acute inhibition of mTORC1 activity suppresses insulin-induced SREBP-1c mRNA expression and lipogenic gene expression, constitutive activation of mTORC1 ironically suppresses SREBP-1c activation.²⁷ Genetic ablation of TSC1 in the liver caused constitutive activation of mTORC1, but suppressed age- and diet-induced hepatic steatosis, possibly due to defective SREBP-1c expression. This unexpected phenotype reflects the complex feedback regulation of the insulin signaling pathway leading to SREBP-1c activation.^{12,40} It has been well established that mTORC1 negatively regulates insulin signaling at multiple steps of the signaling cascade as feedback mechanisms.^{48–50} Indeed, AKT was markedly suppressed in TSC1 deficient hepatocytes, which might contribute to the decreased expression of SREBP-1c and lipogenic genes. An important question that remains to be answered is what ultimately increases SREBP-1c transcription in response to insulin stimulation. So far, liver X receptors (LXRs) and SREBP-1c itself are known to activate SREBP-1c promoter.^{51–55} The relative contribution of LXR and SREBP-1c in the insulin-induced transcriptional activation of SREBP-1c remains to be further investigated.

Liver X receptors are members of the nuclear hormone receptor superfamily that play a critical role in cholesterol efflux, excretion, and absorption.⁵⁶ LXR α also plays an important role in fatty acid and triglyceride synthesis, as it induces SREBP-1c expression via an LXR response element on its promoter.^{51,53,57} The lipogenic activity of LXR α was abrogated in SREBP-1c deficient mice, indicating that LXR promotes lipogenesis through SREBP-1c.⁴ LXR α – deficient mice exhibit reduced expression of SREBP-1c and lipogenic genes such as SCD1 and FAS in the liver.^{53,54,58,59} In contrast, a high-cholesterol diet or the LXR α agonist TO-901317 increase SREBP-1c and stimulate lipogenesis in the liver.^{53,54,58,59} Importantly, disruption of LXR-binding sites on the SREBP-1c promoter abolished the induction of promoter activity by insulin or TO-901317, suggesting that LXR α is responsible for SREBP-1c induction in response to insulin.⁵¹ However, it is not known how insulin activates LXR α . The role of LXR α in the insulin signaling cascade appears to be specific to SREBP-1c because insulin does not induce other LXR α target genes.^{60,61} It has been reported that insulin modestly increases LXR α mRNA in cultured rat hepatocytes.⁶² It is also possible that

insulin stimulates the production of LXR α ligands to activate LXR α .

Regulation of SREBP Protein Stability

The nuclear form of SREBP protein is highly unstable, as it is degraded via ubiquitin-dependent proteasomal degradation pathways.^{63,64} Treatment of proteasome inhibitors increases the amount of nuclear SREBP, but not the precursor form, indicating that only the processed nuclear forms of SREBPs are subjected to proteasomal degradation. Ubiquitination and degradation of SREBPs are closely associated with their transcriptional activities.⁶⁵ Inhibition of transcriptional activity of SREBPs by mutating critical functional domains, or by treating with RNA polymerase inhibitor prevented the degradation of SREBPs. It is conceivable that the proteasomal degradation of SREBPs portrays a feedback regulation of SREBP activity to fine tune the transcriptional response of lipogenesis. Ubiquitination of SREBP could be suppressed by SREBP coactivators, CREB-binding protein (CBP) and p300, which competitively acetylate the lysine residue that is also targeted by ubiquitination, leading to stabilization of SREBP and the induction of SREBP target genes (LDLR, HMG-CoA reductase) and sterol synthesis.⁶⁶

Phosphorylation of SREBP is critically required for its ubiquitination. F-box and WD repeat domain-containing 7 (Fbw7) is a cullin-RING type E3 ubiquitin ligase that has emerged as the major ubiquitin ligase for SREBPs. Phosphorylation of SREBP induces its interaction with Fbw7, and thus facilitates its ubiquitination and degradation.^{67,68} GSK3 phosphorylates SREBP-1a at T426 and S430 residues, which resemble Cdc4 phosphodegron (CPD) motif, a recognition site for Fbw7. SREBP-1c and SREBP-2 are also similarly ubiquitinated by Fbw7. DNA binding of SREBP facilitates the recruitment of GSK3 to the promoter, and the subsequent interaction between SREBP1 and GSK3.⁶⁹ Insulin regulates the stability of SREBP by controlling its phosphorylation by GSK3 and interaction with Fbw7.⁷⁰ Insulin-mediated AKT activation induces Ser-9 phosphorylation of GSK3, leading to the suppression of its kinase activity.⁷¹ Consequently, insulin suppresses SREBP phosphorylation and the following Fbw7-dependent degradation. Cyclin-dependent kinase 8 (CDK8) can also phosphorylate SREBP-1c, and thus trigger its ubiquitination by Fbw7 and proteasomal degradation.⁷² As CDK8 expression is suppressed by insulin, CDK8-triggered SREBP-1c ubiquitination/degradation would constitute a regulatory mechanism of the lipogenesis program. Indeed, knockdown of CDK8 in mouse liver increased SREBP-1c target genes (FAS, ACS, and SCD1) and hepatic triglyceride levels.⁷²

Fbw7 deficiency stabilizes nuclear SREBPs and enhances the expression of their target genes, leading to increased synthesis of fatty acids, TG, and cholesterol, and increased receptor-mediated uptake of low-density lipoprotein (LDL).⁷³ Liver-specific deletion of Fbw7 in vivo increased the expression of hepatic SREBP-1c and lipogenic genes, which was accompanied by massive lipid deposition and the occurrence of nonalcoholic steatohepatitis (NASH) in the mutant mice.⁷³ These findings establish Fbw7 as an important regulator of SREBP protein stability and lipid metabolism.

MicroRNA–SREBP Connection in Lipid Metabolism

MicroRNAs (miRNAs) are small nonprotein-coding RNAs of ~23 nt in length that are produced from longer primary miRNA transcripts via sequential processing by DROSHA and DICER ribonucleases.⁷⁴ miRNAs bind to the 3' untranslated regions of target mRNAs, and thereby either promote the degradation or suppress the translation of target mRNAs.⁷⁴ Given that a single miRNA can control the expression of multiple target genes in the same pathway, miRNAs have emerged as critical regulators of a variety of biological processes, including nutrient metabolism.^{75,76}

Interestingly, recent reports revealed that SREBP genes (SREBF1 and SREBF2) harbor miRNAs within introns that are consequently cotranscribed with the respective SREBP genes. In humans, miR-33a is located in intron 16 of the SREBF2 gene (encoding SREBP-2), and miR-33b is within intron 17 of the SREBF1 gene (encoding SREBP-1a and SREBP-1c).^{77–81} Mature miR-33a and miR-33b have similar nucleotide sequences and hence would be expected to regulate overlapping target mRNAs. Although miR-33a is evolutionarily conserved in multiple animal species, miR-33b exists in the human, but is absent in the rodent genome.^{79,81} Reminiscent of the critical role of SREBP proteins in lipid metabolism, miR-33a and miR-33b also regulate cholesterol and fatty acid homeostasis.^{77–81} miRNA target sequence analysis predicted that miR-33a and miR-33b target adenosine triphosphate-binding cassette A1 (ABCA1) mRNA, which encodes a cholesterol transporter that plays a crucial role in cholesterol efflux. Indeed, silencing or genetic ablation of miR-33 markedly increased ABCA1 expression both in cultured hepatocytes and macrophages, and increased plasma high-density lipoprotein (HDL) levels.^{77–82}

Given the beneficial effects of miR-33 antagonism in increasing plasma HDL levels, miR-33 inhibition aroused great interest as a potential therapeutic approach to treat cardiovascular diseases.⁸³ Indeed, Rayner et al demonstrated that anti-miR-33 treatment promoted reverse cholesterol transport and reduced atherosclerotic plaques in LDL-receptor knockout (*Ldlr*^{-/-}) mice.⁸⁴ Similarly, genetic loss of miR-33 in ApoE null mice increased circulating HDL-cholesterol levels and reduced plaque size.⁸⁵ A recent study in nonhuman primates also reported the increase of HDL cholesterol by anti-miR-33 therapy, highlighting the strong potential of anti-miR-33 as a new therapy for coronary heart disease.⁸⁶ However, subsequent independent studies using anti-miR-33 antisense oligonucleotides or locked nucleic acids reported somewhat inconsistent effects of miR-33 inhibition on HDL cholesterol levels and atherosclerotic lesion development in *Ldlr*^{-/-} mice.^{87,88} For example, both studies found that anti-miR-33 had no effect on HDL cholesterol levels in *Ldlr*^{-/-} mice fed a Western diet, although animals on a chow diet exhibited increased HDL cholesterol by anti-miR-33 treatment. Nonetheless, Rotllan et al demonstrated that anti-miR-33 therapy significantly reduced atherosclerotic lesions and macrophage infiltration.⁸⁸ In contrast, Marquart et al failed to detect any significant changes in the size or composition of atheroscle-

rotic plaques in anti-miR-33 treated mice, while plasma TG levels were significantly increased.⁸⁷ Further studies should address the effectiveness of anti-miR-33 treatment as a therapeutic approach, and identify the full spectrum of miR-33 target mRNAs.

A recent elegant study identified a pair of microRNAs that are transcriptionally induced by SREBPs, and in turn suppress SREBPs, constituting a negative feedback loop.⁸⁹ Sterol regulatory element binding proteins directly activate the transcription of a primary miRNA transcript that is processed to three miRNAs; miR-96, miR-182, and miR-183. Interestingly, miR-96 and miR-182 suppressed the expression of the processed SREBPs and the synthesis of fatty acids and cholesterol, suggesting that these miRNAs regulate the processing or stability of SREBPs. Target sequence analysis predicted that *Insig-2* and *Fbw7*, which regulate the processing and proteasomal degradation of SREBPs, might be regulated by miR-96 and miR-182, respectively. Indeed, miR-96 and miR-182 suppressed the synthesis of *Insig-2* and *Fbw7*, and increased the processed SREBP1 and SREBP2 protein levels. This study reveals a new layer of regulatory mechanisms in lipid metabolism.

Carbohydrate Response Element Binding Protein

Carbohydrate response element binding protein (ChREBP) was first identified as a glucose responsive transcription factor, which regulates glycolytic, gluconeogenic, and lipogenic gene expression.^{6,7} Transcriptional targets of ChREBP encode important enzymes in these pathways including L-pyruvate kinase (L-PK) for glycolysis, glucose 6 phosphatase catalytic subunit (G6PC) for gluconeogenesis, fatty acid synthase (FAS), acetyl coA carboxylase 1 (ACC1), and stearyl coA desaturase 1 (SCD 1) for lipogenesis.⁶ Carbohydrate-response elements (ChoREs) have been identified in promoters of these genes, which are composed of two E-box (CACGTG) or E-box-like sequences separated by five nucleotides.^{90,91} Carbohydrate response element binding protein and its interaction partner Max-like protein X (MLX) form heterodimers and bind to the ChoREs to induce the expression of its target genes (**Fig. 4**).^{92,93}

Carbohydrate response element binding protein contains two nuclear export signals and one nuclear localization signal near the N-terminal, proline-rich domains, a basic loop-helix-leucine-zipper (b/HLH/Zip), and a leucine-zipper-like (Zip-like) domain.^{7,94}

Posttranslational modification of ChREBP is required for its activation. The phosphorylation/dephosphorylation of ChREBP has been proposed to be important for ChREBP nuclear translocation and activation. Under basal conditions, like starvation or low-glucose concentrations, ChREBP is phosphorylated on Ser-196, Ser-626, and Thr-66 by cAMP-dependent protein kinase (PKA), on Ser568 by AMP-activated protein kinase (AMPK) and localized in the cytosol.^{8,95} Upon high-glucose stimulation, xylulose 5-phosphate (X5P), an intermediate of the pentose phosphate pathway, activates protein phosphatase 2A (PP2A) and dephosphorylates

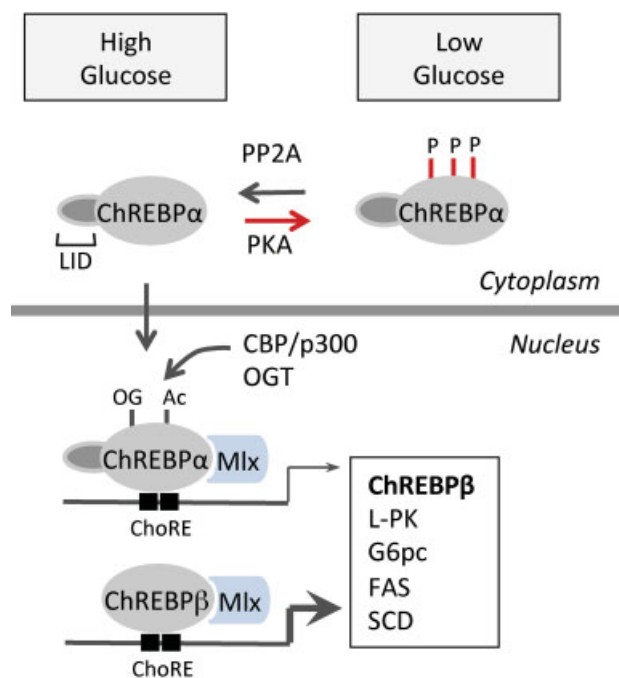


Fig. 4 Regulation of carbohydrate response element binding protein (ChREBP) activity. The phosphorylation/dephosphorylation of ChREBP α by PKA/protein phosphatase 2A (PP2A) is involved in ChREBP α nuclear translocation and activation. Acetylation by coactivator CBP/P300 and O-GlcNAcylation by O-GlcNAc transferase (OGT) also contribute to ChREBP α transcriptional activities. ChREBP α forms a heterodimer with Max-like protein X (MLX) and binds to the carbohydrate-response elements (ChoREs) in the nucleus to induce its target genes involved in glycolytic and lipogenic pathways. In adipose tissue, active ChREBP α induces expression of ChREBP β , a new ChREBP isoform that lacks the low-glucose inhibitory domain (LID), and is hence constitutively active regardless of glucose concentration.

ChREBP, allowing its translocation into the nucleus and activation.⁹⁶ However, some studies show that mutation of one or several PKA phosphorylation sites did not affect the responsiveness of ChREBP to high-glucose levels, suggesting a more complex mechanism could be involved.⁹⁷ Transactivity of ChREBP can also be modulated through acetylation on Lys672 by histone acetyl-transferase (HAT) and its coactivator p300⁹⁸ and by O-linked- β -N-acetylation.^{99,100}

Alternatively, intramolecular interaction has been proposed to be another way to modulate ChREBP activity. Carbohydrate response element binding protein contains a glucose-sensing module near the N-terminus, which consists of a low-glucose inhibitory domain (LID) and a glucose response activation conserved element (GRACE). Due to the inhibition of the LID domain on GRACE, ChREBP is restrained in an unfavorable conformation for DNA binding and activation, which is reversed by high glucose.^{94,101–103} In line with this model, deletion of the LID domain produced a constitutively active ChREBP even under low-glucose conditions.¹⁰² The involvement of the glucose-sensing module and conformational modulation has been implicated in the regulation of ChREBP activity by glucose metabolites, such as glucose 6 phosphate (G6P).^{104,105}

The mechanism of carbohydrate-mediated ChREBP activation may involve feed-forward regulation because changes

of ChREBP activity can also be reflected on ChREBP mRNA levels.^{106,107} Recently, the self-regulation of ChREBP in adipose tissue has been revealed with the discovery of a novel ChREBP isoform, ChREBP β .¹⁰⁸ ChREBP β is transcribed from an alternative promoter, differing from the previously identified ChREBP, or ChREBP α . ChREBP β protein does not contain LID and nucleus export signals, therefore exhibits constitutively higher transactivation ability than ChREBP α with increased nuclear localization,¹⁰² regardless of the glucose concentration.¹⁰⁸ ChREBP β expression was markedly increased by cotransfection of ChREBP α and LMX in a glucose dose-dependent manner. The ChoREs are also identified in the promoter region of ChREBP β and the deletion of these elements completely abolished the responsiveness of the ChREBP β promoter to ChREBP α /MLX.¹⁰⁸ Therefore, ChREBP α may be activated by high-glucose concentrations as previously reported, and induce ChREBP β expression as a feed-forward regulation. It remains to be determined if this feed-forward regulation of ChREBP also occurs in the liver and other tissues.

As ChREBP directly regulates genes involved in both glucose and lipid metabolism that indirectly influence each other, genetic manipulation of ChREBP expression *in vivo* results in rather complex metabolic changes. ChREBP $^{-/-}$ mice have impaired glycolytic and lipogenic pathways in the liver and show moderate glucose intolerance.⁶ Global or liver-specific deletion of ChREBP greatly ameliorated fatty liver diseases, and improved overall glucose tolerance and insulin sensitivity in *ob/ob* mice, possibly through decreasing *de novo* lipogenesis.^{109,110} Overexpression of ChREBP in the liver increased hepatic steatosis associated with the increased expression of genes regulating fatty acid and TG synthesis in the liver. Interestingly, ChREBP transgenic mice exhibited elevated monounsaturated fatty acids in the liver, which conferred improved glucose tolerance and insulin sensitivity upon high-fat-diet feeding despite greater hepatic steatosis.¹¹¹

Carbohydrate response element binding protein expression is induced during adipocyte differentiation, and by refeeding with a high-carbohydrate diet in adipose tissue, suggesting it might have a metabolic function in adipocytes.¹¹² Indeed, a recent study demonstrated that ChREBP regulates the *de novo* lipogenesis program in response to glucose flux in adipocytes, and that adipose tissue ChREBP levels correlate well with glucose tolerance and insulin sensitivity in humans.¹⁰⁸ Carbohydrate response element binding protein is also expressed in pancreatic β cells. Glucose stimulated the expression of ChREBP target genes in β cells,^{107,113} and activation of ChREBP promoted glucose stimulated β -cell proliferation.¹¹⁴

Concluding Remarks

In this review, we summarized the complex signaling network that controls the hepatic lipogenesis transcriptional program activated directly or indirectly by carbohydrate ingestion. Sterol regulatory element binding protein and ChREBP are major transcriptional regulators that are activated by carbohydrate signal, and stimulate *de novo* hepatic

lipogenesis. Recent studies revealed that AKT/mTORC1 signaling pathways are critically involved not only in the transcriptional activation, but also in the posttranslational processing of SREBP-1c. Uncontrolled de novo lipogenesis causes hepatic steatosis, which is closely associated with the onset of obesity, insulin resistance, and type 2 diabetes. Excessive lipogenesis induced by transgenic overexpression of SREBP-1, ingestion of a high-fructose diet, or leptin deficiency causes hepatic steatosis. Under insulin-resistant states, hyperinsulinemia can also activate SREBP-1 to induce hepatic steatosis, with the loss of insulin-mediated suppression of gluconeogenesis.¹¹⁵ On the other hand, inhibition of SREBP-1 could be a potential therapeutic approach to treat dyslipidemia and the metabolic syndrome. A better understanding of the signaling pathways controlling lipogenesis may lead to the identification of novel targets for metabolic diseases.

The role of ChREBP in sensing glucose and regulating nutrient homeostasis, especially lipid synthesis, is of great interest, considering its therapeutic potential in the treatment of diabetes and metabolic syndrome. With the discovery of ChREBP α and β isoforms, tissue-specific distributions of ChREBP should be more carefully considered when studying its regulation on lipid metabolism and systemic glucose homeostasis.

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