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SREBPs in Lipid Metabolism, Insulin Signaling, and Beyond

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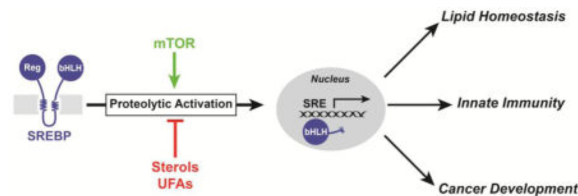
Abstract

SREBPs are a family of membrane-bound transcription factors that activate genes encoding enzymes required for synthesis of cholesterol and unsaturated fatty acids. SREBPs are controlled by multiple mechanisms at the level of mRNA synthesis, proteolytic activation, and transcriptional activity. In this review, we summarize the recent findings that contribute to the current understanding of the regulation of SREBPs and their physiologic roles in maintenance of lipid homeostasis, insulin signaling, innate immunity, and cancer development.

Graphical Abstract



Introduction of the SREBP pathway

The synthesis of cholesterol and unsaturated fatty acids (UFAs), two key components of animal cell membranes, is coordinated by the sterol regulatory element-binding protein (SREBP) family of transcription factors [1]. The three SREBP proteins are encoded by two different genes. The *SREBP-1* gene gives rise to SREBP-1a and SREBP-1c, which are derived from utilization of alternate promoters that yield transcripts in which distinct first exons are spliced to a common second exon. SREBP-2 is derived from a separate gene. SREBPs are synthesized as inactive precursors that are anchored to membranes of the endoplasmic reticulum (ER) (Figure 1) [2–5]. The N-terminal domain of SREBPs are basic helix-loop-helix-leucine zipper (bHLH-Zip) transcription factors, whereas the C-terminal regulatory domain mediates association with a membrane protein called Scap that plays a critical role in SREBP activation [6–8] (see below). The N- and C-termini of SREBPs project into the cytosol and are separated by a short loop that projects into the ER lumen [5].

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In lipid-deprived cells, Scap facilitates incorporation of SREBP into COPII-coated vesicle that bud from ER membranes and deliver cargo to the Golgi [9–11]. In the Golgi, SREBPs encounter two proteases: a serine protease called the Site-1 protease (S1P) [12, 13] and a zinc metalloprotease called Site-2 protease (S2P) [14]. S1P cleaves SREBPs within the lumenal loop, separating the protein into two membrane-bound halves (Figure 1) [15]. Upon Site-1 cleavage, the N-terminal half of SREBP becomes a substrate for S2P, which cleaves the protein three residues within the membrane [16]. This two-step cleavage process, which has been designated as regulated intramembrane proteolysis (RIP) [17], releases the transcriptionally active fragment of SREBP into the cytosol from which it migrates into the nucleus. Nuclear SREBPs bind to sterol regulatory element (SRE) sequences in the promoter of several genes encoding enzymes required for lipid synthesis and uptake [18]. SREBP-1a and SREBP-1c preferentially activates transcription of genes required for fatty acid and triglyceride synthesis, which includes acetyl CoA carboxylase, fatty acid synthase, stearoyl CoA desaturase, and glycerol 3-phosphate acyltransferase. On the other hand, SREBP-2 modulates transcription of genes required for cholesterol synthesis and uptake such as HMG CoA synthase, HMG CoA reductase, farnesyl diphosphate synthase, squalene synthase, and the low-density lipoprotein (LDL) receptor. The RIP of SREBPs results in increased synthesis and uptake of cholesterol and UFAs in lipid-deprived cells [19]. Here, we will summarize the recent understanding of the regulation of the SREBP pathway and discuss emerging data that establish new roles for the lipid-regulated pathway in inflammation and cancer.

Lipid-mediated regulation of the SREBP pathway

Although cholesterol and UFAs are key components of cellular membranes and serve as precursors for a variety of biologically active molecules, their synthesis must be tightly controlled. The overaccumulation of cholesterol and UFAs can be toxic at both the level of the individual cell and the whole animal. Homeostatic control of these lipids is achieved in part, through transcriptional and post-transcriptional mechanisms that modulate the SREBP pathway.

Transcriptional regulation of SREBPs

In the liver, a major site of cholesterol and UFA synthesis, the SREBP pathway is subject to transcriptional regulation through several mechanisms. The genes encoding SREBP-1c and SREBP-2, the predominant SREBP isoforms expressed in the liver, contain SREs within their promoters that mediate feed-forward transcriptional regulation [20, 21]. The significance of this regulation is highlighted by the observation that mRNAs encoding SREBPs are elevated in livers of animals that express constitutively activated SREBPs [18]. Conversely, SREBP mRNAs are reduced in livers of mice that are deficient in S1P or Scap [18, 22, 23].

Feed-forward regulation of SREBPs also leads to the expression of two closely-related microRNAs designated miR-33a and miR-33b that are encoded within introns of the human SREBP-2 and SREBP-1 genes, respectively [24, 25]. These two microRNAs target the mRNA for ABCA1, an ATP-binding cassette transporter that mediates efflux of cholesterol

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from cells to lipid-poor apolipoproteins (apoA1 and apoE), which then form nascent high-density lipoproteins (HDLs) [26]. Thus, feed forward transcription not only increases levels of SREBP precursors for proteolytic activation and enhanced synthesis of cholesterol, but also reduces efflux of newly synthesized cholesterol from cells by inhibiting expression of ABCA1 through the action of miR-33a and miR-33b.

A second mechanism for transcriptional control of SREBPs is mediated by Liver X receptors (LXR)-α and -β, nuclear receptors that heterodimerize with retinoic X receptors [27]. LXRs become activated by a variety of sterols including oxysterols, derivatives of cholesterol that contain a hydroxyl group in the side chain, and selectively enhance transcription of the SREBP-1c gene in mice [27, 28]. In livers of mice deficient for LXRα and LXRβ, synthetic agonists of LXR no longer enhance expression of SREBP-1c and FA biosynthetic genes [27]. Moreover, FA biosynthetic genes are not activated in livers of SREBP-1c knockout mice treated with LXR agonists or fed a high cholesterol diet [29]. LXR-mediated regulation of SREBP-1c ensures continued synthesis of FAs that are utilized in the esterification of free cholesterol, protecting cells from toxic effects of its overaccumulation. It should be noted that UFAs exert feedback inhibition on the expression of SREBP-1c by antagonizing the action of LXR [30], thereby guarding against their overaccumulation.

Lipid-mediated regulation of proteolytic activation of SREBPs

In addition to its role in ER-to-Golgi transport of SREBPs, Scap is the target for sterol-mediated suppression of the reaction [6]. This suppression is mediated by the hydrophobic N-terminal domain of Scap, which contains 8 transmembrane helices separated by 7 loops and precedes a cytosolic C-terminal domain with multiple WD-repeat sequences that mediate association with SREBPs [7]. Current models suggest that in the sterol-deprived state, Scap assumes a conformation in which two large lumenal loops (designated Loops 1 and 7) associate, allowing COPII proteins to bind to a hexapeptide MELADL sequence in cytosolic Loop 6 [31–33] (Figure 2). When cholesterol accumulates to more than 5 mol% of ER lipids, it binds to Loop 1 of Scap and this causes a conformational change in a region of Scap called the sterol-sensing domain (SSD), which encompasses transmembrane helices 2–6 [32, 34–36]. As a result of this conformational change, Scap binds to ER membrane proteins called Insig-1 and Insig-2 [37, 38] (Figure 2). Cholesterol-induced binding of Insig to the SSD of Scap triggers dissociation of Loop 1 from Loop 7, inducing a second conformational change that prevents binding of COPII to the MELADL sequence and abolishes transport of Scap to the Golgi [11, 31–33, 39] (Figure 2). These two conformational changes in Scap are experimentally separable. However, it should be noted that the first, cholesterol-induced conformational change in Scap occurs regardless of the presence or absence of Insigs. In contrast, the second conformational change, which prevents binding of Scap to COPII and Golgi transport, requires the interaction with Insigs.

Oxysterols are derivatives of cholesterol that contain a hydroxyl group in the side chain, which increase the solubility of the molecules in aqueous solution. The oxysterol 25-hydroxycholesterol (25-HC) potently stimulates binding of Scap to Insigs and blocks proteolytic processing of SREBPs. However, 25-HC and other oxysterols fail to bind to

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Scap, indicating they block SREBP processing through a mechanism distinct from that of cholesterol [34, 40]. Indeed, subsequent studies revealed that 25-HC directly binds to Insigs *in vitro* [41]. This binding was competitively inhibited by other oxysterols that block SREBP processing, but not by inactive oxysterols [41]. Mutations in Insigs that abolish binding to 25-HC also prevent the protein from binding to Scap and promoting its retention in ER membranes. Although sensing of cholesterol and oxysterols occurs through distinct mechanisms, both reactions result in binding of Insigs to Scap and sequestration of the MELADL sequence from exposure to COPII proteins.

UFAs, but not saturated FAs, inhibit proteolytic activation of SREBPs through a mechanism distinct from that of sterols. In cells deprived of UFAs, Insig-1 associates with gp78, an E3 ubiquitin ligase that facilitates its ubiquitination [42]. Insig-1 also binds to Ubxd8, a membrane-bound protein containing a UAS domain that binds to UFAs and a UBX domain that associates with the ER-associated degradation (ERAD) co-factor p97 (Figure 3) [43]. In cells depleted of UFAs, ubiquitination and p97 recruitment lead to the rapid ERAD of Insig-1 by 26S proteasome, which renders sterols less effective in inhibiting cleavage of SREBP [43]. UFAs, but not saturated FAs, bind to the UAS domain of Ubxd8, trigger polymerization of the protein [44]. This polymerization causes Ubxd8 to dissociate from Insig-1, thereby stabilizing the protein. Stabilized Insig-1 then binds to the Scap/SREBP complex and retains it in the ER to block SREBP activation (Figure 3) [43]. In cultured cells, Insig-2 is not rapidly degraded and its stability is not influenced by UFAs [43, 45].

It should be noted that proteolytic activation of SREBP-1 and SREBP-2 is inhibited by both sterols and UFAs. However, SREBP-1 activation appears to be more responsive to UFA-mediated inhibition than that of SREBP-2, which is more responsive to sterols (28). This phenomenon is also observed in the mouse liver. For example, cholesterol feeding has a more pronounced effect in blocking activation of hepatic SREBP-2 than SREBP-1 [46]. In mice deficient for endogenous UFAs, levels of nuclear SREBP-1 was increased 4-fold [47]; levels of nuclear SREBP-2 remained constant in these animals. When UFAs are restored by dietary supplementation, nuclear SREBP-1 levels decline to levels similar to that in chow-fed wild type counterparts.

Insulin-mediated regulation of the SREBP pathway

Insulin-mediated regulation of SREBP-1c transcription

The hormone insulin activates transcription of genes encoding FA biosynthetic enzymes in the liver. Transcription of these genes declines when mice are fasted, which suppresses insulin levels, and elevated following refeeding, which restores insulin levels. Importantly, insulin-induced activation of FA synthetic genes is abolished in livers of mice lacking SREBP-1c or Scap [22, 29]. Subsequent studies using freshly isolated rat hepatocytes and various inhibitors of the insulin signaling pathway revealed that insulin activates transcription of SREBP-1c through the mammalian target of rapamycin (mTOR), but not its substrate S6 Kinase-1 (S6K1) (Figure 4) [48]. In addition, insulin-mediated induction of SREBP-1c transcription requires LXR in complex with C/EBPβ [49]. Insulin does not appear to promote formation of the LXR-C/EBPβ complex, but rather activates the complex bound to LXR elements in the SREBP-1c promoter. It remains to be established whether

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mTOR-dependent phosphorylation leads to recruitment of an LXR-C/EBPβ coactivator or production of an LXR ligand to activate SREBP-1c transcription.

The mTOR complex also regulates the transcriptional activity of SREBPs through its phosphorylation of lipin-1 [50], a phosphatidate phosphatase involved in production of diacylglycerol during synthesis of triglycerides and phospholipids [51]. Lipin-1 also functions in the nucleus as a transcriptional co-regulator. The mTOR-mediated phosphorylation of lipin-1 prevents its nuclear localization [50]. In the absence of insulin, lipin-1 is no longer phosphorylated and migrates to the nucleus where it promotes down-regulation of nuclear SREBP-1 and SREBP-2 transcriptional activity (Figure 4). The mechanism for lipin-1-mediated inhibition of SREBP transcriptional activity is unknown.

Insulin-mediated regulation of SREBP-1c proteolysis

In addition to stimulating transcription of the SREBP-1c gene, insulin stimulates processing of the membrane-bound SREBP-1c precursor. This conclusion was drawn from studies employing transgenic rats that express epitope-tagged SREBP-1c under control of a constitutive liver-specific promoter, which allows effects of insulin on processing to be differentiated from those of the hormone on transcription of SREBP-1c. Characterization of these transgenic rats revealed that mTOR mediates the effects of insulin on processing of SREBP-1c [52]. In contrast to the induction of SREBP-1c mRNA, the insulin-mediated stimulation of SREBP-1c processing is blocked by inhibitors of S6K1 (Figure 4). Importantly, evidence was provided that insulin does not block proteasomal degradation of nuclear SREBP-1c, indicating that insulin enhances production of the protein. The results of these studies indicate that the insulin-induced pathway regulating SREBP-1c in the liver branches at a point distal to mTOR (Figure 4). One branch stimulates processing of the SREBP-1c precursor and requires the mTOR-mediated activation of S6K1. The target of S6K1 that triggers Scap-dependent cleavage of SREBP-1c has yet to be determined. The second branch leads to enhanced transcription of SREBP-1c and is mediated by an unknown target of mTOR. The third branch enhances transcriptional activity of SREBPs through mTOR-mediated phosphorylation of Lipin-1.

Insulin-mediated regulation of Insigs

Another post-transcriptional mechanism that modulates the SREBP pathway involves Insigs. The Insig-1 gene is a target of SREBPs and the transcription rate of the gene parallels the nuclear content of processed SREBPs [37]. Transcription of the Insig-2 gene is controlled by alternative promoters that generate two Insig-2 transcripts designated Insig-2a and Insig-2b [53]. The nucleotide sequences of the Insig-2a and Insig-2b transcripts differ in their non-coding first exons – Insig-2a contains exon 1a, whereas Insig-2b contains exon 1b. These non-coding first exons are spliced into a common second exon. Thus, the Insig-2a and Insig-2b transcripts encode for the same protein; however, their expression is differential regulated. The Insig-2b transcript is constitutive and expressed at low levels in the liver. Insig-2a transcripts are expressed exclusively in the liver and only when insulin levels are low [53]. The Insig-2a transcript is strongly down-regulated by insulin. Transcription driven by alternative promoters gives rise to this difference and allows for differential regulation of Insig-2a and Insig-2b transcripts.

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In fasted mice, insulin levels are low and as a result, transcription of the SREBP1c gene is reduced and Insig-1 mRNA and protein levels are low. At the same time, Insig-2 protein accumulates owing to enhanced expression of the Insig-2a transcript [53, 54]. When insulin levels are restored by refeeding, Insig-2 mRNA and protein rapidly disappears because of the insulin-mediated repression of Insig-2a transcription. In contrast, insulin induces expression of SREBP1c, which restores transcription of the Insig-1 gene and expression of Insig-1 protein [53, 54]. Although the precise function of the Insig-2 to Insig-1 switch that occurs during refeeding is unknown, it is tempting to speculate the reaction allows continued processing of SREBP1c under conditions of nutrient excess in the refed state.

SREBP pathway in innate immunity

Recent studies have demonstrated the critical importance of SREBP pathway in preventing overproduction of proinflammatory cytokines from macrophages activated by lipopolysaccharides (LPS). LPS stimulates mTOR activity [55], generating a signal stimulating cleavage of SREBPs (see above). LPS also markedly activates transcription of cholesterol-25-hydroxylase (Ch25h), which catalyzes production of 25-HC. In turn, 25-HC overrides the stimulatory effect of mTOR on SREBP-2 activation and transcription of SREBP-2 target genes, which are required for cholesterol synthesis, becomes suppressed in LPS-activated macrophages [55]. In contrast, LPS stimulates transcription of SREBP-2 target genes in macrophages from Ch25h deficient mice, owing to the absence of LPS-induced generation of 25-HC. The increased production of cholesterol in these cells causes overaccumulation of cholesterol in mitochondria, leading to leakage of mitochondria DNA into cytosol [55]. The leaked DNA triggers secretion of proinflammatory cytokine IL-1β by stimulating formation of inflammasome that converts IL-1β precursor into its active form [56]. Thus, 25-HC-mediated suppression of SREBP-2 cleavage prevents overproduction of IL-1β by blocking leakage of mitochondria DNA caused by overaccumulation of cholesterol in mitochondria [55, 57].

In contrast to SREBP-2, cleavage of SREBP-1 is not inhibited in LPS-activated macrophages [58]. This observation is consistent with previous studies showing that cleavage of SREBP-2 in cultured cells was fully suppressed by 25-HC, whereas that of SREBP-1 required the addition to cells of both 25-HC and UFAs [43, 59]. Activation of SREBP-1 leads to increased transcription of genes required for production of anti-inflammatory UFAs, which play an important role in preventing overproduction of proinflammatory cytokine from macrophages subjected to prolonged exposure to LPS [58]. As a result, SREBP-1 deficient macrophages overproduce proinflammatory cytokines upon exposure to LPS [58].

Recent studies also suggest that SREBP pathway may be involved in production of type-1 interferon in response to viral infection, but the conclusion remains controversial. One study reported that macrophages deficient in Scap constitutively produced interferon-β even in the absence of viral infection, and this production depended on Stimulator of interferon genes (STING) [60], which activates transcription of interferon-β in response to cytosolic DNA [61]. In contrast, another report demonstrated that viral infection-induced production of interferon-β was impaired in Scap-deficient macrophages [62]. Data from this study suggest

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that Scap directly regulates STING in a mechanism similar to that of SREBPs: upon sensing cytosolic DNA, Scap escorts STING from ER to Golgi-like compartment where STING is active to assemble a complex eventually leading to transcriptional activation of interferon-β [62]. These observations suggest that Scap may control interferon production directly by regulating STING localization, and indirectly through regulating cholesterol metabolism. This dual mechanism makes it difficult to interpret results of interferon production from Scap-deficient macrophages, and may explain the discrepancy shown above.

SREBP pathway in cancer

Cancer cells must produce enough cholesterol and phospholipids so that new membranes can be synthesized to support their constant replication. As a result, SREBPs are frequently activated in cancer cells [63]. Cleavage of SREBPs has been shown to be stimulated by the PI3K-Akt-mTOR pathway (see above), which is the most frequently activated oncogenic signaling pathway found in various cancers [64]. Mutation of p53, which is the most frequently mutated genes in all cancers, also contributes to activation of the SREBP pathway. Unlike most tumor suppressors the expression of which is silenced in tumor cells, most p53 mutations are point mutations, and the mutant proteins are expressed in tumor cells [65]. It has been reported that the mutant p53 actually gains the function as a transcriptional coactivator for SREBP-2 to stimulate transcription of genes involved in cholesterol biosynthesis [66].

Recent lipidomics analyses have revealed that compared to benign tissues, lipids produced through the SREBP pathway such as cholesterol, oxysterols, UFAs, and triglycerides accumulate to high levels in several cancers including prostate cancer [67, 68], clear cell renal cell carcinoma (ccRCC) [69, 70], breast cancer [71] and glioblastoma [72]. These observations suggest that in addition to providing sufficient lipids for cell proliferation, activation of the SREBP pathway may also generate lipids signaling for oncogenic transformation. For example, 27-hydroxycholesterol accumulated in breast cancers has been identified as an agonist for estrogen receptor α (ERα) that selectively actives transcription of genes promoting cell proliferation, thus facilitating growth of the tumors that express ERα [71, 73]. UFAs have been demonstrated to accumulate in the most aggressive form of ccRCC, and these fatty acids stimulate tumor growth by stabilizing β-catenin [74], the aberrant accumulation of which triggers development of various cancers [75]. In order to produce massive amount of the lipids that facilitate tumor growth generated through the SREBP pathway, it is likely that feedback inhibition in SREBP activation may be impaired in some cancer cells. This can be achieved through mutations in Scap, Insig-1 and SREBP-2 in cultured CHO cells [6, 76, 77]. It remains to be determined whether these mutations occur in tumors, the growth of which is stimulated by oncogenic lipids produced by the SREBP pathway.

Concluding Remarks

SREBPs were originally identified as transcription factors controlling cholesterol homeostasis in mammalian cells. Studies over the past two decades have revealed the molecular machinery through which sterols are sensed by the cells to inhibit proteolytic

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activation of SREBPs. Recent studies have expanded the functions of SREBPs, establishing the family of transcription factors as master regulators of lipogenesis coupling lipid metabolism with various physiological and pathological processes such as insulin-induced signal transduction, innate immunity and cancer development. SREBPs are activated through the PI3K-Akt-mTOR axis in these processes, but the molecular mechanism remains sketchy. Understanding how SREBPs are activated and the functional significance of such activation under these new settings will open a new chapter in the study of the field (see Outstanding Questions).

Outstanding Questions

1. How does mTOR activate SREBPs? In particular, what are the targets of mTOR and S6K1 that are required for activation of SREBPs?
2. What is the functional significance of insulin-induced Insig-2 to Insig-1 switch in livers?
3. How does the SREBP pathway regulate production of type-1 interferons?
4. Is lipid-mediated feedback inhibition in SREBP activation impaired in lipid-accumulating cancer cells? If so which somatic mutations lead to such impairment?

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Highlights

- Cholesterol, oxysterols, and UFAs regulate the SREBP pathway through their binding to Scap (cholesterol), Insigs (oxysterols), and Ubxd8 (UFAs). These binding events influence the ER-to-Golgi trafficking of Scap-SREBP complexes and thereby modulate the proteolytic activation of SREBPs.
- Insulin modulates the SREBP pathway through transcriptional and post-transcriptional mechanisms that emerge from the mTOR pathway.
- Modulation of the SREBP pathway contributes to innate immunity and development of cancer.

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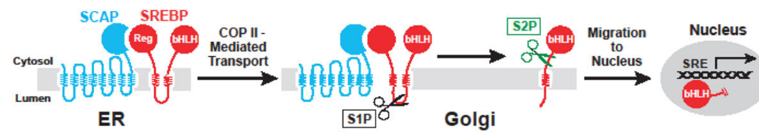

Figure 1. Proteolytic activation of SREBPs

Following synthesis on ER membranes, SREBP precursors bind to Scap through an interaction mediated by the C-terminal regulatory domain of the SREBP and the cytosolic C-terminal domain of Scap, which contains WD repeats. When cells are deprived of sterols, Scap escorts SREBPs from the ER to the Golgi by binding to the Sar1/Sec23/Sec24 complex of the COPII protein coat. In the Golgi, SREBP first encounters the Site-1 protease (S1P), which cleaves the SREBP in the lumenal loop, separating the protein into two membrane-bound halves. S1P-mediated cleavage renders the N-terminal half of SREBP a substrate for the Site-2 protease (S2P), which releases the bHLH-containing domain of SREBP by cleaving the intermediate near the cytosol-membrane boundary. Once released from membranes, transcriptionally active fragments of SREBPs migrate to the nucleus and bind to SREs in promotors of target genes to activate transcription.

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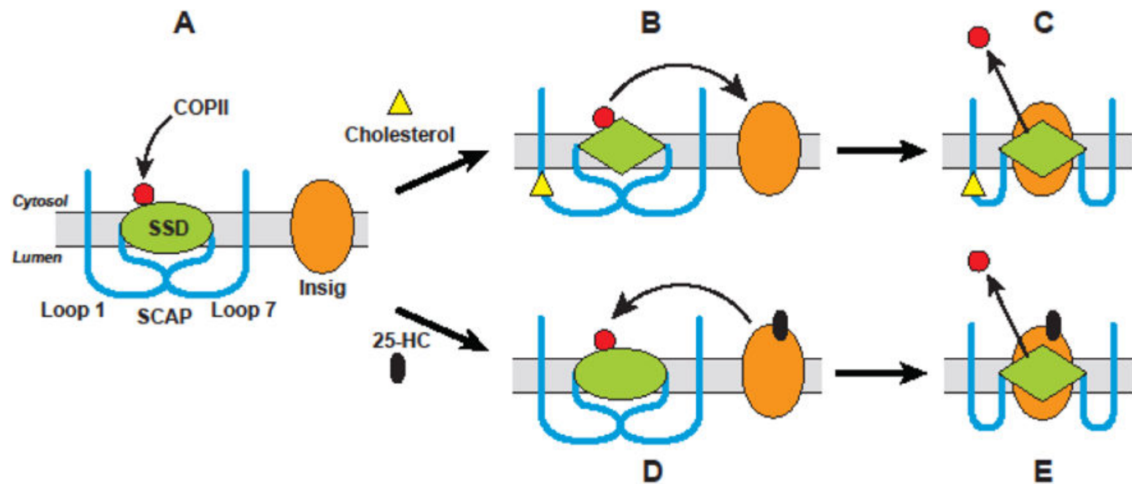

Figure 2. Sterol-mediated regulation of ER-to-Golgi transport of Scap

In sterol-deprived cells, Loop 1 of Scap is bound to Loop 7 (A). This conformation allows Scap to bind to COPII, which triggers transport of Scap from ER to Golgi. When cholesterol accumulates in ER membranes, it binds to Loop 1 and induces a conformation change in the Scap SSD that increases its affinity for Insig (B). The binding of Insig triggers a second conformational change in the Scap SSD that leads to dissociation of Loop 1 and Loop 7. As a result, Scap no longer binds COPII and becomes trapped in ER membranes (C). 25-HC also promotes binding of Scap to Insigs, but through a distinct mechanism. 25-HC directly binds to Insig (D), increasing its affinity for the SSD of Scap. Binding to Insig induces a conformational change in Scap that prevents binding to COPII (E).

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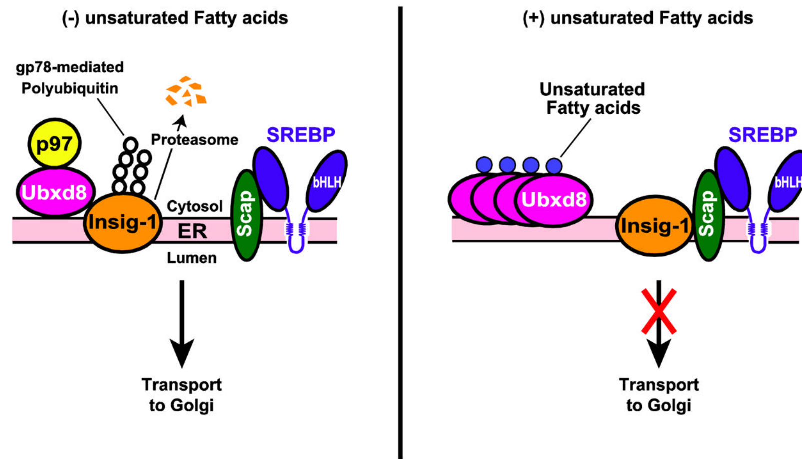

Figure 3. UFA-mediated inhibition of ER-to-Golgi transport of Scap/SREBP

In cells deprived of UFAs, Insig-1 is poly-ubiquitinated by gp78 and bound by the p97 adapter Ubxd8. Ubiquitinated Insig-1 is extracted from ER membranes through p97 and delivered to proteasomes for degradation. In the absence of Insig-1, the Scap escorts SREBP from the ER to the Golgi for proteolytic activation. Ubxd8 polymerizes upon binding to UFAs, resulting in its dissociation from Insig-1. As a result, Insig-1 becomes stabilized and blocks proteolytic activation of SREBPs by binding to Scap.

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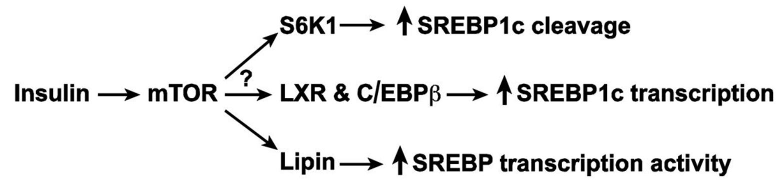

Figure 4. Insulin-mediated regulation of the SREBP pathway

Insulin modulates the SREBP pathway through transcriptional and post-transcriptional mechanisms that require the action of mTOR. Insulin-mediate activation of mTOR leads to phosphorylation of S6K1, which enhances cleavage of SREBP-1c by activating an unknown target. At the same time, mTOR activates an unknown factor that in turn modulates the LXR/C-EBPβ complex to enhance transcription of the SREBP-1c gene. Finally, mTOR phosphorylates Lipin-1, relieving its inhibition of SREBP transcriptional activity.