SREBPs: sterol-regulated transcription factors

Peter J. Espenshade

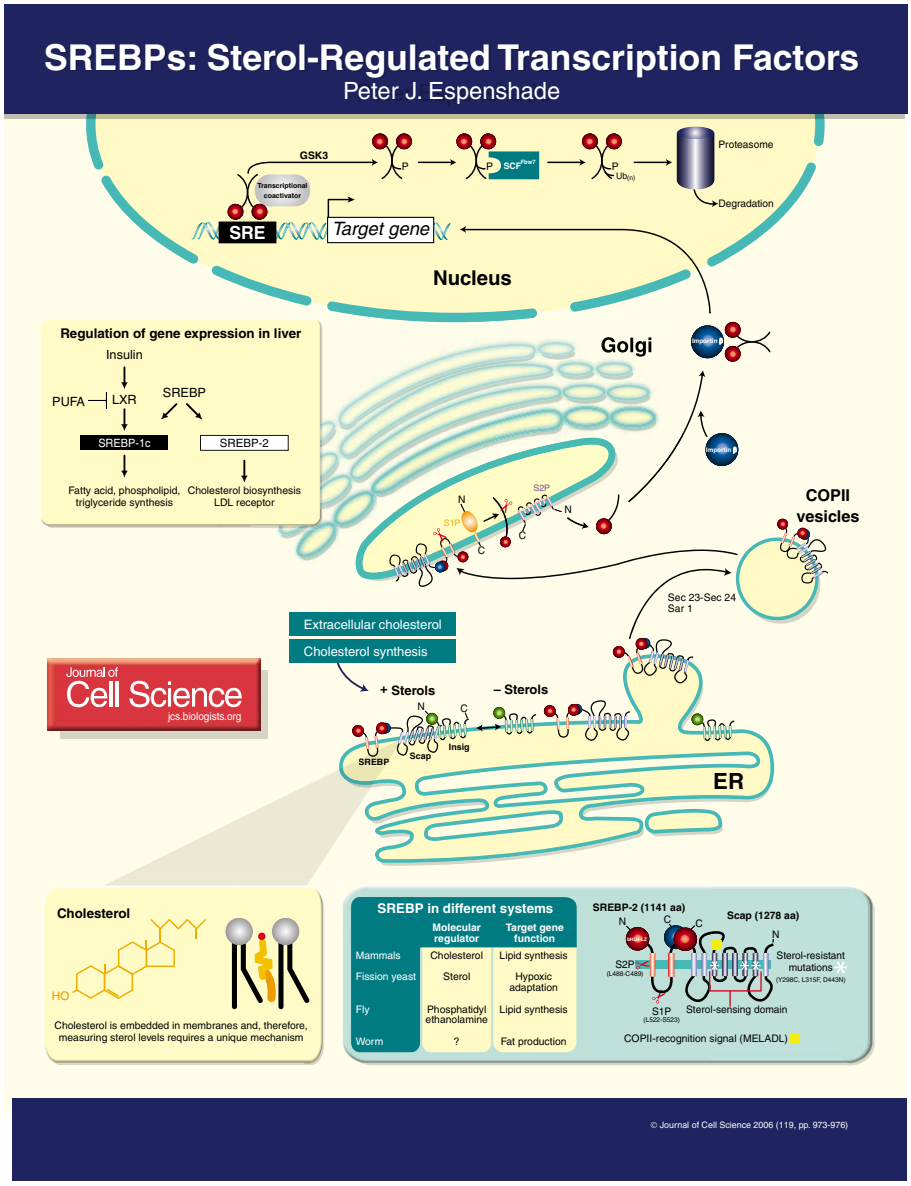
Johns Hopkins University School of Medicine, 725 N. Wolfe Street, Baltimore, MA 21205, USA
e-mail: peter.espenshade@jhmi.edu

Journal of Cell Science 119, 973-976
Published by The Company of Biologists 2006
doi:10.1242/jcs02866

Studies of the feedback regulation of cholesterol synthesis in animals have led to the identification of a unique family of membrane-bound transcription factors, sterol regulatory element binding proteins (SREBPs) (Brown and Goldstein, 1997). In the presence of cholesterol, SREBPs are sequestered in the endoplasmic reticulum (ER). In the absence of a sterol signal, however, SREBPs undergo specific proteolytic events that lead to activation of distinct sets of target genes that control lipid metabolism. Characterization of this sterol-regulated pathway has revealed new paradigms for metabolic signal transduction.

Sterol regulatory element binding proteins

Transcription of LDL receptor and genes required for cholesterol and fatty acid synthesis are controlled by membrane-bound transcription factors called SREBPs (Horton et al., 2002). The N-terminal domain of SREBP is a basic helix-loop-helix leucine zipper transcription factor. The C-terminus forms a tight complex with SREBP-cleavage-activating protein (Scap), which functions as the sterol sensor in this system. Two genes encode three SREBP isoforms (~1150 residues): SREBP-1a, SREBP-1c/ADD1 and SREBP-2 (Tontonoz et al., 1993; Brown and Goldstein, 1997). The predominant forms in the liver are SREBP-2 and SREBP-1c/ADD1, which preferentially regulate genes involved in sterol biosynthesis and fatty acid synthesis, respectively (Horton et al., 2002). SREBP-1a activates all SREBP-responsive genes. To date, SREBPs are known to enhance directly transcription of more than 30 genes needed for uptake and synthesis of cholesterol, fatty acids, triglycerides, and phospholipids (Horton et al., 2003). Despite acting in diverse biosynthetic pathways, the activity of each SREBP isoform is regulated by sterols and Scap (Matsuda et al., 2001). This mechanism is shown in the poster.



(See poster insert)

Regulation of SREBPs

In sterol-replete cells, Scap binds to cholesterol in the ER membrane and assumes a conformation that promotes binding to the ER-resident protein Insig (for insulin-induced gene) (Peng et al., 1997). This retains the SREBP-Scap complex in the ER by preventing interaction of Scap with the COPII vesicle-formation proteins Sar1, Sec23 and Sec24 (Rawson, 2003). In sterol-depleted cells, binding to Insig is disrupted and SREBP-Scap is sorted in COPII-coated transport vesicles. Scap then escorts SREBP to the Golgi, where two sequential proteolytic cleavage events, mediated by the site 1 (S1P) and site 2 (S2P) proteases, release the N-terminal transcription factor domain from the membrane (Rawson, 2003). Released SREBP is transported into the nucleus as a dimer by importin β through interactions with the helix-loop-helix domain (Lee et al., 2003). In the nucleus, SREBP activates transcription by binding to sterol regulatory element (SRE) sequences in the promoters of target genes. The resultant increase in synthesis and uptake of cholesterol then

feeds back to inhibit activation of SREBP. Finally, nuclear residence of SREBPs is limited by ubiquitin-dependent proteasomal degradation.

Scap

Scap (1278 residues) contains two functional domains: the N-terminus consists of eight transmembrane segments and the C-terminus contains multiple WD repeats that mediate binding to SREBP. Genetic and biochemical experiments define transmembrane segments 2-6 (TM2-TM6) of Scap as a sterol-sensing domain. Point mutations in TM2-TM6 (Y298C, L315F or D443N) prevent binding of Scap to Insig, resulting in constitutive ER-to-Golgi transport of SREBP-Scap that is resistant to sterol inhibition (Rawson, 2003). In addition, recombinant Scap TM1-TM8 binds to cholesterol in vitro and undergoes a cholesterol-dependent conformational change (Brown et al., 2002; Radhakrishnan et al., 2004). In the absence of sterols, Scap forms a complex with the COPII cargo-selection proteins Sec23 and Sec24 (Espenshade et al., 2002; Antonny and Schekman, 2001). COPII binding requires the sequence MELADL between TM6 and TM7 in Scap, whereas sterol inhibition of COPII binding requires Insig (Sun et al., 2005).

Interestingly, HMG-CoA reductase, which catalyzes the first committed step in sterol synthesis, also contains a sterol-sensing domain and is negatively regulated by binding to Insig (Sever et al., 2003). However, in the case of HMG-CoA reductase, sterol-regulated binding to Insig accelerates ubiquitylation and proteasomal degradation of the enzyme (Song et al., 2005). Sterol-sensing domains are also found in other proteins, such as the Neimann-Pick type C disease gene *NPC1* and the Hedgehog receptor Patched. However, the role of Insig in regulation of these proteins remains to be tested (Kuwabara and Labouesse, 2002).

Insig

Insigs are ER-resident proteins that contain six transmembrane segments and negatively regulate Scap and HMG-CoA reductase (Yang et al., 2002). Humans have two proteins, Insig-1 (277 aa) and Insig-2 (225 aa), that differ in the length of their cytosolic N-termini (Yabe et al., 2002). Studies using mice and cultured cells lacking Insig-1 as well as Insig-2 demonstrate that they are essential mediators of cholesterol feedback regulation, controlling both activation of SREBP through ER retention and sterol-accelerated degradation of HMG-CoA reductase (Lee et al., 2005; Engelking et al., 2005). Although the two proteins appear functionally equivalent, expression of Insig-1 and expression of Insig-2 is inversely regulated by insulin in the liver (Yabe et al., 2003; Attie, 2004). Insig-1 is an SREBP target and is highly expressed in livers of mice fed a normal diet owing to elevated insulin and SREBP-1c levels. Upon fasting, insulin falls, decreasing Insig-1 and increasing Insig-2 expression. The situation is reversed upon refeeding animals, when insulin levels rise, upregulating Insig-1 and downregulating Insig-2 (Yabe et al., 2003).

S1P and S2P

SREBP is sequentially processed by S1P (1052 residues) and S2P (519 residues) (Rawson, 2003). S1P (also called SKI-1), a member of the subtilisin/kexin family of serine proteases, cleaves after a leucine residue in the consensus sequence RxxL in the luminal loop of SREBPs (Duncan et al., 1997; Espenshade et al., 1999). The zinc metalloprotease S2P cleaves a Leu-Cys bond predicted to lie within the lipid bilayer by a process known as regulated intramembrane proteolysis (RIP) (Brown et al., 2000). SREBP is not the only substrate for these proteases: the two proteins function in tandem to activate the stress response transcription factor ATF6 (Ye et al., 2000).

SREBP transcriptional regulation

SREBPs function as master regulators of cholesterol and fatty acid synthesis. SREBP-2 upregulates expression of most cholesterol biosynthetic enzymes and the LDL receptor, whereas SREBP-1c stimulates transcription of genes required for fatty acid synthesis, such as acetyl-CoA carboxylase and fatty acid synthase (Horton et al., 2002). SREBPs cooperate with other DNA-binding transcription factors and coactivators. Maximal transcriptional activation requires additional DNA-binding proteins: NF-Y and CREB for the HMG-CoA reductase gene, and Sp1 for the LDL receptor gene (Edwards et al., 2000). In addition, SREBPs recruit the coactivators CBP/p300 and the mediator complex to stimulate transcription (Edwards et al., 2000; Toth et al., 2004). The coactivator PGC-1β, induced by a fat-rich diet, also binds SREBP-1c and is required for SREBP-mediated lipogenic gene expression (Lin et al., 2005). Importantly, these factors permit modulation of SREBP activity independently of sterol-regulated proteolytic processing.

Regulation of SREBP activity

The central role that SREBPs play in control of lipid synthesis is highlighted by the multiple inputs to SREBP activity from other signaling pathways. The nuclear hormone receptors RXR and LXR function as a heterodimer to upregulate SREBP-1c in response to cholesterol overloading, possibly to increase the supply of unsaturated fatty acids needed for cholesterol esterification and storage (Repa et al., 2000). In the liver, transcription of both SREBP-1c and SREBP-2 is stimulated by SREBPs in a feed-forward mechanism that requires SRE sequences in the promoters of these genes (Horton et al., 2002).

One function of the liver is to convert excess carbohydrates to fatty acids for storage as triglycerides. Insulin stimulates this fatty acid synthesis in response to excess carbohydrate (Horton et al., 2002). Importantly, these lipogenic effects of insulin in the liver are mediated by SREBP-1c (Eberle et al., 2004). Insulin increases SREBP-1c mRNA levels and SREBP-1c target gene expression in both the liver and tissue culture cells (Shimomura et al., 1999; Foretz et al., 1999). Although a complete description of insulin action on SREBP-1c requires further experimentation, a recent study suggests that control of SREBP-1c transcription by insulin is mediated by RXR-LXR (Chen et al., 2004). Interestingly, polyunsaturated fatty acids (PUFA) inhibit SREBP-1c and fatty acid synthesis activity by antagonizing LXR-dependent activation of SREBP-1c. LXR may thus integrate

these two dietary signals (Ou et al., 2001).

Control of protein degradation

Mature nuclear SREBP is highly unstable owing to its ubiquitin-dependent degradation (Wang et al., 1994). Although this is not sterol regulated (Hirano et al., 2001), phosphorylation of SREBP promotes its binding to the E3 ubiquitin ligase SCF^{Fbw7} and thus its ubiquitylation and degradation (Sundqvist et al., 2005). This can be mediated by GSK3, whose activity is inhibited by insulin signaling, which suggests a non-transcriptional mechanism by which insulin may stimulate SREBP activity (Frame and Cohen, 2001).

SREBP in non-mammalian systems

Homologs of SREBP have been identified and characterized in fission yeast, flies and worms. In *S. pombe*, SREBP is activated in response to sterol depletion as a consequence of low oxygen levels (Hughes et al., 2005). Yeast SREBP is required for anaerobic growth and activates genes required for adaptation to low oxygen levels. In *D. melanogaster*, a cholesterol auxotroph, SREBP is not regulated by sterols, but instead activation is controlled by phosphatidylethanolamine (Rawson, 2003). In response to reduced levels of this lipid, *Drosophila* SREBP activates lipogenic enzymes. Lastly, *C. elegans* SREBP is highly expressed in the intestine, where it is required for expression of lipogenic enzymes and fat production (McKay et al., 2003). What regulates *C. elegans* SREBP is unknown. Analysis of SREBP function in these organisms promises to give insights into the molecular mechanisms of sterol sensing and the evolution of this regulatory system.

Outlook

Despite the increasing clarity of the mechanisms controlling SREBP activity, many questions still remain. How does cholesterol-dependent binding of Scap to Insig prevent COPII binding? What are the functional differences between Insig-1 and Insig-2? How does differential regulation of these genes by insulin affect regulation of Scap in the liver? Is regulation of SREBP degradation a major control point in the liver? Finally, how can Scap sense both cholesterol in mammals and phosphatidylethanolamine in insects? Answers to these questions should reveal new paradigms for signal transduction and control of lipid homeostasis.

Thanks to members of the Espenshade Lab and Susan Michaelis for comments on the manuscript. I apologize to those whose work was not discussed due to space limitations. P.J.E. is supported by a Career Award in the Biomedical Sciences from the Burroughs Wellcome Fund and by a grant from NIH (HL77588).

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