ELSEVIER

Sterol Regulatory Element-binding Protein-1 as a Dominant Transcription Factor for Gene Regulation of Lipogenic Enzymes in the Liver

Hitoshi Shimano*

Sterol regulatory element-binding proteins (SREBPs) are basic helix-loop-helix (bHLH) type transcription factors that control expression of genes involved in biosynthesis of cholesterol and fatty acids. Dietary studies with normal, transgenic, and knockout mice have established SREBP-1 as a dominant transcription factor regulating gene expression of lipogenic enzyme in the liver. Polyunsaturated fatty acids inhibit hepatic lipogenic enzymes through suppressing SREBP-1. Whereas SREBP-2 exerts sterol regulation through cleavage of the membrane-bound precursor protein to liberate the active nuclear form into the nucleus, SREBP-1 controls lipogenic enzymes by self-regulating its own transcription level. Promoter analysis of the SREBP-1 gene will be important to clarify the mechanism of nutritional regulation of lipogenic genes. (Trends Cardiovasc Med 2000;10:275–278). *© 2001, Elsevier Science Inc.*

Sterol regulatory element-binding proteins (SREBPs) are transcription factors that belong to the basic helix-loop-helix leucine zipper (bHLH-Zip) family (Brown and Goldstein 1997 and 1999, Brown et al. 2000). In contrast to other members of the bHLH-Zip family, SREBPs are synthesized as precursor proteins that remain bound to the endoplasmic reticulum and the nuclear envelope in the presence of sufficient sterol concentrations. Upon sterol deprivation, the precursor protein undergoes a sequential two-step cleavage process to release the NH_2 terminal portion (Sakai et al. 1996). This mature SREBP then enters the nucleus and activates the transcription of genes involved in cholesterol and fatty acid synthesis by binding to sterol regulatory elements (SREs) or to palindromic sequences called E-boxes within their promoter regions (Kim et al. 1995, Wang et al. 1993). To date, three SREBPs have been identified; SREBP-1a and SREBP-1c (also known as ADD1) are produced from a single gene through the use of alternate promoters, and SREBP-2 is transcribed from a separate gene (Hua et al. 1993, Tontonoz et al. 1993, Yokoyama et al. 1993). It has been shown that all cultured cells analyzed to date mainly express SREBP-2 and the -1a isoform of SREBP-1 whereas most organs, including the liver and adipose tissue, express predominantly SREBP-2 and the -1c isoform of SREBP-1 (Shimomura et al. 1997). Although SREBP-1a and -1c share the same bHLH and regulatory domains, SREBP-1a is a stronger activator than SREBP-1c owing to a longer amino-terminal transactivation domain and has a wider range of target genes involved both in cholesterol and fatty acid synthesis (Shimano et al. 1996, 1997). Cumulative lines of evidence, including studies with transgenic mice and diet studies with normal mice, established that SREBP-1c plays a role in regulating the transcription of genes involved in fatty acid synthesis whereas SREBP-2 is involved in the transcription of cholesterogenic enzymes (Horton et al. 1998b).

Hitoshi Shimano is from the Department of Internal Medicine, Institute of Clinical Medicine, Tsukuba, Ibaraki, Japan.

*Address correspondence to: Hitoshi Shimano, Department of Internal Medicine, Institute of Clinical Medicine, 1-1-1 Tennodai, Tsukuba, Ibaraki, 305-8575 Japan. Fax: +81-298-53-3051; e-mail address: shimanotky@umin.ac.jp.

• Studies from SREBP-1 Knockout Mice

Lipogenic enzymes which are involved in energy storage through synthesis of fatty acids and triglycerides are coordinately regulated at the transcriptional level during different metabolic states (Goodridge 1987, Hillgartner et al. 1995). Recent in vivo studies suggest that SREBP-1c plays a crucial role in the dietary regulation of most hepatic lipogenic genes. These include studies of the effects of the over-expression of SREBP-1a and -1c on hepatic lipogenic gene expression in transgenic mice (Shimano et al. 1997, 1999), as well as physiological changes of SREBP-1c protein in normal mice after dietary manipulation such as placement on fasting–refeeding regimens (Horton et al. 1998a). The similarly coordinated changes in SREBP-1c and lipogenic gene expression at fasting and

refeeding were also observed in adipose tissue (Kim et al. 1998).

To obtain a conclusive estimate of the role of SREBP-1 in dietary regulation of hepatic lipogenic genes, we assessed the effects of absence of SREBP-1a and -1c proteins on the expression of these genes in SREBP-1 knockout mice (Shimano et al. 1999). Initially we used a fasting–refeeding treatment which is an established dietary manipulation for the induction of lipogenic enzymes. In the fasted state, the mRNA levels of all lipogenic enzymes were consistently low in both wild-type and SREBP-1 null mice. However, absence of SREBP-1 severely impaired the marked induction of hepatic mRNAs of fatty acid synthetic genes such as acetyl-CoA carboxylase, fatty acid synthase (FAS), and stearoyl-CoA desaturase-1 (SCD-1) that was observed upon refeeding in the wild-type mice. Furthermore, the refeeding responses of other lipogenic enzymes (glycerol-3-phosphate acyltransferase, ATP citrate lyase, malic enzyme, glucose-6-phosphate dehydrogenase, and Spot14 (S14) mRNAs) were completely abolished in SREBP-1 knockout mice. In contrast, mRNA levels for cholesterol biosynthetic genes were paradoxically elevated in the refed SREBP-1 knockout livers, accompanied by an increase in nuclear SREBP-2 protein. When fed a high carbohydrate diet for 14 days, the mRNA levels for these lipogenic enzymes were also strikingly lower in SREBP-1 deficient mice than in wild-type mice (see Figure 1). These data basically confirmed that SREBP-1 plays a crucial role in the induction of lipogenesis, but not cholesterol biosynthesis, in liver when excess energy by carbohydrates is consumed. However, the extent to which the gene regulation depends upon SREBP-1 considerably differs among the lipogenic genes and organs. For instance, glycolytic enzymes such as pyruvate kinase and glucokinase are sometimes classified as lipogenic enzymes, but are not highly regulated by SREBP-1. Gene expression of FAS and SCD-1 is highly controlled by SREBP-1, but other factors such as USFs for FAS regulation also seem to be involved. The cis-elements which SREBPs bind and activate in the promoters of lipogenic genes show a considerable diversity and do not completely match classic sterol regulatory elements, but show some similarities to SRE and/or E-boxes, and are currently

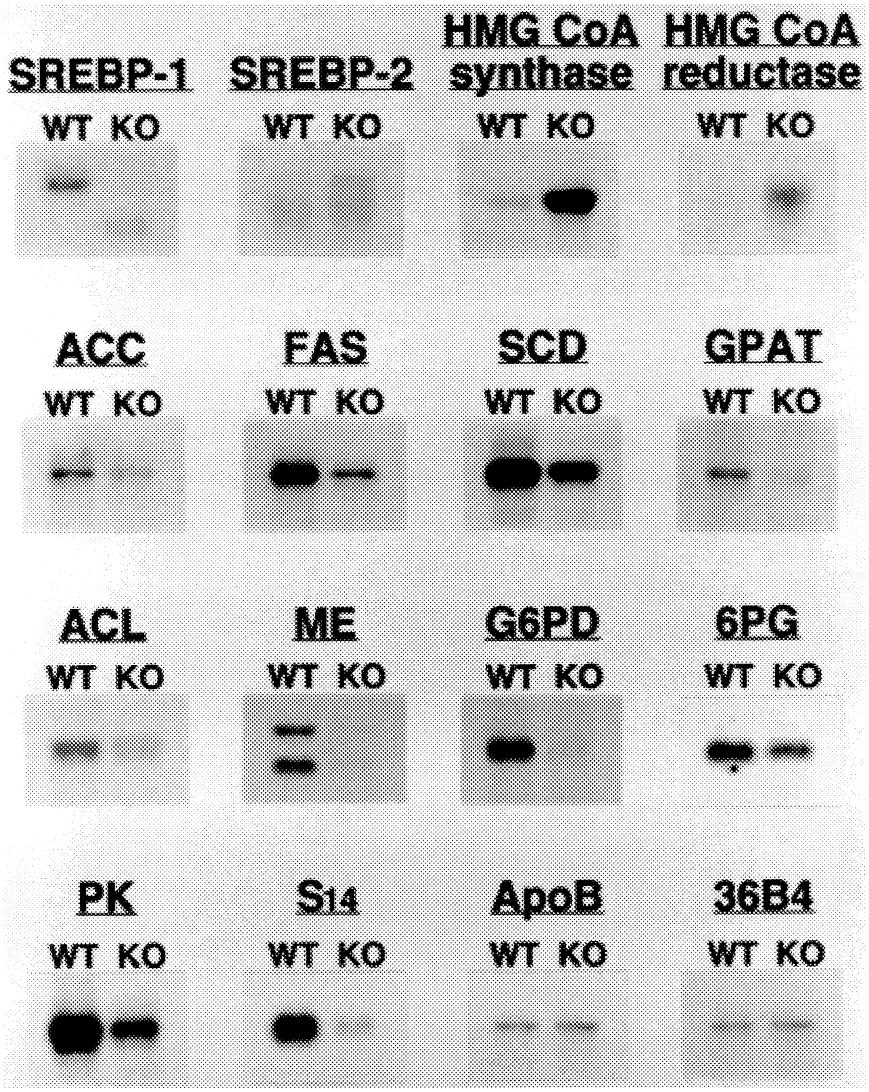

Figure 1. RNA levels of various genes involved in lipogenesis and cholesterogenesis as measured by Northern blot analysis of total RNA of livers from wild-type (WT) and SREBP-1 null (KO) mice fed a high carbohydrate diet (70% sucrose diet) for 2 weeks. A smaller SREBP-1 transcript from KO mice was derived from the disrupted SREBP-1 gene and produced an aberrant non-functional protein. Expression of lipogenic genes were highly induced by high carbohydrate diet. This induction was severely impaired in glycerol-3-phasphate acyltransferase (GPAT), glucose-6-phasphate dehydrogenase (G6PD), and S14 gene by SREBP-1 disruption. Expression of acetyl-CoA carboxylase (ACC), fatty acid synthase (FAS), ATP citrate lyase (ACL) stearoyl-CoA desaturase (SCD), 6-phosphogluconate dehydrogenase (6PG), and pyruvate kinase (PK) was less, but considerably impaired. In contrast, cholesterogenic genes including SREBP-2, HMG CoA synthase, and reductase were roughly three-fold increased in SREBP-1 KO mice as compared to wild-type mice.

designated SRE-like sequences. A detailed promoter analysis is required for these SRE-like sequences in lipogenic gene promoters to clarify the differences in response of each lipogenic gene to SREBPs. Another interesting observation was made in adipose tissue of refed SREBP-1 knockout mice. The absence of SREBP-1 did not severely affect overall gene expression of lipogenic enzymes in fat as observed in the livers, suggesting that control of lipogenic gene expression might show some tissue specificity. The mechanism of the paradoxical induction of cholesterogenic genes in SREBP-1 knockout mice is not fully understood. It is presumably owing to a compensatory activation of SREBP-2 since cholesterol feeding partially suppressed this induction (data not shown). However,

overshooting of cholesterol synthetic genes suggests that endpoint of this regulation might not be cellular cholesterol content.

- **Inhibition of Lipogenic Gene Expression by Polyunsaturated Fatty Acids (PUFA) through Suppression of SREBP-1**

Dietary manipulations are known to regulate gene expression of lipogenic genes. Placement on a fat-free and high carbohydrate diet or refeeding with this diet after fasting markedly induces lipogenic gene expression in the livers. Meanwhile, dietary polyunsaturated fatty acids (PUFA) are negative regulators of hepatic lipogenesis that exert their effects primarily at the level of transcription. Finally, studies with the knockout mice established that SREBP-1 plays a crucial role in the regulation of lipogenic gene expression in the liver (Shimano et al. 1999). To explore the possible involvement of SREBP-1 in the suppression of hepatic lipogenesis by PUFA, we challenged wild-type mice and transgenic mice overexpressing a mature form of SREBP-1 in the liver with dietary PUFA (Yahagi et al. 1999). In the liver of wild-type mice, dietary PUFA drastically decreased the mature, cleaved form of SREBP-1 protein in the nucleus, whereas the precursor, uncleaved form in the membranes was not suppressed. The decreases in mature SREBP-1 paralleled those in mRNAs for lipogenic enzymes such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC). In the transgenic mice, dietary PUFA did not reduce the amount of transgenic SREBP-1 protein, excluding the possibility that PUFA accelerated the degradation of mature SREBP-1. The resulting sustained expression of mature SREBP-1 almost completely canceled the suppression of lipogenic gene expression by PUFA in the SREBP-1 transgenic mice. These results demonstrate that the suppressive effect of PUFA on lipogenic enzyme genes in the liver is caused by a decrease in SREBP-1 expression. There are some other reports describing similar results that diet containing fish oil decreased both hepatic SREBP-1 mRNA and protein levels (Kim et al. 1999, Thewke et al. 1998, Worgall et al. 1998). In the light of suppression of lipogenesis, it does not matter whether the fish oil belongs to n-3 or n-6 family of PUFA. In our studies, linoleate, EPA and DHA were potent suppressors of SREBP-1, in this order of magnitude. Fish oils, especially tuna oil enriched in DHA, were generally stronger than each pure PUFA. It is possible that some unknown component of fish oil might contribute to an additional effect of fish oil on SREBP-1 expression.

Many investigators have analyzed the cis-acting elements in the promoter region for carbohydrate stimulation and PUFA suppression of lipogenic genes. In the case of enzymes such as FAS, ACL, and pyruvate kinase (PK), glucose/insulin response elements overlap with PUFA response regions. Especially the glucose/insulin and PUFA response element in the FAS promoter has been shown to contain an SREBP-binding site. The PUFA response region in the mouse SCD1 promoter is also reported to have an SREBP-binding site. Furthermore, PUFA suppressive element and SREBP binding site in S14 gene promoter were found to be identical. These data are supportive of our finding that carbohydrate stimulation and PUFA suppression are mediated by a common molecule, SREBP-1 (shown in Figure 2).

Because PUFA are known as activators of PPARs, the effects of PUFA through PPARs were also examined. The finding that fenofibrate did not affect the amount of mature SREBP-1 in the liver indicates that the suppressive effect of PUFA on mature SREBP-1 is not mediated by PPARα. This finding is compatible with a previous study using PPARα-null mice which showed that PPAR was not required for the PUFA-mediated inhibition of either FAS or S14 gene expression (Ren et al. 1996). Future studies should focus on the clarification of the mechanism by which PUFA modulates the cleavage of SREBP-1 precursor protein in terms of the regulation of hepatic lipogenesis as well as identification of the PUFA acceptor that mediates PUFA suppression of SREBP-1c promoter activity.

- **SREBP-1c Promoter Analysis and Future Prospects**

Previous reports on the regulation of SREBP-1c have all demonstrated the induction to be at the mRNA level (Foretz

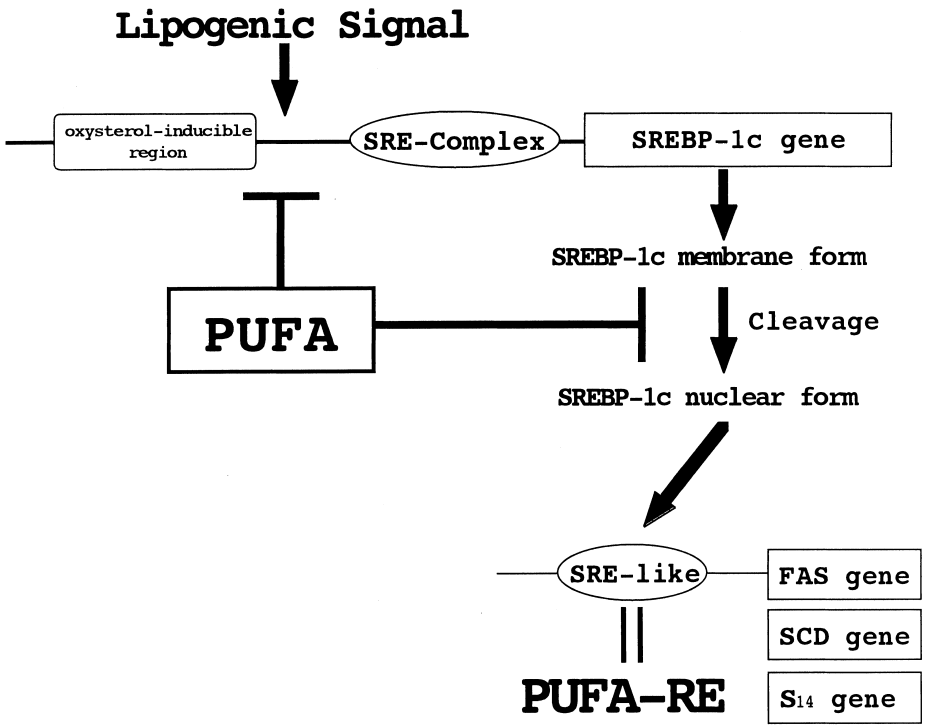

Figure 2. Inhibition of lipogenic genes by PUFA (polyunsaturated fatty acids) through SREBP-1c suppression PUFA suppresses both SREBP-1c gene expression and the activity to cleave SREBP-1c precursor into its nuclear form to activate transcription of lipogenic genes. Consequently, SREBP binding sites (SRE-like sequences) correspond to PUFA responsible elements (PUFA-RE) in these gene promoters. SREBP-1c gene promoter contains SRE complex and oxysterol-inducible region. Neither of exact target sites for lipogenic signal to induce SREBP-1c gene expression nor for PUFA to suppress SREBP-1c gene expression is currently identified.

et al. 1999, Hasty et al. 2000, Horton et al. 1998, Kim et al. 1999, Shimomura et al. 1999, Thewke et al. 1998, Worgall et al. 1998, Yahagi et al. 1999,). In contrast to SREBP-2, which mediates sterol regulation completely at the cleavage level through interaction with SCAP and site-1 protease, SREBP-1c controls the transcriptional regulation of lipogenic enzymes by self-regulating the nuclear concentration of its mature form, which is highly correlated to its precursor and mRNA levels. This raises a possibility that the sterol-regulated cleavage system might not be so specific to SREBP-1c as to SREBP-2, and makes the promoter analysis on SREBP-1c gene important. Our initial analysis of the mouse SREBP-1c promoter identified two functional regions (Amemiya-Kudo et al. 2000). One region is the SRE complex that confers a response to SREBPs. Upstream of this region, there was an oxysterol-inducible region, details of which remains unknown (Figure 2). Further studies are needed to identify the regions of carbohydrate-responsive elements and PUFA-suppressive elements in the SREBP-1c promoter. Recently, we have reported that SREBP-1c is regulated by glucose at the transcriptional level in a well-differentiated hepatic cell line, H2-35 cells (Hasty et al. 2000). Preliminarily, we observed suppression of SREBP-1c nuclear protein in these cells by addition of EPA to the medium. This cell line would help in the understanding of dietary regulation of lipogenic genes through regulation of SREBP-1 expression in the liver.

• Acknowledgments

I greatly thank my cowokers: Naoya Yahagi, Michiyo Amemiya-Kudo, Alyssa H. Hasty, and Tomohiro Yoshikawa for main contributions to this work, Nobuhiro Yamada, Shun Ishibashi, J. L. Goldstein and M.S. Brown for continuous support.

References

Amemiya-Kudo M, et al.: 2000. Promoter analysis of the mouse sterol regulatory element-binding protein (SREBP)-1c gene. J Biol Chem 275:31,078–31,085.

Brown MS, Goldstein JL: 1997. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell 89:331–340.

Brown MS, Goldstein JL: 1999. A proteolytic pathway that controls the cholesterol content of membranes, cells, and blood. Proc Natl Acad Sci USA 96:11,041–11,048.

Brown MS, Ye J, Rawson RB, Goldstein JL: 2000. Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell 100:391–398.

Foretz M, Pacot C, Dugail I et al.: 1999. ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. Mol Cell Biol 19:3760–3768.

Goodridge AG: 1987. Dietary regulation of gene expression: enzymes involved in carbohydrate and lipid metabolism. Annu Rev Nutr 7:157–185.

Hasty AH, Shimano H, Yahagi N, et al.: 2000. Sterol regulatory element-binding-1 protein is regulated by glucose at the transcriptional level. J Biol Chem 275:31,069–31,077.

Hillgartner FB, Salati LM, Goodridge AG: 1995. Physiological and molecular mechanisms involved in nutritional regulation of fatty acid synthesis. Physiol Rev 75:47–76.

Horton JD, Bashmakov Y, Shimomura I, Shimano H: 1998a. Regulation of sterol regulatory element binding proteins in livers of fasted and refed mice. Proc Natl Acad Sci USA 95:5987–5992.

Horton JD, Shimomura I, Brown MS, et al.: 1998b. Activation of cholesterol synthesis in preference to fatty acid synthesis in liver and adipose tissue of transgenic mice overproducing sterol regulatory element-binding protein-2. J Clin Invest 101:2331–2339.

Hua X, Yokoyama C, Wu J, et al.: 1993. SREBP-2, a second basic-helix-loop-helix-leucine zipper protein that stimulates transcription by binding to a sterol regulatory element. Proc Natl Acad Sci USA 90: 11,603–11,607.

Kim HJ, Takahashi M, Ezaki O: 1999. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mrnas. J Biol Chem 274:25,892–25,898.

Kim JB, Spotts GD, Halvorsen YD, et al.: 1995. Dual DNA binding specificity of ADD1/SREBP1 controlled by a single amino acid in the basic helix-loop-helix domain. Mol Cell Biol 15:2582–2588.

Kim JB, Sarraf P, Wright M, et al.: 1998. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J Clin Invest 101:1–9.

Ren B, Thelen A, Jump DB: 1996. Peroxisome proliferator-activated receptor alpha inhibits hepatic S14 gene transcription. Evidence against the peroxisome proliferator-activated receptor alpha as the mediator of polyunsaturated fatty acid regulation of s14 gene transcription. J Biol Chem 271:17,167–17,173.

Sakai J, Duncan EA, Rawson RB, et al.: 1996. Sterol-regulated release of SREBP-2 from cell membranes requires two sequential cleavages, one within a transmembrane segment. Cell 85(7):1037–1046.

Shimano H, Horton JD, Hammer RE, et al.: 1996. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J Clin Invest 7:1575–1584.

Shimano H, Horton JD, Shimomura I, et al.: 1997. Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. J Clin Invest 99:846–854.

Shimano H, Yahagi N, Amemiya-Kudo M, et al.: 1999. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J Biol Chem 274:35,832–35,839.

Shimomura I, Bashmakov Y, Ikemoto S, et al.: 1999. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. Proc Natl Acad Sci USA 96:13,656–13,661.

Shimomura I, Shimano H, Horton JD, et al.: 1997. Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J Clin Invest 99:838–845.

Thewke DP, Panini SR, Sinensky M: 1998. Oleate potentiates oxysterol inhibition of transcription from sterol regulatory element-1-regulated promoters and maturation of sterol regulatory element-binding proteins. J Biol Chem 273:21,402–21,407.

Tontonoz P, Kim JB, Graves RA, Spiegelman BM: 1993. ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. Mol Cell Biol 13:4753–4759.

Wang X, Briggs M, Hua X, et al.: 1993. Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. II. Purification and characterization. J Biol Chem 268:14,497–14,504.

Worgall TS, Sturley SL, Seo T, Osborne TF, Deckelbaum RJ: 1998. Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. J Biol Chem 273:25,537–25,540.

Yahagi N, Shimano H, Hasty A, et al.: 1999. A crucial role of sterol regulatory element-binding protein-1 in the regulation of lipogenic gene expression by polyunsaturated fatty acids. J Biol Chem 274:35,840–35,844.

Yokoyama C, Wang X, Briggs MR, et al.: 1993. SREBP-1, a basic-helix-loop-helix-leucine zipper protein that controls transcription of the low density lipoprotein receptor gene. Cell 75:187–197.

PII S1050-1738(00)00079-7 **TCM**