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BREAKTHROUGHS AND VIEWS

Transcriptional Homeostatic Control of Membrane Lipid Composition[1]

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Plasma membranes have a structural property, commonly referred to as membrane fluidity, that is compositionally regulated. The two main features of plasma membrane lipid composition that determine membrane fluidity are the ratio of cholesterol to phospholipids and the ratio of saturated to unsaturated fatty acids that are incorporated into the phospholipids. These ratios are determined, at least in part, by regulation of membrane lipid biosynthesis—particularly that of cholesterol and oleate. It now appears that cholesterol and oleate biosynthesis are feedback regulated by a common transcriptional mechanism which is governed by the maturation of the SREBP transcription factors. In this article, we briefly review our current understanding of transcriptional regulation of plasma membrane lipid biosynthesis by sterols and oleate. We also discuss studies related to the mechanism by which the physical state of membrane lipids signals the transcriptional regulatory machinery to control the rates of synthesis of these structural components of the lipid bilayer.

Key Words: **membrane fluidity; SREBP; oleate.**

Several excellent reviews on the transcriptional regulation of plasma membrane cholesterol levels have been published (1–3). For this reason we will only provide a limited overview of this process with emphasis on its pertinence to regulation of the fatty acid composition of plasma membranes. The transcriptional control of the biosynthesis of plasma membrane lipid acyl substituents, particularly oleate, has only recently become an active area of investigation. In accord with its structural role in membranes, oleate biosynthesis is responsive to the levels of the other lipid determinants of fluidity, including cholesterol, polyunsaturated fatty acids and oleate itself.

TRANSCRIPTIONAL REGULATION OF MEMBRANE LIPID BIOSYNTHESIS BY STEROLS

The enzymes of the cholesterol biosynthetic pathway, as well as the low density lipoprotein (LDL) receptor, are all regulated at the level of transcription by a common mechanism (4). The promoters of these genes contain members of a family of regulatory regions collectively termed sterol responsive elements (SREs), which function with an adjacent binding site, for another ubiquitous transcription factor, to activate transcription (1–3). The transcription factors which recognize SREs are designated SRE binding proteins 1a, 1c, and 2 (SREBP-1a, SREBP-1c, and SREBP-2) (5, 6). They are comprised of three functional domains: an NH_2-terminal segment which is a transcription factor of the basic-helix-loop-helix-leucine zipper family: a middle segment composed of two membrane spanning domains separated by a hydrophilic loop and a COOH-terminal regulatory segment (5, 6). SREBPs are localized to the membranes of the endoplasmic reticulum (ER) and nuclear envelope, oriented in the membrane in a hairpin fashion, with the amino and carboxyl termini projecting into the cytosol (7). In response to declining sterol levels the NH_2-terminal half of the SREBP is released from the membrane spanning domains by a two step proteolytic process allowing it to translocate into the nucleus (8). SREBP processing is regulated by SREBP cleavage activating protein (SCAP) (9) which appears to function in the trafficking of SREBPs from the ER to compartments within the *trans*Golgi network where the processing proteases (S1P and S2P) reside (10, 11).

In addition to the genes of cholesterol homeostasis, the promoters of several genes of fatty acid biosynthe-

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sis: acetyl CoA carboxylase (ACC), fatty acid synthase (FAS), and stearoyl-CoA desaturase (SCD) also contain sequences which confer sterol regulation (12–15). These sequences differ slightly from the classically defined SRE found in the promoters of cholesterol homeostatic genes, more closely resembling "E" box motifs and SRE half sites. In this regard, it is noteworthy that SREBP-1c was originally cloned from an adipocyte expression library based upon its binding "E" box motifs (16). Further evidence of SREBP regulation of fatty acid biosynthetic genes, has been provided by studies which examined the effects of ectotopically expressing a truncated, constitutively active, SREBP in cultured cells or in transgenic mice (17–19). The general consensus from these studies is that SREBP-1a and particularly SREBP-1c are relatively selective activators of fatty acid synthesis while SREBP-2 is a more potent activator of cholesterol synthesis.

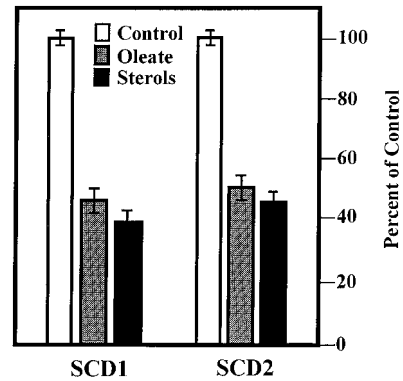

FIG. 1. Regulation of stearoyl-CoA desaturase gene transcription by sterols and oleate in CHO-K1 cells. The vectors, pSCD1-589 and pSCD2-588 (15), contain luciferase reporter genes, each driven by one of the two stearoyl-CoA desaturase gene promoters, SCD1 or SCD2, respectively. CHO-K1 cells stably transfected with pSCD1-589 or pSCD2-588 were incubated for 12 h in Hams' F-12 medium supplemented with 5% organic solvent-delipidized FBS (Control) and either 300 μM oleate (Oleate) or 1 μg/ml 25-hydroxycholesterol + 10 μg/ml cholesterol (Sterols). Results are the mean of triplicate samples ± SEM.

TRANSCRIPTIONAL REGULATION OF MEMBRANE LIPID BIOSYNTHESIS BY FATTY ACIDS

The finding that SREBPs regulate fatty acid synthesis genes, suggest that fatty acid biosynthesis is inhibited by sterols. This presents somewhat of a metabolic paradox. That is, since fatty acids, as precursors to phospholipids, are vital components of the plasma membrane, cell growth would be inhibited when exogenous sterol levels were high but fatty acids were low. Yet this is not the case, as cells cultured in fatty acid free media supplemented with cholesterol have normal proliferation rates. This means that there must be a mechanism by which the regulation of these pathways can be uncoupled in situations when the synthesis of only one of these lipids is required. We were able to provide evidence for such an uncoupling mechanism by demonstrating that the response of reporter constructs bearing classical SREs to sterols is diminished in CHO-K1 cells cultured in the absence of exogenous oleate (20). Even in the absence of sterols, oleate is capable of down regulating reporter constructs containing classical SREs (unpublished data and 21).

Mammalian plasma membranes contain an approximate 1:1 ratio of saturated to unsaturated fatty acids. Altering this ratio produces changes in membrane fluidity and can have dramatic effects on membrane functions, thus maintaining the optimal ratio is of great physiological importance. The predominant saturated fatty acids found in plasma membranes are palmitate and stearate. Endogenous palmitate is the end product of the activities of ACC and FAS, while stearate is produced from palmitate by the fatty acid elongation enzyme complexes associated with microsomes. Palmitate and stearate (as fatty acyl CoAs) are the precursors of the predominant unsaturated fatty acids found in plasma membrane lipids, the monounsaturated fatty acids, palmitoleate and oleate. Monounsaturated fatty acids can be obtained from exogenous sources or synthesized endogenously by SCD which catalyzes the Δ^9-*cis* desaturation of palmitoyl-CoA and stearoyl-CoA, producing palmitoleoyl-CoA and oleoyl-CoA, respectively(reviewed in 22).

SCD is the rate-limiting enzyme in the production of unsaturated fatty acids and has been studied primarily in lipogenic cells and tissues (reviewed in 23 and 24). A prime candidate for feedback regulation of SCD is oleic acid, as the end product of desaturase activity is oleoyl-CoA. However, it has been well documented that in lipogenic tissues (hepatic and adipose), saturated and monounsaturated fatty acids, such as stearate and oleate, have little or no regulatory effect on SCD gene expression.

In contrast, we have found that reporter constructs for the two stearoyl-CoA desaturase genes (SCD1 and SCD2) are repressed by oleate, as well as oxysterol, in CHO-K1 cells (Fig. 1). We suggest that lipogenic tissues may have tissue specific SCD regulatory mechanisms and that the regulation by oleate observed in rapidly proliferating, cultured fibroblasts reflects its use as a substrate for membrane phospholipid synthesis. The importance of oleate in proliferation is underscored by the isolation of a CHO-K1 mutant auxotrophic for oleate (25), demonstrating that oleate synthesis is required for cellular proliferation in the absence of exogenous oleate.

LIPID SIGNALING AND LIPID STRUCTURE

The feedback transcriptional regulation by sterols and fatty acids is reflected in both the rates of synthe-

sis, and more significantly, the actual cellular levels of these membrane lipid components. This is most clearly demonstrated with mutants of CHO-K1 cells (26, 27) that express either constitutively activated SREBPs or are deficient in SREBPs. These mutants are, respectively, defective in transcriptional down-regulation by sterols, or upregulation by sterol starvation, of genes encoding enzymes of cholesterol biosynthesis.

Somatic cell mutants resistant to killing by oxysterols in delipidized media exhibit constitutively activated SREBPs (27) and fall into two classes: class 1 mutants which produce a truncated SREBP-2 (28, 29) and class 2 mutants which are defective in signaling by SCAP (9, 30). CR1, a class 2 mutant isolated by our laboratory (31), exhibits defective regulation of cholesterol biosynthesis and plasma membrane cholesterol levels (31, 32) and a dramatically increased proportion of plasma membrane oleate (32) when cells are grown in medium supplemented with serum.

The CR1 mutant illustrates the regulatory nature of balancing plasma membrane cholesterol and unsaturated fatty acid levels. When grown in medium supplemented with serum—i.e. an exogenous source of both oleate and cholesterol—the increased plasma membrane levels of both lipids is structurally compensatory conserving the order parameter of the membrane lipid bilayer (32). When the CR1 mutant is grown in medium supplemented with cholesterol alone, the membrane lipid ordering is no longer maintained, resulting in altered function of such membrane enzymes as the Na^+/K^+-ATPase (33) and adenylate cyclase (34).

Experiments such as these, suggest that it is the ordering/fluidity of cell membranes, particularly the plasma membrane, which may be the regulated endproduct of membrane lipid biosynthesis rather than the chemical composition of these membranes. This concept, known as "homeoviscous adaptation" has been well described for both prokaryotic and eukaryotic organisms (35). In support of this idea, we have observed (36) that two reagents which fluidize membranes, the quaternary ammonium amphiphile, cetyl trimethylammonium bromide (CTAB) and ethanol, can stimulate cholesterol biosynthesis and membrane cholesterol levels.

A reporter construct for SRE1 can be shown to be up-regulated in response to CTAB treatment (Fig. 2A) consistent with the hypothesis that it regulates sterol biosynthesis transcriptionally (36). Noteworthy, is that this up-regulation does not occur in CR1, which has a defect in SCAP (37), suggesting that CTAB signaling proceeds through SCAP to activate SREBP processing.

Consistent with these findings on CTAB, Lange, Steck and co-workers have demonstrated that a structurally diverse array of amphiphiles (38) can upregulate sterol biosynthesis in cultured cells. These workers have presented evidence (39) that this effect is produced by inhibiting the internalization of plasma membrane cholesterol, thereby reducing the pool of sterol in the endoplasmic reticulum which regulates SREBP maturation. These observations are interpreted to suggest that the plasma membrane contains a "sensor" of the physical state of the plasma membrane lipid bilayer which above a certain threshold level of cholesterol results in a portion of the excess cholesterol being trafficked to the endoplasmic reticulum. A candidate sensor is the multidrug-resistant (MDR) P-glycoprotein, which has been shown by several laboratories to facilitate the internalization of plasma membrane cholesterol (40, 41). Such a model would explain how compounds as chemically diverse, as sterols, unsaturated fatty acid enriched phospholipids and amphiphiles can transcriptionally regulate genes whose promoters contain an SRE. The common regulatory feature is the physical state of the plasma membrane lipid bilayer.

In considering such a model, it should be noted that in many cells, cholesterol is not uniformly distributed in the plasma membrane. Rather, cholesterol is believed to be enriched in detergent-insoluble microdomains of two sorts: rafts and caveolae (for reviews see 41, 42). Both structures have been shown to participate in the internalization of plasma membrane cholesterol (43) to endocompartments. Also noteworthy, are reports (44, 45) that the candidate structural sensor, MDR P-glycoprotein, is enriched in caveolae. Treatment of cells with polyene antibiotics which bind cholesterol, such as filipin, disrupts the caveolar structure (46) and blocks the internalization of molecules which bind to receptors in the caveolae. We have found (Fig. 2B) that filipin treatment, which has previously been shown to block internalization of various receptors, localized to caveolae in CHO cells (47–50), also activates a reporter for SRE1 in these cells. This observation confirms the notion that cholesterol trafficking

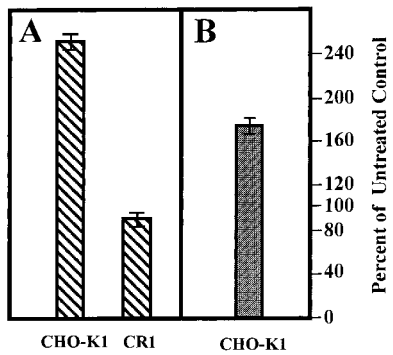

FIG. 2. Activation of transcription from SRE-1 by CTAB (A) or filipin (B). CHO-K1 and CR1 cells were stably transfected with pTK(KX3)LUC, a luciferase reporter gene driven by an artificial promoter bearing three tandem repeats of the LDL receptor SRE-1 inserted into the thymidine kinase promoter. Cells were incubated for 12 h in Hams' F-12 medium supplemented with 5% FBS and either 5 μM CTAB (A) or 1.3 μg/ml filipin (B).

from the plasma membrane to internal membranes can be modulated by structural organization of plasma membrane lipids. It also, suggests that the regulatory structure may be plasma membrane, cholesterol-rich microdomains.

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