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Polyunsaturated Fatty Acid Regulation of Gene Expression

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Abstract

Polyunsaturated fatty acids (PUFAs), specifically the n-3 and n-6 series, play a key role in the progression or prevention of human diseases such as obesity, diabetes, cancer, neurological and heart disease, mainly by affecting cellular membrane lipid composition, metabolism, signal-transduction pathways, and by direct control of gene expression. PUFAs show regulation of gene expression in several tissues, including brain, liver, heart, and adipose. Most recently, research has focused on identifying the mechanisms by which PUFAs regulate lipogenic gene expression. Research to date indicates that PUFA-mediated regulation of the genetic expression and proteolytic maturation of a group of transcription factors termed sterol regulatory element binding proteins (SREBPs) accounts for the suppression of hepatic lipogenic gene expression. However, our recent studies on the transcriptional regulation of the stearoyl-coenzyme A (CoA) desaturase gene, encoding a key enzyme in the cellular synthesis of monounsaturated fatty acids from saturated fatty acids indicates that PUFA can suppress gene transcription by a mechanism independent of SREBP maturation.

Index Entries: Stearoyl-CoA desaturase; PUFA; SREBP; gene expression.

Introduction

The n-3 and n-6 polyunsaturated fatty acids (PUFAs; linolenate and linoleate, respectively) are essential fatty acids that cannot be synthesized by mammals and therefore, must be obtained from plant sources. These fatty acids coordinately repress the expression of genes encoding lipogenic, glycolytic, and cholesterolgenic enzymes while they concomitantly increase expression of genes encoding enzymes involved in hepatic and skeletal muscle β-oxidation (Jump et al., 1994; Clarke et al., 1997; Kim et al., 1999; Xu et al., 1999). The immediate outcome of these events is a decrease in lipogenesis and an increase in fatty acid oxidation and ketogenesis. Thus, PUFAs are effective hypolipidemic agents.

The majority of research in the past few years had focused on PUFA-mediated regulation of gene expression in liver tissue and cultured hepatocytes and most of the original data derived from these studies suggested that the PUFA-mediated regulation of gene expression was liver-specific. However, PUFAs have recently been demonstrated as regulators of gene expression in other tissues as well. PUFAs have been shown to reduce lipogenic gene expression in adipose tissue of lean and obese Zucker rats (Jones et al., 1996) and shown to reduce the expression of stearoyl-coenzyme A (CoA) desaturase (Landschulz et al., 1994; Sessler et al., 1996), S14 (Mater et al., 1999b), and glucose trasporter-4 (Tebbey et al., 1994; Long and Pekela, 1996) genes in cultured 3T3-L1 adipocytes. PUFAs have also been reported to regulate gene expression in cells of the immune system (Tebbey and Buttke, 1993; Finstad et al., 1994; Deglon et al., 1995), the small intestine (Niot et al., 1997), pancreas (Brun et al., 1997), and brain of neonatal mice (DeWille and Farmer, 1993). Such diversity in sites of action suggests that PUFA regulation of gene expression is much more widespread than originally thought and therefore several mechanisms may be involved.

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The PUFA-mediated induction of genes of β-oxidation (i.e., acyl-CoA oxidase, carnitine palmitoyltransferase) has been shown by several research groups to be regulated by a common transcription factor termed peroxisome proliferator-activated receptor alpha (PPAR-α) (Gottlicher 1992; Mater et al., 1999a; Sessler and Ntambi, 1998). Both peroxisome proliferators and PUFAs repress lipogenic genes, such as S14 and fatty acid synthase (Blake and Clarke, 1990, Jump et al., 1995, Bing et al., 1996). It was then hypothesized that PUFAs activate PPARα and along with its heterodimer partner retinoid X receptor (RXR), exert positive and negative effects, on fatty acid β-oxidation and lipogenic genes, respectively. However, several lines of evidence including studies using the PPARα null mouse indicate that while PPARα mediates PUFA-induction of fatty acid β-oxidation gene transcription, it does not mediate PUFA-mediated repression of lipogenic gene transcription (Miller and Ntambi, 1996; Bing et al., 1997; Clarke et al., 1997; Sessler and Ntambi, 1998). The search for PUFA-specific transcription factor has now become an important focus in PUFA research.

Sterol Regulatory Element Binding Proteins (SREBPs) Mediate PUFA Repression of Gene Transcription

Recently, transcription factors termed the sterol regulatory element binding proteins (SREBPs) have been implicated in PUFA-mediated suppression of lipogenic gene transcription (Yahagi et al., 1999; Mater et al., 1999a). SREBPs are transcription factors that were first isolated as a result of their ability to bind to the sterol response element (SRE), therefore conferring sterol regulation to the genes involved in cholesterol synthesis (Brown and Goldstein, 1977). Transcriptional activation of genes containing SREs is known to be under the regulation of sterols through modulation of the proteolytic maturation of the SREBP-1 and SREBP-2 (Brown and Goldstein, 1977; Wang et al., 1994). Novel sequences in the PUFA-RE of several lipogenic genes that function as an SRE have been identified (Tabor et al., 1998; Mater et al., 1999a; Ntambi, 1999). Some of these sequences, although distinct from previously described SREs, have been shown to bind purified SREBP.

The SREBPs are inserted into the membrane of the endoplasmic reticulum envelope in a wide variety of tissues. In sterol-deficient cells, proteolytic cleavage of SREBPs releases their N-terminal mature forms from the membrane, enabling them to enter the nucleus, where they bind to the SREs and activate genes involved in cholesterol, triglyceride, and fatty acid biosynthesis (Brown and Goldstein, 1977; Wang et al., 1994; Korn et al., 1998; Shimomura et al., 1998). It has been shown in vitro that in the presence of sterols or PUFAs, the proteolytic cleavage of the SREBPs is inhibited, and the expression of promoters with SREs is reduced because little SREBP is available to activate their transcription (Tabor et al., 1998; Worgall, 1998). In addition, overexpression of SREBP significantly reduced PUFA-inhibition of SRE-dependent genes (Worgall et al., 1998; Mater et al., 1999b).

There are in vivo observations that implicate SREBPs in the PUFA-mediated repression of lipogenic gene expression. The nuclear abundance of SREBP-1 and mRNA have been found to be reduced by fasting and greatly increased by refeeding a high carbohydrate diet (Horton et al., 1998a). In addition, changes in the nuclear content of SREBP-1 resulting from starving-refeeding displayed a temporal pattern that is similar to the pattern observed for several lipogenic genes (Horton et al., 1998b). In light of these observations, it was postulated that PUFAs coordinately suppress the transcription of hepatic genes by suppressing the expression of SREBP-1 (Xu et al., 1999). When rats were fed fat-free diets supplemented with n-6 and n-3 PUFAs the hepatic levels of SREBP as well as that of several lipogenic genes were dramatically reduced (Kim et al., 1999; Xu et al., 1999). In addition, it was demonstrated that upon feeding transgenic mice overexpressing SREBPs with diets enriched in fish oils, the mRNA expression of the SREBP-activated lipogenic genes was no longer completely responsive to PUFA repression (Yahagi et al., 1999). However, experiments indicate that PUFA-mediated suppression of the SREBP gene occurs at a post-transcriptional level, possibly at the level of mRNA stability (Xu et al., 1999). In light of both the in vivo and in vitro observations, it is postulated that PUFAs coordinately suppress the transcription of hepatic genes by suppressing the expression of SREBP-1 as well as its maturation (Fig 1).

It may, however, be premature to conclude that PUFA-mediated suppression of lipogenic gene expression is entirely SREBP-dependent. Recently, we have generated evidence using the stearoyl-CoA desaturase gene that indicates that PUFA-mediated suppression of lipogenic gene transcription can occur through a mechanism independent of SREBP maturation. Stearoyl-CoA desaturase (SCD) is the rate-limiting enzyme in the cellular synthesis of monounsaturated fatty acids from saturated fatty

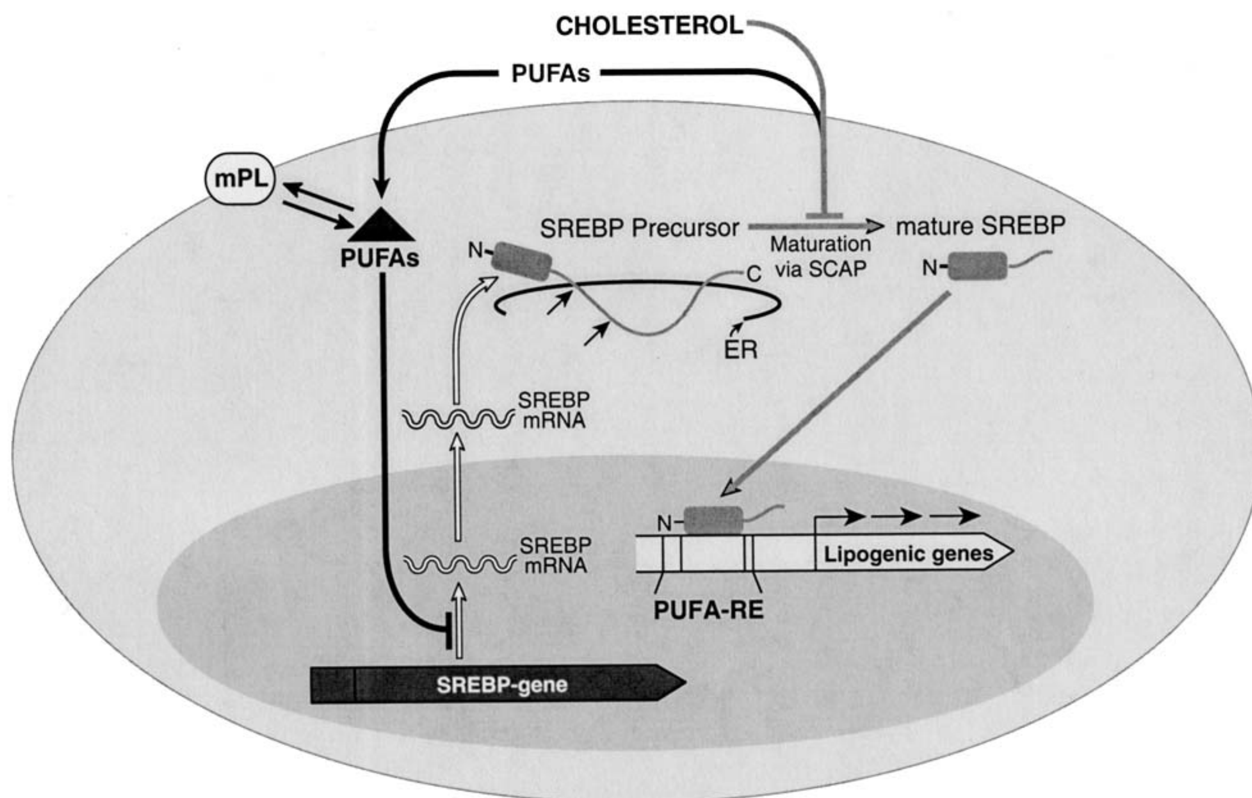

Fig. 1. Pathways of lipogenic regulation by PUFA and cholesterol. PUFAs either from the diet or membrane phospholipid (mPL) independently repress the expression of the SREBP mRNA at a post-transcriptional level. In addition, PUFAs and cholesterol repress the maturation of the sterol regulatory element binding protein (SREBP), via the SREBP cleavage-activating protein (SCAP), that would otherwise translocate from the endoplasmic reticulum (ER) into the nucleus to activate the transcription of the lipogenic genes by binding to the sterol regulatory element (SRE).

acids. We showed previously that linoleic acid, arachidonic acid (AA), and eicosapentanoic acid (EPA) repress the transcription of the SCD1 gene in HepG2 cells (Waters et al., 1997) and recently an SRE has been identified in the PUFA-RE present in the promoter region of the SCD1 and SCD2 genes (Tabor et al., 1998; 1999). To observe the regulatory effects of mature SREBP and PUFAs on the activity of SCD1 gene promoter, we transiently co-transfected 20, 50, and 150 ng (per 6-cm dish) of plasmid DNA containing the sequence-encoding mature SREBP-1a and 5 µg 600-SCD1/luciferase promoter/reporter construct (Tabor et al., 1999) into HepG2 cells. The transfections were carried out in the presence of cholesterol to inhibit the maturation of the endogenous SREBP and thus ensure that there was little mature form of the endogenous SREBP present in the cells. After transfection, the cells were rinsed twice with PBS and treated in Williams E media supplemented with AA, EPA, and docosahexanoic acid (DHA) as albumin complexes and luciferase activity was assayed (Dynex Technologies MLX Microtiter Plate Luminometer). Data were normalized to cell extracts expressing the *Renilla* luciferase as an internal control. If PUFAs inhibit binding of mature form of SREBP to the SRE or act through some other post-translational mechanism, the suppressing effects of PUFAs on the SCD1 promoter activity would not diminish in the SREBP-transfected cells. Cells were co-transfected with increasing levels of mature SREBP-1a expression plasmid, which led to an increase in SCD1 promoter activities by 40–300-fold (Fig 2A), consistent with the results of Tabor et al. (1999). What we found is that in these cells, when treated with 200 µ*M* DHA, promoter activity continued to show the ~40% repression seen in the absence of exogenous SREBP 1a. We then expanded the treatments to include several fatty acids at different levels. AA, EPA, or DHA continued to repress SCD1 promoter activity when added to SREBP-1a co-transfected cells in a dose-dependent manner (Fig 2B). Thus, SREBP maturation does not seem to exhibit

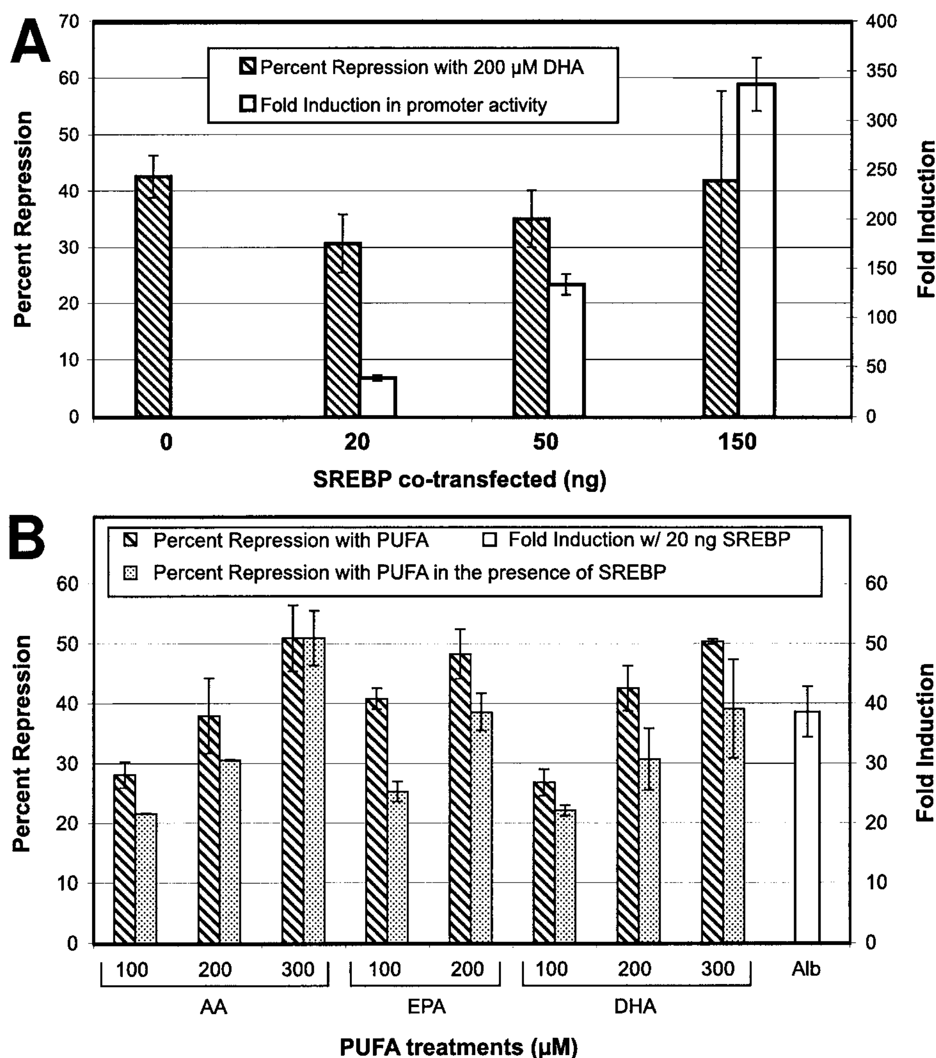

Fig 2. Evidence for an SREBP-independent mechanism for PUFA-mediated suppression of stearoyl-CoA desaturase gene. **(A)** HepG2 cells were transfected with 600 mSCD1-luciferase pGL2 (5 µg/plate) and pCI-mSREBP1a (0, 20, 50, and 150 ng/plate). Cells were treated with 200 µ*M* DHA as a complex with albumin for 24 h. **(B)** HepG2 cells were transfected with 600 mSCD1-luciferase pGL2 (5 µg/plate) and pCI-mSREBP1a (0, 20 ng/plate). Cells were treated with PUFAs at the indicated concentration for 24 h. Following transfection, luciferase activity was assayed using a luminometer. The open bar shows the fold induction of mSCD1 promoter activity upon co-transfection with SREBP-1a. mSCD1, mouse stearoyl-CoA desaturase 1; Alb, albumin treatment alone.

the selectivity required to explain PUFA control of SCD1 gene transcription, suggesting that PUFAs may utilize a different protein in addition to the SREBP to repress SCD1 transcription. Therefore, although there are indications that SREBP maturation as well as binding of the SREBP to the PUFA-RE are involved in PUFA repression of lipogenic genes, there is strong evidence for the existence of an SREBP-independent mechanism through which PUFAs repress the expression of SCD1 gene transcription and possibly other lipogenic genes.

Dietary saturated and monounsaturated fatty acids cannot traverse the blood-brain barrier (BBB) for use by the brain cells and therefore have to be synthesized *de novo* (Marbois et al., 1992). The high level of lipid biosynthesis by the brain is correlated with high expression of SREBP mRNA (Hua et al., 1993). Despite the high levels of SREBP, PUFA feeding in form of fish oil (DHA) and safflower oil has no effect on expression of SCD2 in adult mouse brain (Miyazaki and Ntambi, unpublished). However, in nursing pup mice, SCD2 gene expression in the brain

was reduced by maternal feeding of an essential fatty acid-deficient diet compared with control-fed mothers (DeWille and Farmer, 1992), suggesting upregulation of SCD2 gene expression by PUFAs. High levels of delta 6-desaturase mRNA have also been reported in rat brain (Cho et al., 1999) indicating that the brain actively desaturates fatty acids despite high levels of PUFAs in the form of DHA and EPA.

Conclusions

The mechanisms by which PUFAs control gene expression in different tissues are still being unraveled. The involvement of the SREBPs in the regulation of genes involved in fatty acid biosynthesis suggests molecular interactions between fatty acid and cholesterol metabolism. The PUFA regulation of SREBP that accounts for PUFA-mediated suppression of gene expression seems to be gene- and liver-specific, suggesting that PUFAs use other mechanisms to regulate tissue-specific gene expression. The reasons for the insensitivity of the adult brain to PUFA-mediated repression of gene expression are still unclear. One possible explanation of this could be that during periods of high myelination (mouse day 20), that incorporation of PUFAs into myelin accounts for the de-repression of lipogenic genes seen (Garbay et al., 1992), and that in adults a baseline expression is maintained at least partially due to the specific maintenance of PUFA levels. Continued research on the effects of PUFAs on gene expression should provide insight into diseases such as obesity, diabetes, atherosclerosis, hypertension, cancer, and other disorders associated with fat metabolism.

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