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THE REGULATION OF STEAROYL-CoA DESATURASE (SCD)

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I. INTRODUCTION

Saturated fatty acids are known to be the precursors of unsaturated fatty acids in higher organisms. However, the control mechanisms that govern the conversion of saturated to unsaturated fatty acids are largely unexplored. The balance of these fatty acids forms the composition of stored triacylglycerides and impacts on the physical properties of membranes. Regulation of unsaturated fatty acids is also important because they play a role in cellular activity, metabolism and nuclear events that govern gene transcription. A critical committed step in the biosynthesis of unsaturated fatty acids is the introduction of the first cis-double bond in the Δ^9 position (between carbons 9 and 10). This oxidative reaction is catalyzed by the iron-containing stearoyl-CoA desaturase enzyme (Fig. 1). During desaturation, the electrons flow sequentially from NAD(P)H, through NADH-cytochrome b_5 reductase, to cytochrome b_5, to the terminal Δ^9 desaturase (stearoyl-CoA desaturase), and finally to active oxygen which is reduced to H_2O. Stearoyl-CoA desaturase, thus, catalyzes the Δ^9-*cis* desaturation of a spectrum of methylene-interrupted fatty acyl-CoA substrates.[1,2,3] The preferred substrates, palmitoyl- and stearoyl-CoA, are converted into palmitoleoyl- and oleoyl-CoA, respectively.[3] Palmitoleic and oleic acids are the major constituents of membrane phospholipids and triacylglycerol stores found in adipocytes.[4] The ratio of stearic acid to oleic acid is one of the factors influencing cell membrane fluidity. Alteration of this ratio is important in various diseases such as aging, cancer, diabetes, obesity, hypertension and neurological, vascular and heart diseases.[5,6]

Many investigators have attempted to elucidate the metabolic regulation of the stearoyl-CoA desaturase enzyme. Early studies focused on the characterization of the microsomal stearoyl-CoA desaturase of several mammalian tissues employing spectral and isotopic methods and partially purified components.[1,7–28] It was not until the three enzyme components of the desaturase system (NADH-cytochrome b_5 reductase, cytochrome b_5, and the terminal desaturase) were purified from rat liver[2,29,30] that substrate specificity, enzyme–substrate interactions, the role of non-substrate lipids, tissue distribution, physiological factors affecting rates, and mechanisms of desaturation and electron transport were elucidated.[3,30–34] Further studies, conducted mainly in rats, revealed that, of the three enzyme components of the desaturase system, only the terminal desaturase's activity was sensitive to changes in diet, hormonal balance, developmental processes, temperature changes, metals, alcohol, peroxisomal proliferators and phenolic compounds.[35–54,127–129]

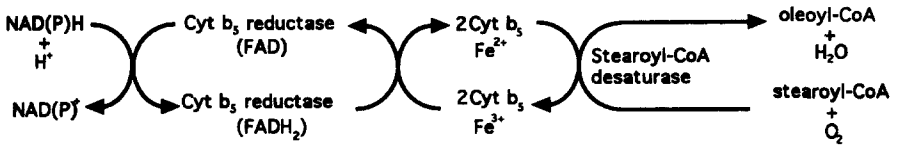

Fig. 1. The pathway of electron transfer in the desaturation of fatty acids by stearoyl-CoA desaturase.

Due to the difficulty of purifying mammalian desaturases[38] (possibly because they are integral membrane proteins), few mechanistic studies utilizing purified desaturases have been reported. Recently, overexpression of the active enzyme in bacteria has overcome these difficulties, allowing further studies of the enzyme and its mechanism of action.[55] This approach has been feasible because of the availability of cloned rat stearoyl-CoA desaturase cDNAs.[56] The cDNAs for the mouse and human stearoyl-CoA desaturase have also recently been cloned.[57–59,61] The availability of these cDNAs has led to the isolation and characterization of the rat and mouse stearoyl-CoA desaturase genes and has revealed the amino acid sequences of the mouse, rat and human proteins. Study of the genes *in vivo* and in tissue culture systems has permitted the measurement of mRNA levels and gene transcription rates during preadipocyte cell differentiation and in response to hormonal and dietary treatments.[57–61] The promoter regions of these genes have been isolated, and studies of the interaction of specific *cis*-acting DNA elements with *trans*-acting factors that mediate preadipocyte cell differentiation as well as hormone and dietary responses have begun.

It is the goal of this review to provide an overview of what is known about the structure and the regulation of the stearoyl-CoA desaturase gene(s) during differentiation and in response to diet and hormones.

II. ISOLATION AND CHARACTERIZATION OF SCD CDNAS AND GENES

Several years ago, a number of cDNAs which increase in abundance upon differentiation of 3T3-L1 preadipocytes into adipocytes were isolated.[61] Based on both nucleotide and amino acid sequence homology to the cloned rat liver stearoyl-CoA desaturase,[56] one of the cDNA clones was determined to encode the mouse homologue of stearoyl-CoA desaturase (SCD1; 57). Genomic Southern blot analysis using SCD1 cDNA fragments as radioactive probes indicated the existence of another closely-related gene.[57] The cDNA corresponding to the second stearoyl-CoA desaturase gene (SCD2) was then isolated.[58] Recently, a segment of 712 bases coding for part of the human stearoyl-CoA desaturase gene was made from human adipose tissue cDNA by the polymerase chain reaction (PCR) using primers based on published rat cDNA sequences.[59] When compared, the coding regions of the human, rat and the mouse cDNA sequences were strikingly similar, with over 80% nucleotide sequence identity. The sequences are also highly conserved at the protein level. The similarities of deduced human peptide sequence with the mouse SCD1 and SCD2 are 93% and 92% identical, respectively,[59] showing a highly conserved carboxyl-terminal half of the enzyme.

The mouse and rat cDNAs contain unusually long 3'-untranslated sequences that are derived from single exons and range from 3457 to 3658 nucleotides.[56–58] Usually, such long 3'-non-coding stretches contain multiple polyadenylation signals that result in mRNAs of differing lengths.[62–65] However, the rat and mouse cDNAs have a single functional polyadenylation signal sequence (AAUAAA), 24–30 nucleotides from the poly(A)$^+$ track.[56–58] The functional role of such a large untranslated sequence at the 3'-end of the desaturase genes is presently unknown. It is possible that this sequence influences the mRNA stability and thereby plays a role in the regulation of expression of the desaturase genes.

Using the cDNAs as probes to screen genomic or cosmid libraries, genes encoding both SCD1 and SCD2 have been isolated from the rat and mouse and characterized.[57,58,60] Both genes are structurally similar, having 5 exons and 6 introns (Fig. 2). The promoter regions of both the mouse and rat SCD1 genes contain a sequence CTGAGGAAA at positions −74 to −66.

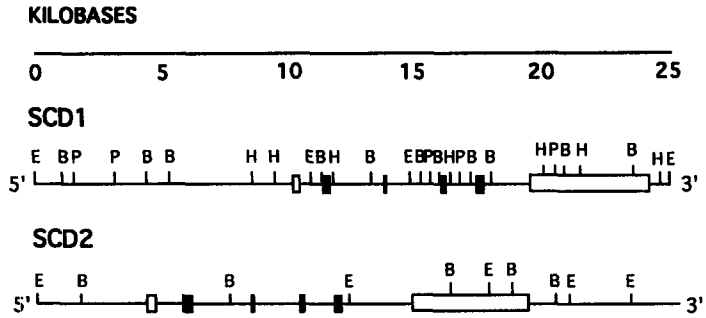

Fig. 2. Structural organization of the mouse SCD1 and SCD2 genes (from Refs 57 and 58).

This sequence is 75% identical to the fat-specific element 2 that has been found in the 5'-flanking regions of several genes activated during differentiation of preadipocytes.[66-68] In addition, within the first 200 bp of the 5'-flanking region of the mouse SCD1 gene, there is a pyrimidine-rich region containing 12 tandem repeats of the sequence TCCC. The mouse SCD2 gene completely lacks this repeated sequence, while the rat SCD1 has only six of these repeats. The significance of this unusual sequence is as yet unknown. Although certain regions of the promoter of the two mouse genes differ markedly, there is one region with high nucleotide sequence homology. The sequence between −201 and −54 in the SCD2 gene is 77% identical to the sequence between −472 and −325 in the SCD1 gene, including conservation of the two CCAAT "boxes". This marked sequence homology suggests that duplication occurred during evolution of the SCD gene family. Presumably, either a sequence insertion or deletion occurred after gene duplication to yield the different promoters for SCD1 and SCD2 genes. In addition, it appears that within the mouse and rat genomes there may be a third related gene, as the SCD1 and SCD2 genes do not account for all bands detected in genomic Southern blots.[57,58,60] Furthermore, a TaqI restriction fragment length polymorphism in the stearoyl-CoA desaturase gene in DNA from purebred Japanese black cattle has been reported.[130]

The yeast desaturase gene has also been cloned,[69] and has greater than 50% amino acid sequence similarlity with the mouse and rat desaturases within the coding region.[70] However, the yeast SCD promoter region has no homology with the promoters of the mammalian SCD genes. Very little homology exists between the plant and the known animal SCD genes, except for the conservation of the Histidine-containing motifs that have been identified within their coding regions.[71]

III. REGULATION OF MOUSE SCD GENES DURING 3T3-L1 PREADIPOCYTE DIFFERENTIATION

The mouse embryo-derived 3T3-L1 preadipocytes first described by Green and Kehinde[72-74] represent a useful model system for studying the mechanisms of cellular differentiation and development. Under the appropriate stimuli, these cells differentiate in culture, acquiring the morphological and biochemical characteristics of adipocytes.[75-79] This differentiation is accompanied by a dramatic increase in the cellular levels of the stearoyl-CoA desaturase,[4] as well as other lipogenic proteins, such as the insulin receptor,[80] characteristic of adipocytes. This increase in stearoyl-CoA desaturase activity has been shown to result from an increased rate of transcription of the SCD1 gene.[81] Addition of retinoic acid to 3T3-L1 cells, concomitant with the adipogenic agents that induce differentiation, inhibits 3T3-L1 cell differentiation and represses the transcription of the SCD1 gene.[82]

Transfection experiments using chimeric SCD1 promoter-chloramphenicol acetyltransferase (CAT) gene constructs in 3T3-L1 preadipocytes showed that the first 363 bp of the SCD1 5'-flanking region possessed promoter activity.[57] This activity was enhanced by cAMP, one of the adipogenic agents known to induce differentiation of 3T3-L1 preadipocytes.[57] However, the expression of the SCD1.CAT construct did not mimic the kinetics of expression of the

endogenous SCD1 gene, as measured by RNA accumulation during the course of differentiation.[57,61] One possible explanation for this discrepancy is that a combination of or the spatial arrangement of regulatory elements in the endogenous SCD1 gene (some of which may not be present in the 363 bp promoter fragment) are determinants not only for the level of expression, but also for the time when the gene is expressed during the differentiation program. The other possibility is that the 363 bp sequence is not sufficient for differentiation-dependent expression in 3T3-L1 preadipocytes. Larger chimeric gene constructs have been made and are now being tested for their differentiation-dependent expression in 3T3-L1 cells.

Another approach to defining the mechanism responsible for activating the expression of the SCD1 gene is looking for the binding of nuclear factors to the promoter. DNase1 footprinting and gel retardation assays have identified two binding regions within the SCD1 promoter that interact with nuclear factors present in differentiated 3T3-L1 adipocyte extracts.[83] One of the differentiation-induced factors was identified as C/EBPα, a nuclear DNA-binding factor first isolated from rat liver. The identity of the second SCD1 nuclear DNA-binding protein, designated SCD1/BP, is presently unknown. Co-transfection of SCD1 promoter-CAT constructs with a C/EBPα expression vector into 3T3-L1 preadipocytes and their use in a cell-free transcription system with recombinant C/EBPα demonstrated that C/EBPα can transactivate the SCD1 promoter and other adipocyte-specific gene promoters.[83–86] These studies implicated C/EBPα in the coordinate transcriptional activation of differentiation-specific gene expression. The SCD2 gene also has sequences with homology to the C/EBPα binding sequence[58] but protein binding and transactivation studies have not yet been carried out. C/EBPα seems to be a general transcription factor, since it binds to many non-adipocyte genes, including the thymidine kinase gene promoter and the murine sarcoma virus (MSV) LTR.[87,88] Thus other factors are likely to impart a differentiation-specific expression of the SCD1 gene and of other adipocyte-specific genes.

Deletion analysis has revealed the presence of a negative regulatory domain in the promoter region of the SCD1 gene (J. M. Ntambi, unpublished results). Deletion of this region results in the constitutive expression of the gene in 3T3-L1 preadipocytes, even in the absence of the adipogenic agents (methylisobutylxanthine, dexamethasone and insulin) that are used to induce differentiation. Another study using transient transfection with chimeric SCD2 promoter-CAT gene constructs has revealed a preadipocyte repressor element (PRE) capable of repressing transcription of the reporter gene in preadipocytes, but not in adipocytes.[89] Furthermore, a 58 kDa nuclear protein, present in 3T3-L1 preadipocytes and Hela cells, but lacking or inactive in adipocytes, binds to this localized element between −435 and −410 bp in the promoter.[89] A sequence homologous to the PRE has been identified recently in the SCD1 promoter.[106] Therefore, the PRE and its binding protein may regulate transcription of the SCD1 and SCD2 genes, and possibly other adipocyte-specific genes, by maintaining them in the repressed state until adipocyte differentiation is initiated (Fig. 3). The repression is relieved only upon treatment of cells with exogenous adipogenic agents that are suspected of promoting dissociation of the repressor protein and the negative regulatory domain. This could happen by either repressing the synthesis of the repressor or by promoting the *de novo* synthesis of a factor that leads to removal of a repressor. Once the repressor is removed, C/EBPα, SCD1/BP and other unidentified proteins bind to the SCD genes and activate their transcription during the differentiation of 3T3-L1 preadipocytes into adipocytes. The generality of this model is uncertain as PRE-like sequences have not been identified in the promoters of other adipocyte-specific genes.

IV. TISSUE-SPECIFIC EXPRESSION OF SCD GENES

Under normal dietary conditions, mouse and rat SCD1 mRNAs are expressed constitutively in adipose tissue but are not expressed in liver.[57,58,60] Their expression is markedly induced in liver in response to a fat-free, high carbohydrate diet, but to a lesser

extent in kidney, lung, spleen and heart. SCD1 mRNA expression is also subject to negative control in liver by feeding chow diet or a fat-free, high carbohydrate diet supplemented with polyunsaturated fatty acids.[90] Studies in humans showed that human SCD1 mRNA is expressed in colonic and esophageal carcinomas and in hepatocellular adenoma; however no consistent trend was seen in hepatocellular carcinoma, suggesting that certain classes of tumors may exhibit increased levels of SCD1 mRNA.[59] The expression of SCD1 mRNA in certain tumors is in agreement with accumulating evidence supporting the hypothesis that the ratio of stearic to oleic acid is important in cancer promotion.[93] This may be consistent with the previous finding that oleic acid is associated with certain tumors. Higher levels of oleic acid in malignant cells accounts for the increased membrane fluidity. In general, increased fluidity leads to increased cell metabolism and higher division rates, characteristic of cancer cells. More work will be needed to elucidate the role of the desaturase enzyme in cell membrane fluidity in normal and neoplastic cells.

The tissue distribution and the dietary alteration of the mouse and rat SCD2 mRNAs differs markedly from that of SCD1, being constitutive in brain and induced in kidney, lung, spleen and adipose tissue in response to a high carbohydrate diet. They are not expressed in liver under either dietary condition.[58,59] The SCD2 expression in brain is induced during the development of neonatal mice,[91] reduced in quaking brain of neonatal mice[124] and down-regulated during the development of mouse lymphocytes.[92]

The differences in the tissue-specific expression and dietary induction demand distinct control mechanisms for expression of each of the two genes, and the differences are currently being exploited to study tissue-specific gene expression.

V. HEPATIC REGULATION OF SCD1 GENE EXPRESSION BY CARBOHYDRATE

Hepatic stearoyl-CoA desaturase levels in rats can be increased 40-fold by a shift from a natural diet, containing unsaturated triacylglycerides, to a regimen of fasting followed by refeeding a fat-free, high carbohydrate diet.[94] This increase in enzyme activity is paralleled by an increase in SCD1 mRNA in total liver mRNA.[56] Hepatic SCD1 mRNA levels in mice are similarly induced by this feeding schedule.[57] SCD2 mRNA is, however, not expressed during either of these dietary conditions in liver.[58] Figure 4 shows that the induction of SCD1

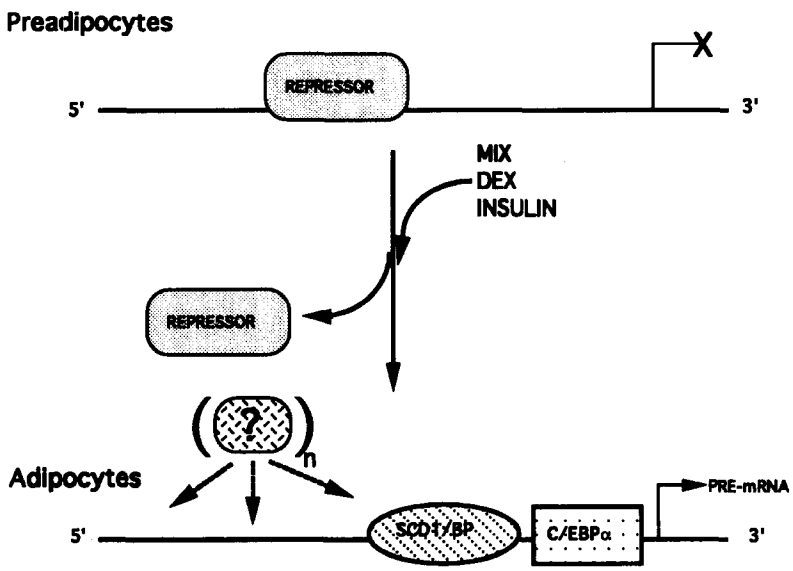

Fig. 3. Proposed mechanism of induction of SCD1 gene expression during 3T3-L1 preadipocyte cell differentiation. MIX, methylisobutylxanthine; DEX, dexamethasone. MIX, DEX and insulin are the adipogenic agents used to induce differentiation of 3T3-L1 preadipocytes into adipocytes in culture. C/EBPα, CCAAT box enhancer-binding protein; SCD1/BP, unidentified SCD1 binding protein; n, unidentified protein factors; X, no SCD1 mRNA expression.

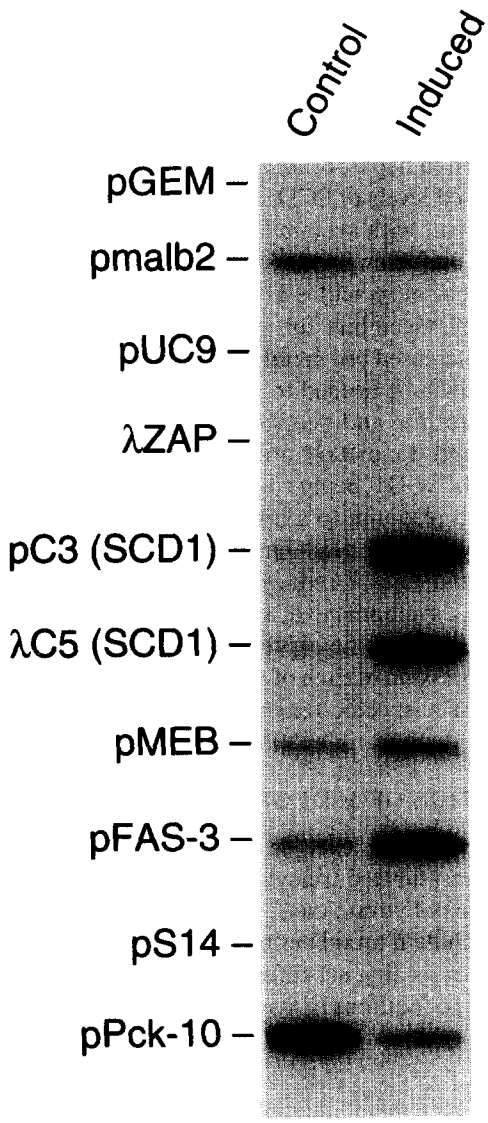

Fig. 4. Effect of a fat-free diet on the transcriptional rate of the SCD1 gene in mouse liver. Four control and four mice that had been fed a fat-free, high carbohydrate diet were sacrificed after 24 h and liver nuclei were isolated for *in vitro* run-on transcription. pGEM, pUC9 and λZAP, vectors; pmalb2, cDNA for mouse liver albumin; pC3 and λC5, are overlapping cDNA probes spanning the entire SCD1 gene (57); pFAS-3, cDNA for mouse liver fatty acid synthase; pMEB, cDNA for rat liver malic enzyme; S14, cDNA for rat liver S14 protein; pPck-10, cDNA for rat liver cytosolic phosphoenolpyruvate carboxykinase. The data are representative of three different experiments yielding essentially identical results (from Ref. 90).

mRNA by a fat-free high carbohydrate diet results from increased rates of gene transcription.[90] This induction is slow compared to that of the mouse fatty acid synthase gene and the rat S14 protein gene, whose transcriptional rates increase rapidly in response to feeding a fat-free, high carbohydrate diet.[95,96] These differences suggest that distinct mechanisms are involved in the dietary regulation of various hepatic genes involved in lipid metabolism.

In a recent study, it has been shown that unlike glucose, fructose induces a 3-fold increase in SCD1 gene transcription in a diabetic mouse *in vivo*.[97] The 10-fold induction of SCD1 gene transcription observed previously in the normal mouse fed a fat-free, high carbohydrate diet,[90] could not be achieved by fructose alone, but instead required a combination of fructose and insulin or glucose and insulin. This suggests that both insulin and carbohydrate metabolism are involved in the regulation of stearoyl-CoA desaturation. The exact

mechanism by which dietary fructose regulates the expression of the SCD1 gene is still unclear. One possibility is that the low insulin levels associated with the diabetic state result in greatly reduced levels of glucokinase activity while fructokinase and triosekinase are unaffected.[98] Consequently, fructose, unlike glucose, can stimulate lipogenesis in liver tissue.[99] It is also possible that dietary fructose activates glucokinase by binding to its regulatory protein in liver.[100] By this mechanism, glucose would be metabolized in the diabetic state in the absence of insulin. Therefore, the molecule that is regulating SCD1 could be a common metabolite of glucose and fructose.

Transcription studies on the genes encoding S14 protein and pyruvate kinase, using primary rat hepatocyte cultures, have led to the identification of a carbohydrate responsive element within their promoters.[101–103] Protein factors of the *c-myc* family that bind to this carbohydrate responsive element have been described.[104] Since it is not yet known whether a similar element exists in the SCD1 gene and the other genes that are regulated by a fat-free, high-carbohydrate diet, the role of this putative regulatory element in carbohydrate control of gene expression is still unknown. The differences present in the promoter sequences of the SCD1 and SCD2 genes that we reported previously,[58] may be responsible for the differences in the expression of these two genes in response to a fat-free diet in mouse liver. These differences are now being exploited in the identification of carbohydrate regulatory DNA sequences, and other *cis*-regulatory elements that may be involved in carbohydrate activation of gene expression.

VI. REGULATION OF STEAROYL-COA DESATURASE GENES BY UNSATURATED FATTY ACIDS

Dietary polyunsaturated fatty acids (PUFAs) have been shown to suppress the activity of stearoyl-CoA desaturase.[105] Concurrently, very low levels of hepatic SCD1 mRNA are expressed when rats and mice are fed on a regular chow diet.[56–58,90] It is possible that a fat component in the regular chow diet may be repressing the expression of the SCD1 gene, and upon its removal (i.e. by feeding a fat-free diet), the expression of the gene is increased. In support of this hypothesis, levels of SCD1 mRNA decrease rapidly to normal levels within 24 hr when mice are switched from a fat-free, high carbohydrate diet to chow diet.[90] Figure 5 shows that the fat-free, high carbohydrate diet, supplemented with various triacylglycerides containing polyunsaturated fatty acids, decreases the levels of SCD1 gene transcription whereas triacylglycerides containing saturated and monounsaturated fatty acids have very little effect.[90] Of the polyunsaturated triacylglycerides tested, triarachidonin (20:4*n*-6) had the most dramatic effect. Similar results have been obtained recently using primary cultured rat hepatocytes.[106] Unsaturated fatty acids have been shown to repress the transcription of the yeast stearoyl-CoA desaturase gene as well.[69,70,107,125] In yeast, the transcriptional control was attributed to the monounsaturated and polyunsaturated fatty acids that contained a double bond between carbon 9 and 10 in their acyl chain.[107] Monounsaturated fatty acids containing double bonds in the Δ-10, 11 or 5 positions had no repressive activity.[107] As in the mouse studies, 18:2(*n*-6) was more potent than 18:1(*n*-9); although it should be noted that the 18:1(*n*-9) had some repressive activity in yeast[107] but not in mice.[90] Taken together, these observations seem to indicate that the repression is related to the length, structure and number of double bonds in the fatty acid chains.

Dietary PUFAs have also been shown to decrease mRNA abundance and transcription of several rat hepatic lipogenic genes such as S14 protein, glucose-6-phosphate dehydrogenase and fatty acid synthase.[108,109,125] This observation suggests that PUFAs, or their metabolic products, regulate the expression of several genes involved in lipid metabolism, and that these genes may share common *cis*-regulatory elements and/or *trans*-acting factors that mediate PUFA repression of gene transcription. While the molecular mechanisms of PUFA-mediated inhibition of gene transcription remain unclear, our understanding of the regulation of SCD1 gene expression makes this an attractive model for examining specific aspects of PUFA action at the molecular level. It is possible that,

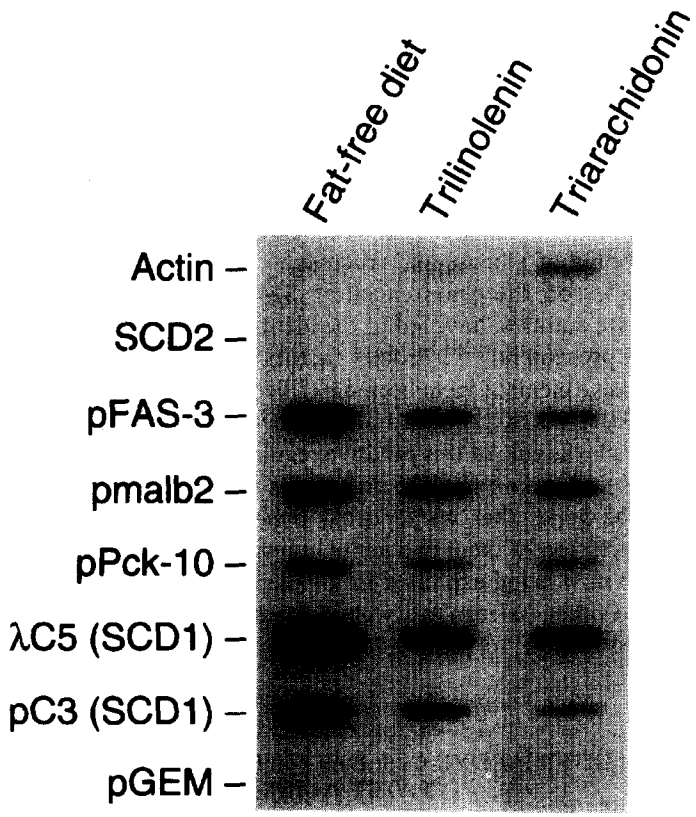

Fig. 5. Effect of triacylglycerides on SCD1 gene expression. Nuclei were isolated from livers of starved mice that had been refed a fat-free, high carbohydrate diet supplemented with 10% trilinolenin and 1% triarachidonin. Run-on transcription and hybridization with cDNA probes and vector sequences were carried out. pmalb2, mouse albumin; pFAS-3, mouse liver fatty acid synthase; SCD2, mouse SCD2; pC3 and λC5, are overlapping cDNA probes spanning the entire SCD1 gene (57); Actin, human actin; pPck-10, rat liver cytosolic phosphoenolpyruvate carboxykinase; pGEM, pGEM vector. The data are representative of three different experiments yielding essentially identical results (from Ref. 90).

because the stearoyl-CoA desaturase enzyme mediates the desaturation of fatty acids synthesized by the fatty acid synthase enzyme system, repression of SCD1 gene expression by PUFAs would lead to the accumulation of palmitoyl-CoA and stearoyl-CoA. This would be expected to feedback inhibit fatty acid synthase and acetyl-CoA carboxylase enzyme activity (and possibly gene expression) thereby inhibiting fatty acid biosynthesis. Moreover, as suggested by Clark and Jump,[110] reduction in the availability of Δ^9 fatty acids, and their subsequent incorporation in biological membranes, would allow the biological processes dependent on essential fatty acids to continue. We are currently studying mechanisms by which the PUFAs and their metabolites act to repress the transcription of the mouse SCD1 gene both *in vivo* and *in vitro*. If a *cis*-regulatory DNA element exists in the SCD1 gene, it could be a target for manipulation of SCD1 gene transcription and, eventually, modification of general hepatic fatty acid synthesis.

Recently, Tebbey and Buttke[92] have found that arachidonic acid has a rapid inhibitory effect on SCD2 gene transcription in the mouse cell line BW5147. This repression is probably due to arachidonic acid itself, since the inhibitory effect did not require oxidation of arachidonic acid by either the lipoxygenase or cycloxygenase pathways and was not observed with metabolites of arachidonic acid.[111] Because PUFAs increase SCD2 mRNA levels in neonatal mice brains,[126] this observation suggests that PUFAs regulate the expression of specific genes in non-hepatic tissues as well. Positive and negative regulation of SCD gene expression by fatty acids in different tissues and species demonstrates that the regulatory effects of fatty acids on gene expression are more widespread than was originally thought.

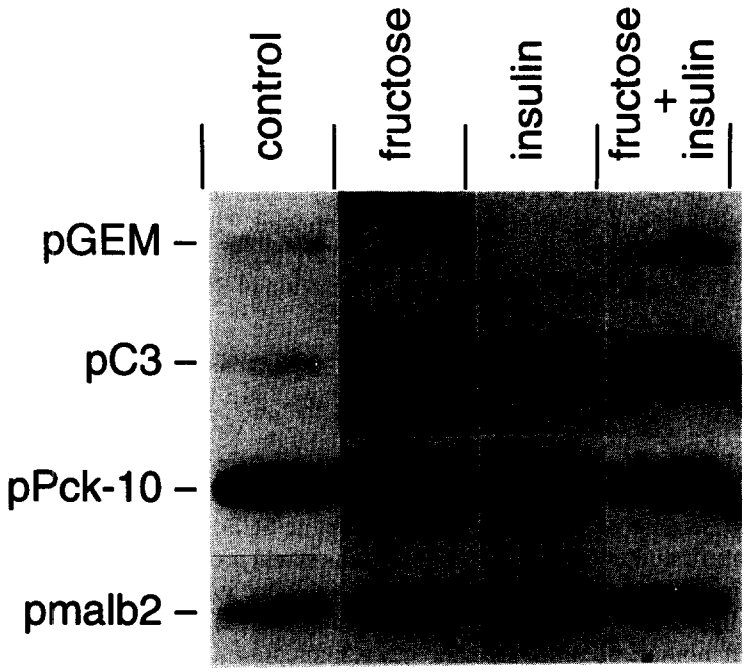

Fig. 6. Effect of insulin and dietary fructose on the transcription of the SCD1 gene in diabetic mouse liver. Gene transcription activity was determined by nuclear run-on assay. Diabetic mice were fasted and then refed fructose or injected with insulin for 24 h. ^{32}P-labeled mRNA was isolated and hybridizations to 5 μg of immobilized DNA probes were carried out. pGEM, pGEM plasmid; pmalb2, cDNA for mouse liver albumin; pC3, cDNA for mouse SCD1; pPck-10, cDNA for rat liver cytosolic phosphoenolpyruvate carboxykinase (from Ref. 97).

VII. HORMONAL CONTROL OF SCD GENE EXPRESSION

Insulin, T3, estradiol and dexamethasone are examples of hormones that have been shown to stimulate the activity of stearoyl-CoA desaturase both in animal and tissue culture studies.[41,53,112–117] Recently, we have started to study the role of these hormones in the regulation and expression of the SCD1 gene in mouse liver and 3T3-L1 adipocytes. Our recent studies have focused on the role of insulin in the regulation of the SCD1 gene in mouse liver. Because the carbohydrate-mediated induction of SCD1 gene transcription in the fasted normal mouse can, at least in part, be explained by an increase in the circulating levels of insulin, we used a diabetic mouse model to study the role of insulin and carbohydrate in the regulation of SCD1 gene expression in liver. As described above, and as Fig. 6 shows, a combination of fructose and insulin enhances the transcription of the SCD1 gene, yet only to a small extent when each is administered independently.[97] However, full induction of SCD1 mRNA could not be solely accounted for by transcription of the SCD1 gene, implicating regulatory roles for both carbohydrate and insulin at post-transcriptional levels. The kinetics of induction of the SCD1 mRNA by fructose and insulin differs from that of S14 and fatty acid synthase, but is similar to that reported for the pyruvate kinase and malic enzyme gene expression[118] which also require insulin to observe significant transcriptional activation upon carbohydrate feeding. Whether the SCD1, pyruvate kinase, and malic enzyme genes are regulated by similar mechanisms remains to be established.

Insulin-stimulated expression of the SCD1 gene is blocked by cAMP analogs and theophylline.[97] This suggests that the repression of SCD1 in the diabetic state is due, at least in part, to glucagon-mediated activation of cAMP-dependent protein kinases. As for other insulin-sensitive genes,[119] the SCD1 induction by insulin also requires protein synthesis.[97] This is in contrast to the action of insulin on phosphoenolpyruvate carboxykinase. In this case, expression of the gene is inhibited within 15 min and protein synthesis is not required.[120] Therefore, the action of insulin on SCD1 gene expression may not be direct. Insulin (or its messenger) may stimulate synthesis of some unknown protein with a very rapid turnover,

and this protein may, in turn, stimulate transcription of the SCD1 gene. In tissue culture, insulin activates the transcription of the stearoyl-CoA desaturase gene in differentiated 3T3-L1 adipocytes.[121] Recently, several insulin-responsive elements (IREs) have been mapped in lipogenic genes, including the fatty acid synthase gene, and the protein factors present in adipose and liver tissues that bind to these DNA sequences have been described.[122,123] Whether similar factors mediate the regulation of SCD1 gene expression by insulin in liver and adipose tissue has not been determined. The mechanisms by which other hormones act to regulate the expression of the SCD1 gene also remain unknown. We are continuing our investigations of the hormonal regulation of SCD1 gene using liver cells and 3T3-L1 cells in culture.

VIII. SUMMARY

The stearoyl-CoA desaturase gene family encodes stearoyl-CoA desaturase, the key enzyme involved in the biosynthesis of unsaturated fatty acids, as well as in the regulation of this process. Because of the important role that the SCD gene product plays in fat cell metabolism, future studies on SCD1 gene expression could provide new insights into the role of fatty acids in cellular regulation, metabolism, and gene expression both in normal and disease states. In addition, the SCD gene family can be used as a model to study mechanisms of cellular differentiation, tissue-specific gene expression, and dietary and hormonal regulation of gene expression.

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