

James M. Ntambi *Editor*

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Preface

This is the first edition of this book that provides the reader with current information on the stearoyl-CoA desaturase gene family that encode stearoyl-CoA desaturase, a previously unknown enzyme and its role in metabolism in different organisms. Whereas today many genes and gene families are defined and analyzed bioinformatically, early events using the murine 3T3-L1 system provided the foothold that have enabled our current understanding of what has become one of the most interesting and important loci in cellular metabolism. In addition, the reader will learn about the important and widely diversified field of fatty acids and their health implications in humans. However since the precise role of fatty acids in the etiology of the various metabolic disorders has yet to be delineated it is not the intention of the book to present a unified view on the healthy implications of monounsaturated fatty acids or provide guidelines for fatty acid consumption.

Work over the last 20+ years has focused on identifying and analyzing numerous SCD isoforms in a variety of species that are in turn controlled by multiple signaling systems. Given the diverse role of monounsaturated fatty acids in metabolic processes it is not surprising that multiple genes exist and that multiple regulatory elements control isoform expression. In addition, tissue-specific dietary control of isoform expression occurs via a series of complex signal transduction schemes making SCD one of the most highly studied gene families. Conditional alleles and corresponding tissue-specific knockout models for many of the murine SCD genes have provided a wealth of information on not only tissue-specific fatty acid metabolism but also the key transcription factors that regulate SCD expression under a variety of metabolic and genetic backgrounds. Our studies using mice with whole-body and tissue-specific deletions of one of the mouse SCD isoforms (SCD1), together with several human studies have all agreed that the expression of the SCD1 gene isoform represents a key step in partitioning of lipids between storage and oxidation. High SCD1 expression (high oleate levels) favors fat storage leading to obesity while reduced SCD1 (low oleate levels) expression favors fat burning and leanness. However, as we understand more about the detrimental roles of cellular saturated fatty acids, it is becoming clear that unqualified complete inhibition of SCD1 activity, whether systemic or restricted to specific organ systems, may not

come without a plethora of undesirable side effects, including an increased propensity to inflammatory disease. A proper ratio of saturated/monounsaturated fat may be required for normal health and physiological function. Nevertheless, novel insights gained into the multifaceted roles of these intrinsic loci in cellular metabolism may yet lead to new models for pharmacological interventions for the treatment of metabolic disease.

I am very grateful to many people who helped me make possible the creation of the first edition on the role of SCD genes in metabolism. First of all I would like to thank my early research mentors, Dr. Paul Englund and M. Daniel Lane both at the Johns Hopkins University School of Medicine who introduced me to research in biochemistry and molecular biology. David Bernlohr presently at the University of Minnesota handed me the PAL122 cDNA clone that I used as a probe to isolate the first murine SCD gene (mSCD1). I would like to express my sincere appreciation to all the contributors of the diverse chapters on SCD genes for their cooperation and excellent work. Without their participation, this initial project would not have been possible. I would like to thank Anna Marsicek who has edited the various chapters of the book. I would also like to thank Springer for their assistance and support during the course of this project. They saw the potential for this project and helped me in many ways to craft this book. Finally I wish to thank my family for their encouragement, support, and patience over the past many years.

Madison, WI, USA

James M. Ntambi, Ph.D.

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Chapter 1

Early Studies on Role of Stearoyl-CoA Desaturase During Preadipocyte Differentiation

David A. Bernlohr and M. Daniel Lane

Background

Having extensively investigated the catalytic mechanism of action of acetyl-CoA carboxylase (ACC) (Polakis et al. 1972, 1974; Guchhait et al. 1971, 1974a, b; Moss and Lane 1971) and its allosteric regulation by citrate (Moss and Lane 1972; Gregolin et al. 1966; Ryder et al. 1967; Beaty and Lane 1983a, b), in the late 1970s and early 1980s, our lab decided to determine how expression of ACC is controlled. To conduct such studies we needed a lipogenic cell line that could be cultured for extended periods of time (i.e., greater than a week), during which gene expression and protein translation could be assessed. About this time Howard Green and colleagues, who had recently moved from the New York University Medical School to the Massachusetts Institute of Technology (MIT), had established several preadipocyte cell lines—notably the 3T3-L1 and 3T3F442 cell lines (Green and Kehinde 1974, 1975, 1976)—for the study of adipocyte biology. These lines could be induced to differentiate into cells with the phenotype of adipocytes including high rates of lipogenesis (Mackall et al. 1976; Mackall and Lane 1977; Coleman et al. 1978; Student et al. 1980) and responsiveness to insulin (Reed et al. 1977, 1981; Reed and Lane 1980). Since then the 3T3-L1 preadipocyte line has become the “gold standard” for studies on adipocyte differentiation. We (Student et al. 1980) and others (Lai et al. 1982) developed protocols to induce differentiation and demonstrated that expression of fatty acid synthase (FAS), ACC, and numerous other lipogenic proteins paralleled the acquisition of adipocyte morphology. These early observations

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verified the system and established the primary parameters linked to the utilization of the 3T3-L1 model including (1) an accumulation of cytoplasmic fat as revealed by Oil Red O staining (Mackall et al. 1976), (2) a dramatic increase in the rate of fatty acid synthesis (Mackall et al. 1976; Student et al. 1980), (3) a coordinate increase in the enzymatic activities of ACC (Mackall et al. 1976), FAS, and complex lipids synthesis (triacylglycerol and phospholipids) (Coleman et al. 1978), and (4) hormonally regulated lipid metabolism including insulin-stimulated glucose uptake and lipogenesis and catecholamine-stimulated lipolysis (Reed et al. 1977, 1981; Reed and Lane 1980).

The most widely used procedure for induction of adipocyte differentiation (Student et al. 1980) involves: allowing 3T3-L1 preadipocytes to proliferate until growth arrest at confluence (G_1 stage of the cell cycle) and after two additional days in G_1 , treating the cells with an induction “cocktail” consisting of cAMP (or methylisobutylxanthine), dexamethasone, and a high level of insulin (or a low level of IGF1). Following withdrawal of cAMP and dexamethasone the induced cells synchronously reenter the cell cycle, undergo ~2 rounds of division (mitotic clonal expansion) (Tang et al. 2003), and return to the state of G_1 growth arrest. At this point expression of the lipogenic proteins/enzymes of the adipocyte differentiation program occurs synchronously (Tang et al. 2003; Tang and Lane 2012). The use of such established preadipocyte lines have been indispensable for the identification and characterization of the key steps in the differentiation program. A large body of evidence (Tang and Lane 2012) shows that these preadipocyte models faithfully recapitulate differentiation of mouse embryonic fibroblasts (MEFs) in cell culture (Tang et al. 2003; Zhang et al. 2003). Likewise, the authenticity of model systems has been validated *in vivo* by demonstrating that these preadipocyte lines give rise to normal adipose tissue when implanted subcutaneously into athymic mice without exogenous inducers (Mandrup et al. 1997).

Gene Expression During Adipocyte Differentiation

The utilization of the 3T3-L1 cell line in culture represents a useful model system for investigating the mechanisms of cellular differentiation (Tang and Lane 2012). Under appropriate conditions, 3T3-L1 preadipocytes, which initially resemble fibroblast, differentiate readily into adipocytes in cell culture (Green and Kehinde 1975; Mackall et al. 1976). The differentiation process is accompanied by a dramatic increase in the activities of the enzymes associated with *de novo* lipogenesis (Mackall et al. 1976; Mackall and Lane 1977; Coleman et al. 1978; Student et al. 1980) and triglyceride mobilization (Hirsch and Rosen 1984), as well as an increased sensitivity to lipogenic and lipolytic hormones (Kohanski et al. 1986; Hirsch and Rosen 1984; Watkins et al. 1982). The cells accumulate triglyceride (Green and Kehinde 1975; Mackall et al. 1976) and acquire the morphological characteristics of adipocytes (Novikoff et al. 1980) isolated from normal adipose tissue. Strong evidence indicated that for some of the lipogenic enzymes (e.g., FAS), the increases in

activity during differentiation arise from increased rates of enzyme synthesis (Student et al. 1980) suggesting increased mRNA abundance. Moreover, it was subsequently shown that changes in mRNA expression closely correlated with the increased activity and abundance of the proteins that were differentially expressed (Mandrup and Lane 1997; MacDougald and Lane 1995; Bernlohr et al. 1985a, b; Angus et al. 1981). Therefore, it became evident that to understand the genetic mechanisms responsible for these changes in phenotype, it would be necessary to identify genes whose expression is activated during differentiation of 3T3-L1 preadipocytes into adipocytes. This ultimately led to the discovery that one of the key regulatory genes in this process was stearoyl-CoA desaturase (Ntambi et al. 1988).

Identification of mRNAs Whose Expression Is Induced During Differentiation of 3T3-L1 Preadipocytes into Adipocytes

An initial approach towards determining the mechanism of increased expression of key lipogenic and lipolytic enzymes was *in vitro* mRNA translation using ³⁵S-methionine followed by immunoprecipitation (Bernlohr et al. 1985a). In the early 1980s antibodies to several lipogenic enzymes were available (ACC, FAS, malic enzyme, pyruvate carboxylase, etc.) and by using *in vitro* translation of mRNA isolated from either 3T3-L1 preadipocytes or adipocytes followed by immunoprecipitation, one could assess the relative expression of certain mRNAs. William Angus, a PhD student at the time, utilized such an approach and demonstrated increased mRNA levels for pyruvate carboxylase (Angus et al. 1981) and acetyl-CoA carboxylase (MacDougald and Lane 1995). These findings suggested that differentiation of 3T3-L1 preadipocytes in culture likely involved the activation of the entire adipogenic gene expression program. While technically simple, the method was limited by the availability of mono-specific antibodies and was time-consuming and relatively inefficient. This consideration drove the race towards cloning differentially expressed mRNAs (MacDougald and Lane 1995; Cornelius et al. 1994).

A second approach towards analysis of gene expression during differentiation involved the isolation and characterization of cDNAs corresponding to genes that are activated during differentiation. Indeed, during the 1980s the identification of cDNA clones corresponding to differentially expressed genes occupied the focus of a number of laboratories. Toward that end, we constructed and characterized a cDNA library from mRNAs expressed by differentiated 3T3-L1 adipocytes (Bernlohr et al. 1984). A similar approach was taken by Spiegelman and colleagues (Ross et al. 1990) with 3T3-F442A cells, a related mouse preadipocyte cell line. Once a cDNA library was developed a dual-label analysis method was utilized to identify such clones that corresponded to mRNAs that were adipose-specific (Bernlohr et al. 1984). In this method, ³H-labeled cDNA was synthesized using mRNA from preadipocytes and mixed with ³²P-labeled cDNA synthesized from differentiated adipocytes. The mixed cDNA population was used in colony hybridization studies to identify cDNAs that contained differentially expressed mRNA

(Bernlohr et al. 1984). Using such a dual-label screening method we identified several cDNA clones that correspond to mRNAs that increase in abundance upon differentiation. Many of these clones (e.g., pAL122, pAL421, pAL422) represent mRNAs that are detectable primarily in differentiated cells. From such a screen came the identification of a cDNA, pAL122, corresponding to stearoyl-CoA desaturase (Ntambi et al. 1988).

Identification of Stearoyl-CoA Desaturase as a Differentiation-Induced Enzyme During Adipocyte Differentiation

Differential screening led to the identification of a family of adipocyte mRNAs (Ntambi et al. 1988) that included: (1) The pAL422 mRNA (later identified as encoding FABP4, the adipocyte fatty acid-binding protein also known as aP2) of which numerous clones were obtained owing to its abundant expression in differentiated 3T3-L1 adipocytes (Bernlohr et al. 1984, 1985b) and (2) The pAL122 mRNA was later identified as encoding the stearoyl-CoA desaturase one mRNA (Ntambi et al. 1988).

It should be noted that at the time of cloning these cDNAs their identities were unknown. The utilization of databases and searchable sequence repositories was in its infancy and even DNA sequencing was time-consuming and laborious. Indeed, since the original cDNA cloning was accomplished using the GC-tailing method, sequencing of such cloned inserts often proved problematic and required many sequencing runs and overlapping replicates to develop reproducible trustworthy results. The pAL422 cDNA was identified from a protein sequence data base as a putative fatty acid-binding protein, aP2, that exhibited ~60 % amino acid sequence identity to mP2, a myelin fatty acid-binding protein (Bernlohr et al. 1985b). James Ntambi undertook the identification of pAL122. It should be noted that Ntambi had little to go on as the pAL122 cDNA nucleotide sequence contained largely 3'-untranslated sequence and only a small amount of coding information upstream of the translational stop site (Ntambi et al. 1988). Northern blotting had revealed that the pAL122 mRNA sequence was ~5 kb, suggesting that the pAL 122 cDNA clone corresponded to less than 30 % of the complete cDNA.

In an act of heroic proportion James Ntambi began a manual “nucleotide-by-nucleotide” sequence comparison of the pAL122 mRNA with any new sequences that appeared in the literature. Remarkably, in 1986 Ntambi found a match with the nearly identical nucleotide sequence of the rat liver SCD1 from Philip Strittmater’s laboratory that appeared in the *Journal of Biological Chemistry* in 1986 (Thiede et al. 1986). Thus, James’ persistence paid off with the identification of pAL122 as murine stearoyl-CoA desaturase1 (Ntambi et al. 1988). Armed with the knowledge that pAL122 corresponded to SCD1, Ntambi utilized the pAL122 1.4-kb cDNA to isolate a 4.9-kb full-length cDNA clone and determined the complete mouse SCD1 sequence (Ntambi et al. 1988).

Isolation and Structure of the SCD1 Gene

Stearoyl-CoA desaturase is the key enzyme in the synthesis of unsaturated fatty acids and is under complex transcriptional and posttranscriptional regulation. To develop tools for the molecular analysis of SCD1, Ntambi, along with Susan Buhrow, Klaus Kaestner, Eric Sibley, and Robert Christy in our lab, was able to isolate genomic clones corresponding to murine SCD1 and to identify binding sites in the 5'-flanking regions of the gene that are involved in the coordinate differentiation-induced expression in 3T3-L1 preadipocytes (Ntambi et al. 1988; Christy et al. 1989). From these studies emerged the structural organization of the murine SCD1 gene and comparison to the murine 3T3-L1 SCD1 cDNA (Fig. 1.1). At the same time, genomic clones corresponding to pAL422 (Kaestner et al. 1989) had been obtained and armed with sequence information in the 5' upstream region from both genes, control elements that may provide clues towards coordinate regulation during differentiation were identified.

Identification and Cloning of a Second Stearoyl-CoA Desaturase, SCD2

Southern blotting of mouse DNA with the SCD1 cDNA suggested that there was a second similar sequence in the genome, and therefore, potentially a second SCD gene. Armed with the SCD1 cDNA, Klaus Kaestner and James Ntambi screened the 3T3-L1 cDNA library and identified a cDNA clone that encoded a second stearyl-CoA desaturase, SCD2, from 3T3-L1 adipocytes. This second SCD gene was

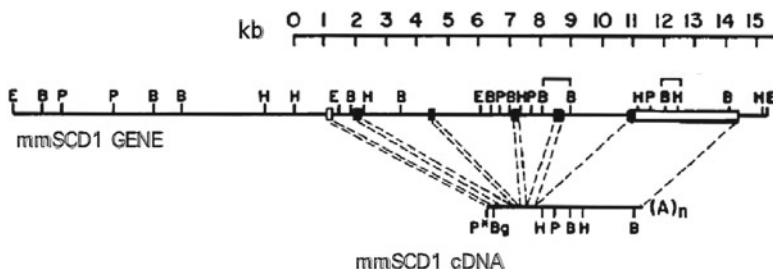


Fig. 1.1 Structural organization of the *Mus musculus* SCD1 gene and cDNA. The *top scale* indicates nucleotides in kilobases. The *middle scale* indicates the organization of the murine SCD1 gene where *boxes* indicate the exonic regions with open regions representing noncoding and filled regions coding sequence. The *lower scale* indicates the organization of the SCD1 cDNA isolated from 3T3-L1 adipocytes. *Single letters* represent restriction sites; *B* BamH1, *E* EcoR1, *H* HindIII, *P* Pst1, *B* BglIII, *kb* kilobase pair. Adapted from (Ntambi et al. 1988)

expressed at tenfold higher levels in 3T3-L1 adipocytes compared to the preadipocytes. The full-length SCD2 cDNA predicted a protein of 358 amino acids with 87 % amino acid sequence identity to murine SCD1 (Christy et al. 1989). Utilizing the SCD2 cDNA the murine SCD2 gene was cloned and analyzed to assess its regulation and expression patterns (Kaestner et al. 1989).

SCD2 mRNA is expressed constitutively at a high level in brain and its expression in kidney, adipose, and lung tissue is increased greatly by shifting mice from a diet containing unsaturated fatty acids to a diet devoid of fat (Landschulz et al. 1994). Presumably, the major function of SCD2 in brain is to supply unsaturated fatty acids constitutively for the synthesis of membrane phospholipids, particularly for myelination. In high carbohydrate, fat-free diets, fatty acids are synthesized *de novo* and are desaturated for membrane phospholipid synthesis and stored as triacylglycerol in the monounsaturated form. Strikingly, SCD2 mRNA is absent from liver regardless of the dietary treatment (Christy et al. 1991). The difference in tissue-specific expression and dietary control of SCD1 and SCD2 expression suggests distinct control mechanisms for the expression of each of the two genes. This in turn led to an analysis of the structure and regulation of SCD2.

The SCD2 gene spans approximately 15 kb pairs and consists of six exons and five introns, with intron–exon junctions similar to those of SCD1 (Kaestner et al. 1989; Christy et al. 1989). As determined by primer extension analysis the start site of transcription maps 300 nucleotides upstream of the initiator methionine codon. Unlike the SCD1 gene, SCD2 lacks a typical “TATA” box in the 5′ flanking region but has two “CCAAT” boxes at positions –90 and –135 relative to the transcription initiation site. The SCD2 promoter contains a 140-base pair sequence (located between nucleotides –54 and –201) that possesses 77 % sequence identity to a region (located between nucleotides –472 and –325) in the SCD1 promoter. There is a GC-rich sequence in the SCD2 promoter (at nucleotide –175) similar to the binding site for the nuclear transcription factor Sp1 as well as an element with homology to the core consensus sequence of the glucocorticoid receptor positioned a –500 and a potential CCAAT box/enhancer binding protein sequence at position –540. Although the promoter of the two genes differs markedly, there is one region with high sequence identity. A nucleotide sequence comparison of the 5′ flanking regions of the SCD2 and SCD1 genes revealed a stretch of 146 bp of striking sequence homology. The sequence between –201 and –54 bp 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 after gene duplication, either a sequence insertion or deletion occurred to yield the different promoters for SCD1 and SCD2. The SCD2 promoter lacks the potential cAMP core regulatory sequence present in the SCD1 promoter, but contains a potential binding site for the CCAAT/enhancer binding protein (Kaestner et al. 1989).

C/EBP α as Transcriptional Inducer of Gene Expression During Preadipocyte Differentiation

In view of the coordinate nature of the increase in expression of the 422/aP2 and SCD1 genes (Bernlohr et al. 1985a; Kaestner et al. 1989), the possibility was considered that a common transcription factor might activate expression of this group of adipocyte genes. Thus, we employed both DNase I footprinting and gel retardation analysis to show that nuclear factors present in differentiated—but not undifferentiated—3T3-L1 adipocytes bind to regions within the 5'-flanking regions of the promoters of the 422/aP2 and SCD1 genes (Christy et al. 1989). Subsequently we searched for and found that a factor present in nuclear extracts of differentiated—but not undifferentiated—3T3-L1 cells “foot-printed” the similar sites in the 5'-flanking regions of several differentiate-induced genes, notably the 422/aP2 and SCD1 genes (Christy et al. 1991). It was also shown that the differentiation-induced nuclear factor was CCAAT/enhancer binding protein alpha (C/EBP α) (Christy et al. 1991), a DNA-binding protein first isolated from rat liver (Landschulz et al. 1988). Bacterially expressed recombinant C/EBP binds to the same site at which the differentiation-specific nuclear factor interacts within the promoter of each gene. Northern analysis with RNA from 3T3-L1 cells shows that C/EBP α mRNA abundance increases markedly during differentiation. Co-transfection of 422(aP2)-chloramphenicol acetyl transferase or SCD1-chloramphenicol acetyl transferase constructs with a C/EBP α expression vector into 3T3-L1 preadipocytes revealed that C/EBP α trans-activated both the 422(aP2) and SCD1 gene promoters, thus implicating C/EBP α in differentiation-specific gene expression. Finally, transient co-transfection studies with a C/EBP α expression vector showed that C/EBP α could serve as trans-activator of both the 422(aP2) and SCD1 gene promoters (Fig. 1.2). Later we found that C/EBP α also served as a transcriptional activator of the leptin (Hwang et al. 1996, 1997; MacDougald et al. 1995) and GLUT4 genes (Kaestner et al. 1990) during the differentiation process (Fig. 1.2).

As C/EBP α appeared to serve as a pleiotropic activator of gene expression during preadipocyte differentiation (Kaestner et al. 1989, 1990; Hwang et al. 1996, 1997; MacDougald et al. 1995; Lin and Lane 1992), it was of interest to determine how the C/EBP α gene itself is controlled during differentiation. Consistent with its suspected role as a pleiotropic activator, we found that the increase in the C/EBP α message level during differentiation was due to an increase in the rate of transcription of the gene (Christy et al. 1991). We then set out to determine the structure of the C/EBP α gene and the nucleotide sequence of its 5' flanking region. DNase I footprinting of the 5' flanking region by nuclear factors from differentiated and undifferentiated 3T3-L1 cells revealed two sites of differential binding (Christy et al. 1991). One site in the gene promoter between nucleotides -252 and -239 binds a nuclear factor(s) present in preadipocytes that is lost or modified upon

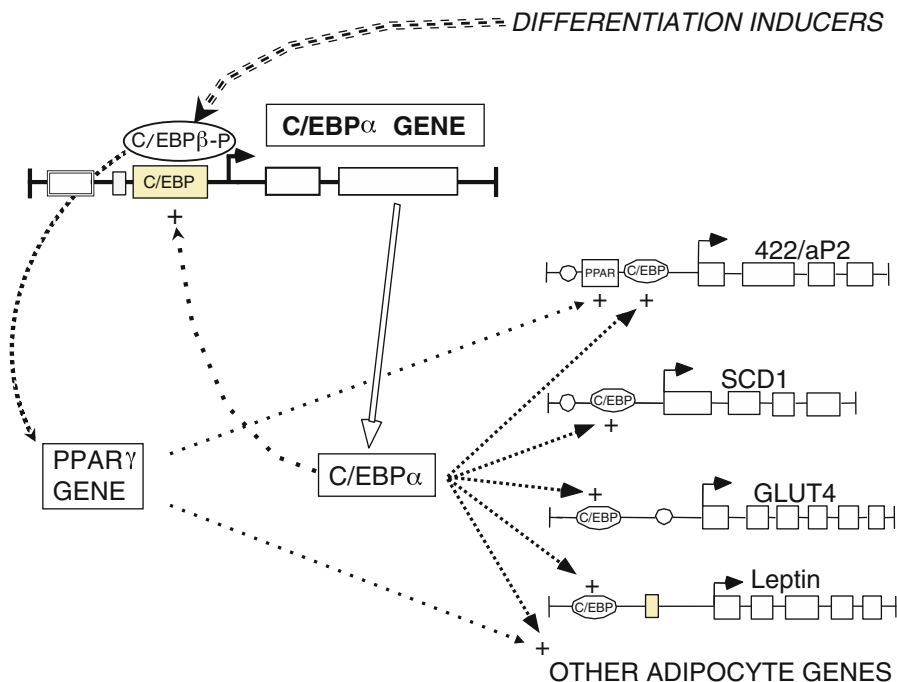


Fig. 1.2 Roles of C/EBP α , C/EBP β , and PPAR γ in the transcriptional activation of adipocyte genes during differentiation of 3T3-L1 preadipocytes. Following induction of differentiation, C/EBP β is expressed that in turn activates the expression of C/EBP α and PPAR γ . C/EBP α and PPAR γ act as pleiotropic activators of many adipocyte genes including those encoding 422/aP2 (aP2 or FABP4), a fatty acid-binding protein; *SCD1* stearoyl-CoA desaturase-1, GLUT4 the insulin-responsive glucose transporter; and the leptin gene. C/EBP α also activates its own gene thereby maintaining the differentiated state. C/EBP refers to CCAAT/enhancer binding protein and PPAR to peroxisome proliferator-activated receptor

differentiation. The second site, located between nucleotides -203 and -176 , exhibited different—but overlapping—footprints by nuclear factors present in differentiated and undifferentiated cells. Gel shift analysis with oligonucleotides corresponding to these sites revealed protein-oligonucleotide complexes containing these differentially expressed nuclear factors. The factor present in differentiated cells that binds at this site was identified as C/EBP presumably C/EBP α (Kaestner et al. 1989; Christy et al. 1991). These findings suggested that C/EBP α may regulate expression of its own gene (Fig. 1.2). It appears that the continued expression of C/EBP α allowed the maintenance of the differentiated state through interaction of the gene product (e.g., C/EBP α) with the promoter of its own gene. It was later found that C/EBP β , a related C/EBP family member, also binds at this site and is responsible for activating expression of the C/EBP α gene following induction much earlier in the differentiation program (Fig. 1.2) (Tang and Lane 2012).

Conclusions

From the simple beginning using the differentiating 3T3-L1 model cell system has come a broad and comprehensive analysis of the SCD gene family in many different organisms. Work over the last 20+ years has focused on identifying and analyzing numerous SCD isoforms in a variety of species that are in turn controlled by multiple signaling systems. The genes for SCD have been cloned from human, mice, rats, sheep, hamster, *Drosophila*, and *C. elegans* (reviewed by Paton and Ntambi 2009). In the mouse, four isoforms (SCD1, 2, 3 and 4) have been identified while in the rat only two isoforms have been characterized. In humans, two genes have been identified; a ubiquitously expressed SCD1 and a brain, pancreas form termed SCD5. Given the diverse role of monounsaturated fatty acids in metabolic processes such as the synthesis of phospholipids, triglycerides, cholesteryl esters, wax esters, and alkyldiacylglycerols, it is not surprising that multiple genes exist and that multiple regulatory elements control isoform expression. In addition, tissue-specific dietary control of isoform expression occurs via a series of complex signal transduction schemes making SCD one of the most highly studied gene families. Conditional alleles and corresponding tissue-specific knockout models for many of the murine SCD genes have provided a wealth of information on not only tissue-specific fatty acid metabolism, but also the key transcription factors that regulate SCD expression under a variety of metabolic and genetic backgrounds. Whereas today many genes and gene families are defined and analyzed bioinformatically, early events using the 3T3-L1 system provided the foothold that enabled our current understanding of one of the most interesting and important loci in cellular metabolism.

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Chapter 2

Skin Stearoyl-CoA Desaturase Genes

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Introduction

The hair follicle and its associated sebaceous gland constitute the skin pilosebaceous unit, the biogenesis of which occurs during embryogenesis. In certain skin areas such as eyelids, nipples, prepuce, and labia minora, sebaceous glands may form and function in the absence of hair follicles, but all hair follicles form and function normally in association with sebaceous glands. Although significant advances have been made in our understanding of hair biology during the last decade, cellular and molecular differences of sebaceous glands between various species and skin sites within an organism are poorly understood. Furthermore, it is currently unknown whether differences in sebaceous glands are related to the heterogeneity of hair follicles. It has been hypothesized that during evolution sebaceous glands arose first in the animal kingdom before hair follicles came into existence (Stenn et al. 2008). If this hypothesis is true, it can be logically concluded that sebaceous glands must have played some crucial role in the evolution of hair follicles. In fact, in vitro and in vivo studies suggest that sebaceous glands are important for normal formation or functioning of hair follicle (Williams and Stenn 1994; Allen et al. 2003). Given the relationship between sebaceous glands and hair follicles, an obvious interesting question that arises is what happens to skin and or hair follicles if sebaceous glands have defects in differentiation and as a result do not function properly. Such questions may be addressed with appropriate animal models (Porter et al. 2002; Lunny et al. 2005; Yager et al. 2012). A spontaneous mutant mouse, asebia (*ab*), is known to exist that serves as an excellent model for sebaceous gland deficiency.

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The recessive asebia mutation originated spontaneously in a colony of BALB/cCrglGa mice (Gates and Karasek 1965). The name asebia (*ab*) was initially given to mutant mice as it was believed that they lacked sebaceous glands; however, subsequent closer examination revealed that in fact sebaceous glands were not absent but hypoplastic, and other related glands (meibomian, perianal glands, and ceruminous glands) are affected to various extents in *ab* mice (Josefowicz and Hardy 1978; Pennycuik et al. 1986; Compton et al. 1989; Sundberg et al. 2000). Studies of dermal-epidermal recombination fetal grafts suggested that the functional defect in mutant mice was in the epidermis and not the dermis (Pennycuik et al. 1986). Other prominent features of *ab* mutant mice are their scant subcutaneous fat and corneal opacities.

Identification of *Scd1* Gene Defect in the Asebia Mouse by Positional Cloning

An allelic spontaneous mutation, *ab^J*, was identified at the Jackson Laboratories and was mapped to mouse Chromosome 19 (Sweet and Lane 1977). Later another allelic mutation, *ab^{2J}*, arose spontaneously at the Jackson Laboratories on the inbred mouse strain DBA/1LacJ and its characterization was reported by Sundberg et al. 2000. Differences in appearance of mutant and normal mice and their skin histology are shown in Fig. 2.1. Whereas well-differentiated sebaceous glands are obvious in normal mouse skin section, they are not apparent in *ab^{2J}* skin section. The gene

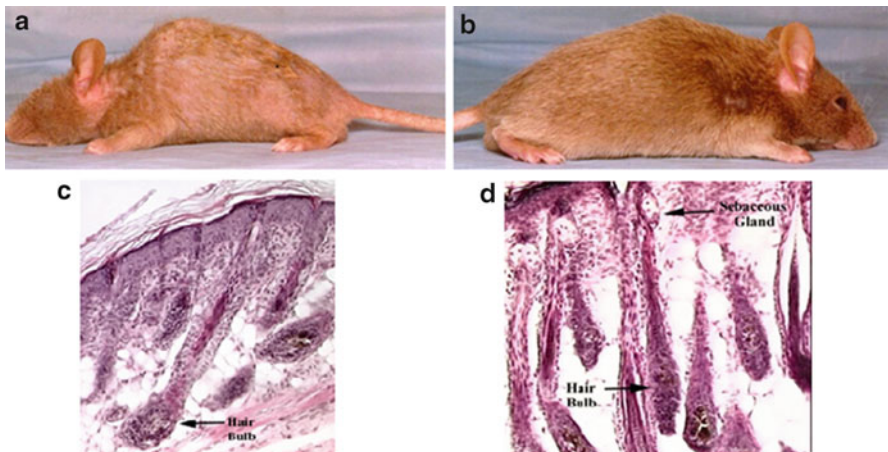


Fig. 2.1 Scaly skin with alopecia in an adult *ab^{2J}* mutant mouse (a) and normal fur of nonmutant littermate (b). Histology (H&E) skin sections of *ab^{2J}* and nonmutant mouse are shown in (c) and (d), respectively. While well-developed sebaceous glands, as indicated, are visible in (d), they are hypoplastic and not obvious in (c)