

Part One
Adipose Tissue Development and Morphology

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Transcriptional Control of Adipogenesis and Fat Cell Gene Expression

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1.1

Introduction

Adipocytes are highly specialized cells that play a major role in energy homeostasis in vertebrate organisms. Excess adipocyte size or number is a hallmark of obesity, which is currently a global epidemic. Obesity is not only the primary disease of fat cells, but a major risk factor for the development of non-insulin-dependent diabetes mellitus, cardiovascular disease, and hypertension. Obesity and its related disorders result in dysregulation of the mechanisms that control the expression of metabolic genes in adipocytes. Therefore, understanding adipocyte differentiation is relevant not only for understanding the pathogenesis of metabolic diseases, but also for identifying proteins or pathways that might be appropriate targets for pharmacological interventions. In the last 15 years, significant advances towards an understanding of the regulatory processes involved in adipocyte differentiation have largely been made by the identification of transcription factors that regulate the differentiation of fat cells and/or are involved in the induction and maintenance of adipocyte gene expression.

Interestingly, the majority of studies that have identified transcriptional regulators of adipogenesis have been performed *in vitro*. These studies have been primarily conducted in the 3T3-L1 or 3T3-F442A preadipocyte cell lines that were originally generated in the laboratory of Howard Green at Harvard University [1, 2]. In the last 32 years, these cell lines have been used by thousands of investigators worldwide. *In vivo*, adipocytes have three primary characteristics, which include lipid storage, insulin sensitivity, and endocrine properties. The 3T3-L1 cells have all three of these notable characteristics of fat cells. In addition, many adipocyte specific genes have been identified using this cell line. Many cell types cannot be adequately studied *in vitro* because the cultured cells do not have all the properties that the cells have *in vivo*. However, the preadipocyte cell lines that were developed by Green have been an extremely useful model system for adipocyte biologists and the data obtained in these cells has been validated from less mechanistic *in vivo* studies in the last decade.

In the late 1980s, a commentary by Steven McKnight and Dan Lane indicated that CCAAT/enhancer-binding protein (C/EBP)- α was a key metabolic regulator of energy metabolism [3]. Numerous studies have since confirmed the role of C/EBP- α and other C/EBP family members in energy balance, and defined roles for these transcription factors in adipocyte differentiation (reviewed in [4]). In 1994, two prominent laboratories independently identified peroxisome proliferator-activated receptor- γ (PPAR)- γ as an important modulator of adipocyte differentiation. Studies by Mitch Lazar's group observed the induction of PPAR- γ during adipogenesis [5], and experiments by Bruce Spiegelman's laboratory revealed that PPAR- γ was a transcriptional factor that bound to an enhancer element in the fatty acid-binding protein, aP2, promoter and conferred its fat-specific expression [6]. The early 1990s was the time that Brown and Goldstein identified sterol regulatory element-binding protein (SREBP)-1 [7], also termed adipocyte differentiation and determination factor (ADD)-1, whose expression was observed to play a role in adipocyte determination by the Spiegelman laboratory [8]. Since this time, several other transcription factors have been found to play an important role in adipocyte differentiation. Section 1.2 focuses on the transcription factors mentioned above and other important transcription factors that are regulated during adipocyte differentiation and are known to contribute to this process. Section 1.3 focuses on target genes that have been shown to be modulated by several of these transcription factors in both differentiating and mature adipocytes.

1.2 Transcriptional Control of Adipogenesis

Studies of the aP2 gene or use of its regulatory sequences have led to significant discoveries in adipocyte biology and metabolic diseases. aP2 is an abundantly expressed adipocyte gene that was first discovered in 1984 [9]. One of the earliest studies on PPAR- γ identified it as a transcription factor that bound to an enhancer element in the aP2 promoter and conferred its fat-specific expression [8]. Since its discovery, the aP2 promoter has been used by hundreds of laboratories to construct transgenes to have fat-specific expression. One lesser known fact is that c-Fos was also shown to bind to the aP2 promoter just 124 bp upstream of the transcriptional start site [10]. As a means of introduction, this was the first study to propose that activating protein (AP)-1 proteins were regulators of adipocyte gene expression.

1.2.1 AP-1 Transcription Factors

Members of the AP-1 family of transcription factors are well-known regulators of cellular proliferation and differentiation. AP-1 is a collective term referring to dimeric transcription factors composed of c-Jun, JunB, JunD and c-Fos, FosB, Fra-1, or Fra-2 subunits that bind to a common DNA site – the AP-1-binding site (reviewed in [11]). As indicated above, studies by the Spiegelman laboratory indicated that c-Fos was

involved in the modulation of aP2 expression [10]. Several years later, it was shown that the expression of c-Jun, c-Fos, Jun-B, Fos-B, and Fra-1 was induced immediately after the induction of adipocyte differentiation [12, 13]. Although the role of individual AP-1 family members in adipogenesis has not been elucidated, there is strong evidence to indicate these transcription factors are important *in vivo*. Transgenic mice were generated that express a dominant-negative protein that prevents the DNA binding of B-ZIP transcription factors of both the C/EBP and Jun families under the control of the adipose-specific aP2 enhancer/promoter. These mice have no white fat tissue throughout life [14]. Collectively, these studies suggest that the induction and expression of AP-1 transcription factors are important in fat cell differentiation.

1.2.2

Signal Transducers and Activators of Transcription

In the last 10 years, several groups have studied the modulation and function of signal transducers and activators of transcription (STAT) proteins during adipogenesis and in mature fat cells. The STAT family of mammalian transcription factors is comprised of seven proteins (STAT-1, -2, -3, -4, -5A, -5B, and -6) that, in response to stimulation of various receptors, mainly those for cytokines, are phosphorylated on tyrosine residues causing their translocation to the nucleus. Each STAT family member shows a distinct pattern of activation by cytokines and upon nuclear translocation can regulate the transcription of particular genes [15]. STATs have been shown to bind distinct DNA sequences and this binding regulates the transcription of specific genes [15, 16]. Since the tissue distribution and function of each STAT is unique, the regulation of tissue-specific genes appears to be a physiological role for these proteins [17]. This hypothesis is supported by numerous reports that demonstrate that specific STATs are activated differently by growth factors and cytokines, and STAT activation can be cell-type dependent. In addition, transgenic knockout experiments have revealed crucial roles for each known mammalian STAT [15] and cell-specific functions for STAT family members have been identified [18].

The first studies on STAT expression in 3T3-L1 cells revealed that STAT-1, -5A, and -5B were highly induced during murine adipogenesis [19]. Similar results were observed during the *in vitro* differentiation of human preadipocytes [20]. In addition, the ectopic expression of C/EBP- β and - δ in nonprecursor cells results in an induction of adipogenesis [21] that is accompanied by an induction in STAT-5A and -5B protein levels [22]. These two STAT proteins are also coordinately regulated with both PPAR- γ and C/EBP- α in differentiating 3T3-L1 cells under a variety of different conditions [23]. In 3T3-F442A preadipocytes, the ability of growth hormone to modulate adipogenesis was attenuated by STAT-5 antisense oligonucleotides [24]. Also, constitutively active STAT-5 is capable of replacing the requirement for growth hormone in adipogenesis of these cells [25]. Moreover, ectopic expression of STAT-5A has been shown to confer adipogenesis in 3T3-L1 preadipocytes [26] and in two different nonprecursor cell lines [27]. Interestingly, STAT-5B was not capable of conferring adipogenesis in nonprecursor cells [27]. Transgenic deletion of STAT-5A, STAT-5B, or

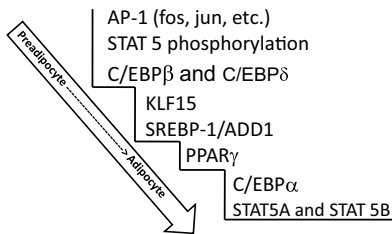


Figure 1.1 Induction of transcription factors during adipogenesis.

both STAT-5 genes in mice resulted in significantly reduced fat pad sizes compared to wild-type mice [28]. Also, in primary cultures of adipose tissue from these animals, growth hormone did not stimulate lipolysis as it did in adipocytes from wild-type animals [29], suggesting that some of the effects of growth hormone on fat metabolism are dependent on STAT-5 proteins. It should be noted that the increased expression of STAT-5 proteins is not typically observed until after the induction of C/EBP- α and PPAR- γ (refer to Figure 1.1), yet the activation of STAT-5 proteins in preadipocytes occurs prior to the induction in expression of PPAR- γ in 3T3-L1 cells [27]. In fact, both STAT-5 proteins are tyrosine phosphorylated and translocate to the nucleus within 15 min after the induction of adipogenesis of preadipocytes [27, 30]. Coupled with the observations in STAT-5 null mice, which have fat pads one-fifth normal size [28], the data suggest that activation of STAT-5 proteins may be an important driver of adipogenesis both *in vitro* and *in vivo*. This hypothesis is also supported by work indicating that one of the PPAR- γ promoters can be modulated by STAT-5 [31], suggesting that STAT-5 activation might drive adipogenesis by inducing PPAR- γ expression. In summary, work by a variety of laboratories has demonstrated that STAT-5 proteins, particularly STAT-5A, are activated and induced during adipogenesis, and play an important role in adipose tissue development.

1.2.3

Krüppel-Like Factors

Krüppel-like zinc finger transcription factors (KLFs) are known to play diverse roles in cell differentiation and development in mammals. One protein in the KLF family, KLF-15, was shown to be highly induced during the differentiation of 3T3-L1 preadipocytes into adipocytes [32]. Inhibition of KLF-15 function or expression with a dominant-negative mutant or via RNA interference results in an inhibition of adipogenesis in 3T3-L1 cells [32]. These studies also revealed that KLF-15 could confer adipogenesis in nonprecursor cells and result in the induction of PPAR- γ expression. Similar to KLF-15, KLF-5 expression is also highly induced during adipocyte differentiation in 3T3-L1 cells and embryonic fibroblasts obtained from heterozygote KLF-5 mice exhibit reduced adipogenesis [33]. Another member of this family, KLF-2, has been shown to be a negative regulator of adipogenesis and has the ability to attenuate PPAR- γ expression [34]. KLF-s are not only involved in lipid accumulation, but also appear to play a role in the ability of the adipocyte to

be insulin-sensitive, as indicated by studies showing that KLF-15 is important in the expression of glucose transporter 4 (GLUT4) [35].

1.2.4

SREBPs

In 1993, studies by the Spiegelman group identified a basic helix–loop–helix transcription factor that was expressed in adipocytes and whose expression was increased during adipogenesis [8]. The protein was called ADD-1. Two months later, this protein was labeled SREBP-1 by the Brown and Goldstein laboratories, who named the transcription factor for its ability to bind sterol-responsive elements within the promoter of the low-density lipoprotein receptor gene [7]. It is now known that there are three SREBP isoforms (SREBP-1a, -1c, and -2) that have been well characterized. SREBP-1a and -1c are transcribed from the same gene, each by a distinct promoter, and the predominant SREBP-1 isoform in liver and adipose tissue is SREBP-1c. SREBP-2 is relatively selective in transcriptionally activating cholesterol biosynthetic genes and SREBP-1c has a greater role in regulating genes associated with fatty acid synthesis (reviewed in [36]). Although there is clear evidence that SREBP is an insulin-modulated transcription factor involved in the regulation of genes associated with cholesterol and lipid metabolism, there are less convincing studies to indicate that SREBPs are critical for adipogenesis. Mice deficient in SREBP-1 do not have a significantly decreased amount of white adipose tissue, but SREBP-2 levels were increased suggesting it might compensate for SREBP-1 in this animal model [37]. These *in vivo* studies are supported by additional transgenic studies where SREBP-1 deficient mice were crossed with *ob/ob* (leptin-deficient) mice and it was found that SREBP-1 was not required for the development of obesity [38]. These observations concluded that SREBP-1 regulation of lipogenesis was highly involved in the development of fatty livers, but was not a determinant of obesity in this animal model [38]. However, ectopic expression of a dominant-negative SREBP-1c was shown to attenuate adipocyte differentiation [39]. In addition, overexpression of SREBP-1c enhanced the adipogenic activity of PPAR- γ [39] and other studies suggest that SREBP-1c contributes to the generation of PPAR- γ ligands [40]. In summary, *in vitro* studies support a role for SREBP-1 in adipogenesis, whereas *in vivo* studies indicate that SREBPs are not required for the production or expansion of adipose tissue.

1.2.5

C/EBP

C/EBP transcription factors were the first family of transcription factors shown to play a critical role in the differentiation of fat cells *in vitro*. Today, we know that transgenic mice lacking both C/EBP- β and C/EBP- δ or C/EBP- α alone have defective adipocyte differentiation [41, 42]. Prior to these *in vivo* observations, the cascade of induction of these three C/EBP family members was revealed by McKnight and collaborators who showed that C/EBP- β and - δ were induced immediately after the

induction of differentiation, whereas C/EBP- α expression did not occur until 4–5 days after the initiation of differentiation [43]. This group also demonstrated that C/EBP- β and - δ were responsible for inducing C/EBP α expression [44]. Ectopic expression studies conducted by several laboratories demonstrated the adipogenic capabilities of C/EBP- α or - β alone, or in the presence of C/EBP- δ [21, 44–46]. Today, C/EBP- α and PPAR- γ are considered the two primary transcription factors that mediate adipogenesis. However, cells lacking C/EBP- α are capable of adipogenesis, but are not insulin-sensitive [47, 48]. There is also evidence to indicate that C/EBPs may play a role in the induction of PPAR- γ ligands [49]. In summary, both *in vitro* and *in vivo* studies indicate a substantial role for C/EBP- β , - δ , and - α in adipogenesis. Although C/EBP- α may not be required for lipid accumulation, this transcription factor clearly plays a role in conferring insulin sensitivity in adipocytes.

1.2.6

PPAR- γ

Although a number of transcription factors, including those mentioned above, have been shown to have profound effects on fat cell differentiation and the expression of adipocyte genes, only one adipocyte transcription factor has been shown to be necessary for adipogenesis. PPAR- γ is a member of the nuclear hormone receptor superfamily that is required for the development of adipocytes, and deletion of PPAR- γ in mice results in placental dysfunction and embryonic lethality [50, 51]. As mentioned above, PPAR- γ was originally identified as a transcription factor induced during differentiation that bound an enhancer element within the aP2 promoter [6]. Since that time, there have been multiple studies on the role of PPAR- γ in adipocytes. A remarkable finding in 1995 was that the insulin-sensitizing drugs thiazolidinediones were ligands for PPAR- γ [52]. These were some of the first molecular studies to indicate the importance of adipose tissue in insulin resistance. Many investigators were surprised to learn that activating a transcription factor whose expression was highly enriched in fat cells could contribute to whole-animal insulin sensitivity. It is now known that adipocytes secrete several hormones that can affect the activity of other tissues; however, the studies of PPAR- γ have revealed that modulation of this transcription factor can contribute to systemic insulin resistance. Although the PPAR- γ null mice are embryonic lethal [51], transgenic mice lacking PPAR- γ specifically in adipose tissue exhibit greatly reduced sized fat pads, and insulin resistance in fat and liver [53]. However, PPAR- γ heterozygote mice have enhanced insulin sensitivity [54]. Together, these studies suggest the amount of PPAR- γ in adipose tissue is physiologically relevant. In the last several years, several studies have examined pathways that are involved in regulating the levels of PPAR- γ . In particular, the ubiquitin–proteasome system has emerged as an important regulator of PPAR- γ proteins [55, 56]. In addition, a role for the ubiquitin-like protein SUMO (small ubiquitin-like modifier) in regulating PPAR- γ has been demonstrated by several groups [57–60]. The phosphorylation of PPAR- γ by mitogen-activated protein kinases is also an important modulator of the activity of this transcription factor (reviewed in [61]). A very recent study confirmed that PPAR- γ knockdown prevented adipocyte differentiation, but also suggested that PPAR- γ was not required for maintenance of the

differentiated state after the cells had undergone adipogenesis [62]. These observations are supported many anecdotal observations that indicate that PPAR- γ is decreased as adipocytes age *in vitro*. In addition, the increase of lifespan via caloric restriction results in the induction of sirtuin (SIRT)-1 – a transcriptional modulator with deacetylase activity that represses PPAR- γ activity *in vivo* [63]. This study also demonstrated that the repression of PPAR- γ by SIRT1 was evident in 3T3-L1 adipocytes. Collectively, studies by numerous laboratories demonstrate the adipogenic capabilities of PPAR- γ . However, the role of this transcription factor in mature adipocytes is not well understood and recent studies suggest that this transcription factor is not required for maintenance of the adipocyte following adipogenesis [62]. Nonetheless, PPAR- γ expression and activity is controlled at multiple levels, including alternative promoter usage, tissue-limited expression, phosphorylation, acetylation, ubiquitylation, and SUMOylation. The multiple levels of regulation of this transcription factor suggest that controlling the amount and activity of PPAR- γ is important. The role of PPAR- γ in the development and treatment of diabetes is well established (reviewed in [64]), and the importance of PPAR- γ in humans is indicated by several loss-of-function mutations in the PPAR- γ gene that cause lipodystrophy and diabetes in humans ([65–67], reviewed in [68]).

In addition to the transcription factors described above that promote adipogenesis, there are several other transcription factors that have been shown to have positive or negative effects on adipocyte differentiation (reviewed in [4]). Section 1.3 reveals specific target genes that have been identified as targets of the positive adipogenic transcriptional activators described above. The identification and characterization of these target genes has provided critical information in understanding the role of these proteins in both differentiating and mature fat cells.

1.3

Identification of Adipocyte Transcription Factor Target Genes

As stated in Section 1.2, adipogenesis occurs as a result of a transcriptional cascade that involves the tightly regulated induction of key transcription factors, including C/EBP- β , C/EBP- δ , C/EBP- α , SREBP-1c, STAT-5, and PPAR- γ . These adipogenic factors, in turn, induce the expression of various adipocyte genes that are important in conferring lipid accumulation, insulin sensitivity, and endocrine properties to mature adipocytes. In addition, many of these adipogenic factors possess the ability to regulate one another's gene expression. These interactions add complexity to the pathways that regulated lipid and glucose metabolism. Hence, it is critical to identify the target genes of these adipogenic transcription factors in order to understand adipocyte gene expression and fat cell function.

1.3.1

C/EBP Target Genes

The three isoforms of the C/EBP family are differentially regulated and expressed at specific times in accordance with each protein's regulatory role in adipogenesis.

C/EBP- β and - δ are expressed very early in adipocyte differentiation in 3T3-L1 adipocytes, in contrast to C/EBP- α , whose expression is limited to the late phase of differentiation [43]. Both C/EBP- β and - δ play important roles in the induction of PPAR- γ gene expression [21], as functional C/EBP regulatory elements have been identified in the promoter of the PPAR- γ gene [69]. In addition, investigators have shown that these C/EBP regulatory elements are also in the promoter of the C/EBP- α gene [70], supporting a role of C/EBP- β and - δ in regulating the expression of C/EBP- α [43, 44]. Hence, C/EBP- β and - δ can modulate the transcriptional regulation of other primary players in adipogenesis (i.e., PPAR- γ and C/EBP- α). C/EBP- β has also been shown to regulate other fat-specific genes, as the binding of C/EBP- β to C/EBP response elements in the adiponectin promoter is necessary for its transcription [71].

C/EBP- α plays a more diverse role in adipocyte gene expression, as it regulates more target genes that are influential not only in adipogenic pathways, but also genes that are important in insulin signaling and the production of endocrine hormones. C/EBP- α has the ability to affect the expression of other transcription factors that are necessary for adipogenesis. For example, C/EBP- α can directly modulate the transcription of PPAR- γ through C/EBP response elements within the PPAR- γ 2 promoter [69, 72]. C/EBP- α can also transactivate the promoters of both aP2 [73, 74] and stearoyl-CoA desaturase (SCD) genes [73] during 3T3-L1 differentiation via specific sequence elements in the promoter. In addition, C/EBP- α can transactivate the insulin-responsive GLUT4 promoter *in vitro* [75] and the insulin receptor gene *in vivo* [76]. These studies clearly demonstrate the importance of this transcription factor in affecting glucose metabolism and insulin signaling. Adipocytes also have important endocrine functions, including the production and secretion of leptin and adiponectin. One study has demonstrated that C/EBP- α is a transcriptional activator of the leptin promoter via a functional C/EBP-binding site [77]. Similar to C/EBP- β , C/EBP- α can activate transcription of the murine adiponectin promoter via C/EBP-binding sites [78] and can modulate adiponectin expression in mature human adipocytes via interactions with C/EBP response elements in an intronic enhancer [79]. In summary, these studies indicate C/EBP- α can modulate the expression of important adipocyte transcription factors, key endocrine products of adipocytes, and genes associated with lipid and glucose metabolism.

1.3.2

SREBP-1 Target Genes

The identification of ADD-1 [8], later termed SREBP-1 due to its importance in cholesterol metabolism [7], is an important modulator of adipocyte gene expression. SREBP-1/ADD-1 appears to be responsible for the differentiation-dependent induction of fatty acid synthase (FAS) during adipogenesis [39]. In addition, one study has shown that C/EBP- β is under the direct control of SREBP-1c in 3T3-L1 adipocytes. In this study, SREBP-1c was shown to activate transcription via SREBP-binding sites in the C/EBP- β promoter [80]. Adiponectin gene expression has also been shown to be directly regulated by SREBP-1c, which binds sterol regulatory elements in the adiponectin promoter in mouse adipocytes *in vitro* and *in vivo* [81]. These data

support SREBP-1c's regulatory involvement in mature adipocytes by mediating the expression of several adipogenic genes. Since SREBP-1 null mice are not defective in adipogenesis [37], the primary function of this transcription factor in adipocytes appears to be the regulation of genes associated with lipid accumulation and the endocrine properties of adipocytes.

1.3.3

PPAR- γ Target Genes

PPAR- γ , which is considered the master regulator of adipogenesis, exerts its effects directly in adipocytes, but is also known to be important in other cell types. PPAR- γ can modulate the expression of a variety of genes that differ in their metabolic function. The first PPAR- γ target gene identified was the aP2 gene. The aP2 gene contains a functional peroxisome proliferator response element (PPRE) within its promoter that plays a central role in its induction during adipogenesis [6]. Several studies also support a role for PPAR- γ in the induction of other adipocyte transcription factors, including C/EBP- α and STAT-5A. It is known that PPAR- γ and C/EBP- α can regulate each other's expression during adipogenesis [48], and the cross-regulation of PPAR- γ and C/EBP- α is required for efficient adipogenesis [82]. Recently, PPAR- γ was also found to regulate expression of STAT-5A through several identified PPREs [83]. Taken together, these studies demonstrate cross-talk between three transcription factors that are highly induced during adipogenesis and are known to play a role in adipose tissue development *in vivo* [28, 42, 50].

Through its transcriptional regulation of various genes, PPAR- γ is functional in many biological processes, including lipid metabolism. PPAR- γ regulates genes involved in lipoprotein metabolism, including lipoprotein lipase (LPL) whose expression is modulated in adipocytes via a PPRE within its promoter [84]. In addition, PPAR- γ can directly modulate the transcription of hormone-sensitive lipase (HSL) [85], which is an important contributor to lipolysis. The malic enzyme, whose product is involved in lipogenesis, is a PPAR- γ target gene [86]. The expression of acyl-CoA synthetase, which converts fatty acids to acyl-CoA for subsequent β -oxidation, is induced in differentiated adipocytes [87] and its expression can be modulated by a PPRE [88]. A functional PPRE in the murine fatty acid transport protein (FATP) gene is modulated by PPAR- γ in 3T3-L1 adipocytes [89]. Gene expression of another fatty acid transporter gene, fatty acid translocase (FAT) [90], is induced by PPAR- γ in adipose tissue *in vivo* [91]. The expression of SCD, an enzyme involved in fatty acid biosynthesis, is modulated by a PPRE promoter element [92] and may be regulated by PPAR- γ in adipose tissue *in vivo* [90]. Two lipid droplet-associating proteins are also known PPAR- γ target genes in adipocytes. A functional PPRE has been characterized in the murine perilipin gene promoter [93] and in the lipid droplet-associating protein, S3-12 [94]. Hence, PPAR- γ regulates genes that play a role in lipogenesis, lipolysis, and lipid droplet formation.

PPAR- γ can also directly control genes whose expression contributes to glucose homeostasis and insulin signaling. The expression of phosphoenolpyruvate

carboxykinase (PEPCK), a key enzyme in gluconeogenesis, is modulated by a PPAR- γ -binding site in its promoter [95]. The insulin-stimulated glucose transporter, GLUT4, is upregulated by PPAR- γ activation in rat adipose tissue *in vivo* [90] and in NIH-3T3 cells [96]. Rev-Erb α (NR1D1) is another nuclear receptor whose expression is also induced during adipocyte differentiation [97]. Rev-Erb α expression is induced by PPAR- γ activation in rat adipose tissues *in vivo* as well as in 3T3-L1 adipocytes *in vitro* and this transcriptional induction occurs via a PPAR response element in the Rev-Erb α promoter [98]. Therefore, Rev-Erb α is a target gene of PPAR- γ in adipose tissue, and is another member of the nuclear receptor superfamily that is modulated during adipogenesis and may contribute to the regulation of adipocyte gene expression. Although many studies on PPAR- γ focus on the identification of target genes in white adipocytes, studies in brown adipocytes have shown that the uncoupling protein (UCP)-1 gene contains a PPRE that is bound and activated by PPAR- γ [99].

It has been known for several years that PPAR- γ ligands can increase the expression and plasma concentrations of adiponectin [100], and more recent study have observed that PPAR- γ induces adiponectin in adipocytes via a PPRE in the human adiponectin promoter [101]. All of these results indicate that PPAR- γ is not only required for regulating transcription factors that drive adipogenesis, but this nuclear receptor is important for modulating the adiponectin gene, which confers an important endocrine function to adipocytes. Overall, the identification of PPAR- γ target genes in adipocytes has revealed that this nuclear receptor regulates a variety of genes that are involved in adipogenesis, insulin signaling, glucose homeostasis, and lipid metabolism.

1.3.4

STAT-5 Target Genes

It is widely accepted that STAT proteins have cell-specific functions. Studies on STAT-5 proteins have revealed their importance in adipogenesis *in vitro* and *in vivo* [27, 28]. Recent studies have focused on the identification of STAT-5 target genes in adipocytes. The promoter for acyl-CoA oxidase, the rate-limiting enzyme in peroxisomal fatty acid β -oxidation, contains a STAT-5-binding site that modulates its gene expression in fat cells [102]. Another study indicates that growth hormone also exerts stimulatory effects on adipogenesis through STAT-5A and -5B by enhancing the transcriptional activity of C/EBP- β/δ and PPAR- γ [31]. Transfection studies have demonstrated that aP2 promoter activity can be activated by STAT-5 [103]. Yet other studies have shown that STAT-5 mediates the inhibition of aP2 expression in rat primary preadipocytes [104]. This was the first study to suggest that STAT-5 proteins could act as transcriptional repressors. This observation has been supported by another study that revealed STAT-5A could act as a transcriptional repressor in adipocytes. A STAT-5A-binding site in the murine FAS promoter has been shown to mediate the repression of FAS transcription that occurs with prolactin treatment [105]. In addition to modulation of genes associated with lipid metabolism, STAT-5 can also modulate a gene associated with glucose metabolism. The gene for

pyruvate dehydrogenase kinase (PDK)-4, a known regulator of glycolysis, is highly induced in adipocytes by growth hormone or prolactin (PRL) in a STAT-5-dependent manner [106]. Under these conditions, the induction of PDK-4 is accompanied by insulin resistance. It is well known that PRL and growth hormone are important modulators of lipid metabolism, and are also potent inducers of STAT-5 in adipocytes [103, 107]. Hence, many of the metabolic actions of these hormones could be mediated by STAT-5's direct modulation of target genes. Unfortunately, relatively few STAT-5 target genes have been identified in adipocytes. Nonetheless, we hypothesize that several other STAT-5A target genes will be identified that play a role in lipid or glucose metabolism.

1.3.5

Summary

It is clear that there are several transcription factors that are highly expressed during adipogenesis and are critical not only for lipid accumulation, but to confer insulin sensitivity and endocrine properties to mature adipocytes. The primary function of these proteins is to directly modulate target genes that contribute to the adipocyte phenotype (reviewed in Table 1.1). Although much emphasis has been placed on C/EBP- α and PPAR- γ , there are clearly several other transcription factors that are important, including STAT-5 proteins and KLFs. However, very few target genes for these transcription factors have been identified. Interestingly, it is still not known which transcription factor(s) confer the adipocyte specific expression leptin. In the future, the identification of new or known adipocyte transcription factors and their target genes will be essential in enhancing our knowledge of adipogenesis and indicating what transcriptional modulators are required for the maintenance of lipid laden, insulin-sensitive, hormone-secreting fat cells.

Table 1.1 Target genes of key adipocyte transcription factors.

Adipogenic transcription factors	Characterized target genes
C/EBP- β	PPAR- γ [21, 69], C/EBP- α [43, 70], adiponectin [71]
C/EBP- δ	PPAR- γ [21, 69], C/EBP- α [43, 70]
C/EBP- α	PPAR- γ [69, 72], aP2 [73, 74], SCD [73], GLUT4 [75], insulin receptor [76], leptin [77], adiponectin [78, 79]
SREBP-1	FAS [39], C/EBP- β [80], adiponectin [81]
PPAR- γ	aP2 [6], C/EBP- α [48], STAT-5A [83], LPL [84], HSL [85], malic enzyme [86], acyl-CoA synthetase [88], FATP [89], FAT [91], SCD [90, 92], perilipin [93], S3-12 [94], PEPCCK [95], GLUT4 [90-96], Rev-Erba [97, 98], UCP-1 [99], adiponectin [100, 101]
STAT-5	acyl-CoA oxidase [102], C/EBP- β/δ [31], PPAR- γ [31], aP2 [103, 104], FAS [105], PDK [106]

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