A detailed understanding of the processes governing adipose tissue formation will be instrumental in combating the obesity epidemic. Much progress has been made in the last two decades in defining transcriptional events controlling the differentiation of mesenchymal stem cells into adipocytes. A complex network of transcription factors and cell-cycle regulators, in concert with specific transcriptional coactivators and corepressors, respond to extracellular stimuli to activate or repress adipocyte differentiation. This review summarizes advances in this field, which constitute a framework for potential antiobesity strategies.

Introduction
Obese individuals are more likely than their lean counterparts to develop cardiovascular disease and type 2 diabetes. The increase in adiposity in these individuals results from an upsurge in both adipocyte number and size of individual fat cells. Additionally, the disproportionate increase in the visceral adipose depots in some individuals is linked to development of certain metabolic disorders. Consequently, understanding the mechanisms regulating adipose formation should provide valuable information in the fight to combat the growing incidence of obesity in the modern world.

During the last several years, investigators have embarked on a detailed and systematic endeavor to define the transcriptional events regulating preadipocyte differentiation (adipogenesis) and adipocyte function. The differentiation of preadipocytes into adipocytes is regulated by an elaborate network of transcription factors that coordinate expression of hundreds of proteins responsible for establishing the mature fat-cell phenotype. At the center of this network are the two principal adipogenic factors, PPARγ and C/EBPα, which oversee the entire terminal differentiation process. PPARγ in particular is considered the master regulator of adipogenesis; without it, precursor cells are incapable of expressing any known aspect of the adipocyte phenotype (Rosen et al., 2002). On the other hand, cells deficient in C/EBPα are capable of adipocyte differentiation; however, these C/EBPα-deficient cells are insulin resistant (El-Jack et al., 1999; Wu et al., 1999). Much of our knowledge of this complex network and the importance of PPARγ and C/EBPα comes from studies performed in established preadipocyte cell lines as well as mesenchyme-derived precursor cells. More recently, data from a variety of knockout mice have confirmed these in vitro studies showing that many components of this network are required regulators of adipocyte development and function.

The 3T3-L1 and 3T3-F422A preadipocyte cell lines originally established by Green and associates have greatly facilitated our knowledge of the molecular mechanisms controlling adipogenesis (Green and Kehinde, 1975, 1976). Although committed to the adipocyte lineage, proliferating 3T3-L1 preadipocytes exert characteristic similarities to those of other 3T3 fibroblasts. Confluent 3T3-L1 preadipocytes differentiate upon exposure to the adipogenic inducers fetal bovine serum (FBS), dexamethasone, isobutylmethylyxantine, and insulin. This cocktail activates an adipogenic program, which occurs in two well-defined phases. The stimulated cells immediately reenter the cell cycle and progress through at least two cell-cycle divisions, a phase often referred to as clonal expansion. During this time, the cells express specific adipogenic transcription factors as well as cell-cycle regulators that together facilitate expression of PPARγ and C/EBPα. Following this event, the committed cells undergo terminal differentiation manifested by production of lipid droplets as well as expression of multiple metabolic programs characteristic of mature fat cells. The validity of this 3T3-L1 system as an appropriate model of adipocyte formation in the animal has been supported by many studies performed in both mouse and human tissue.

The goal of this review is to discuss the transcriptional processes controlling the conversion of progenitor mesenchymal cells into fully functional adipocytes. Emphasis will be given to the transcription factors that have been shown to respond to various effectors and that induce a well-defined component of the adipogenic process. A number of factors attenuate adipogenesis and serve to function as molecular switches in controlling the fate of the progenitors; consequently, the mechanisms by which these negative regulators inhibit the activity of the preadipogenic factors will be discussed. Finally, the review will conclude with a discussion of the recent advances in our understanding of how various coactivators and corepressors control the activity of the adipogenic transcription factors and facilitate their communication with the transcriptional machinery.

Elucidation of the network of transcription factors regulating adipogenesis
PPARγ and C/EBPα: master regulators of adipogenesis
The role of PPARγ as the master regulator of adipogenesis is supported by overwhelming evidence from both in vivo and in vitro studies. Important early evidence of the critical role of PPARγ in regulating adipogenesis came from Spiegelman and collaborators, who had worked for several years to elucidate the transcription factors regulating expression of the adipose-specific fatty acid binding protein apoP2/FABP4. This endeavor resulted in identification of a nuclear factor initially referred to as ARF6 that was later shown through cloning technology to correspond to PPARγ and its heterodimeric partner, RXR (Tononoz et al., 1994a, 1994b). A series of gain-of-function studies in which PPARγ was ectopically expressed in nonadipogenic mouse fibroblasts showed that PPARγ alone can initiate the

entire adipogenic program, giving rise to fat cells that are capable of many of the functions of mature adipocytes (Tontonoz et al., 1994c). In attempting to understand the importance of PPARγ in the development of adipocytes, investigators found that ablation of pparγ in embryonic stem (ES) cells leads to embryonic lethality at E10 due to a defect in placentalization as a result of PPARγ’s participation in formation of the trophoblast (Barak et al., 1999). To circumvent this problem, alternative strategies for obtaining knockout mice were developed that supported a role for PPARγ in the formation of all fat depots, including both brown and white (Barak et al., 1999; Rosen et al., 1999). These mouse models, however, provided only partial information concerning the function of PPARγ in adipocytes since both models were subject to significant limitations. In one case, the conclusions were based on chimeric mice derived from homozygously targeted ES cells (Rosen et al., 1999). In these animals, the knockout cells failed to develop into adipocytes; whereas the wild-type-derived cells gave rise to fully functioning adipose depots. Consequently, it was difficult to assess what impact the absence of PPARγ has on adipose tissue function. The tetraploid embryo strategy of Evans and coworkers (Barak et al., 1999) generated only one mouse, which died soon after birth, but allowed these investigators to observe that PPARγ deficiency in these animals resulted in failure to form adipose tissue. The establishment of white adipose tissue (WAT)-hypomorphic pparγ knockout mice resulted in animals that were severely lipodystrophic; these data authenticate PPARγ as the master regulator of adipogenesis (Koutnikova et al., 2003).

pparγ is expressed as two isoforms, pparγ1 and pparγ2, generated by alternative promoter usage of the same gene, which gives rise to four distinct mRNAs. pparγ1, pparγ3, and pparγ4 mRNAs all encode the PPARγ1 polypeptide, while pparγ2 mRNA encodes the corresponding PPARγ2 polypeptide, which is identical to PPARγ1 with an additional 30 amino acids present at the N terminus (Fajas et al., 1997; Meirhaeghe et al., 2003; Tontonoz et al., 1994b). PPARγ1 is expressed in many tissues, whereas PPARγ2 expression is restricted almost exclusively to adipose. Studies performed in pparγ1−/− mice embryonic fibroblasts (MEFs) demonstrate that ectopic PPARγ1 is as capable of inducing adipogenesis as PPARγ2 (Mueller et al., 2002). Furthermore, adipose-selective knockout of pparγ in the mouse gives rise to insulin-insensitive animals with reduced fat; however, they still contain substantial amounts of adipose tissue, suggesting that PPARγ1 can compensate for many of the adipogenic functions of PPARγ2 (Zhang et al., 2004a). The fact that the PPARγ2-deficient mice are insulin resistant suggests that PPARγ2 may play a selective role in regulating insulin sensitivity.

Recognition that C/EBPβ functions as a principal player in adipogenesis also resulted from gain-of-function studies in cultured cells as well as establishment of appropriate knockout mice. In the former case, Freytag and associates demonstrated that ectopic expression of C/EBPβ in a variety of fibroblastic cells could induce adipogenesis (Freytag et al., 1994). Similar to the PPARγ studies, establishment of C/EBPβ knockout mice was subject to significant setbacks since the animals die soon after birth due to the pups’ inability to produce glucose. This phenotype results from the requirement of C/EBPβ for gluconeogenesis in the liver (Wang et al., 1995). Ablation of c/ebpβ in all tissues except the liver revealed that C/EBPβ is required for formation of WAT. Interestingly, C/EBPβ is not required for the formation of brown adipose tissue (BAT), an observation that currently is not understood (Linhart et al., 2001).

PPARγ can induce adipogenesis in C/EBPβ-deficient MEFs, whereas C/EBPβ is incapable of driving the adipogenic program in the absence of PPARγ (Rosen et al., 2002). This observation suggests that C/EBPβ and PPARγ participate in a single pathway of adipose development, in which PPARγ is the dominant factor. It must be mentioned that C/EBPβ does provide a critical function during terminal adipogenesis since failure to express C/EBPβ results in insulin resistance in cell culture models and an inability to develop WAT in vivo (El-Jack et al., 1999; Linhart et al., 2001; Wu et al., 1999). It has been suggested that, in addition to controlling insulin action, C/EBPβ is required for maintaining expression of PPARγ in the mature fat cell (Wu et al., 1999). It is possible that establishment of the adipogenic phenotype in C/EBPβ-deficient brown adipocytes is due to other mechanisms (possibly other C/EBPs) that function to maintain PPARγ production.

C/EBPβ and C/EBPδ

Well before the discovery of PPARγ as the master regulator of adipogenesis, several investigators attempted to identify the mechanisms responsible for determining the differentiation of precursor cells into adipocytes. It is now established that a cascade of transcription factors eventually leads to expression of PPARγ and C/EBPβ. The first indication of such a network came from the work of McKnight and associates, which suggested that two other members of the C/EBP family, C/EBPβ and C/EBPδ, are expressed earlier than C/EBPβ during adipogenesis in 3T3-L1 cells and that they are responsible for regulating C/EBPβ expression (Cao et al., 1991; Yeh et al., 1995). Specifically, they demonstrated that ectopic expression of C/EBPβ and C/EBPδ in 3T3-L1 preadipocytes induces C/EBPβ expression and the adipogenic program in the absence of extracellular hormones. They also showed that introduction of these C/EBPs into nonadipogenic NIH 3T3 fibroblasts can induce adipogenesis without stimulating C/EBPβ expression.

These studies did not address, however, the mechanisms regulating PPARγ production. Other studies aimed at identifying the early events regulating adipogenesis demonstrated a direct link between the C/EBPs and PPARγ. Specifically, ectopic expression of C/EBPβ in NIH 3T3 fibroblasts, alone or in combination with C/EBPδ, induces expression of PPARγ and, following exposure to PPARγ ligands, in doing so, facilitates the conversion of the cells into adipocytes (Wu et al., 1995, 1996). In agreement with McKnight et al., these studies showed that NIH 3T3 cells do not express C/EBPβ, even though they accumulate abundant amounts of triglyceride in response to activation of PPARγ. Additionally, both groups observed that C/EBPδ alone possesses minimal adipogenic activity. C/EBPβ and C/EBPδ play important roles in inducing expression of C/EBPβ and PPARγ. This was shown by the identification of functional C/EBP regulatory elements in the promoters of c/ebpβ and pparγ (Christy et al., 1991; Clarke et al., 1997).

In an attempt to define the sequence of events leading to terminal adipogenesis, it was proposed that C/EBPβ and C/EBPδ simultaneously control expression of both PPARγ and C/EBPβ. Alternatively, some investigators have suggested that C/EBPβ induces C/EBPβ and that, together, these factors regulate PPARγ expression. More recently, studies have shown that ectopic expression of C/EBPβ in Swiss fibroblasts induces PPARγ as expected but is incapable of inducing C/EBPβ to
any significant extent in the absence of a potent PPARγ ligand. Moreover, retroviral expression of C/EBPβ in pparγ−/− MEFs also shows that C/EBPβ, in the absence of active PPARγ, is incapable of stimulating expression of cebpα (Zuo et al., 2006). It appears, therefore, that the principal pathway of adipogenesis involves induction of C/EBPβ and C/EBPα, which then facilitate expression of PPARγ. PPARγ along with these C/EBPs then activates C/EBPα expression.

The precise role of C/EBPβ and C/EBPα in regulating this cascade of factors has been questioned, however, in knockout mice. Specifically, Tanaka et al. (1997) demonstrated that neonatal mice lacking both C/EBPβ and C/EBPα have a defect in their ability to produce adipose tissue; however, this defect appears to be downstream of both PPARγ and C/EBPα since both factors are expressed in the poorly differentiated adipose tissue. In contrast, MEFs obtained from these knockout mice do not express C/EBPα or PPARγ and are incapable of undergoing adipogenesis in culture when compared to wild-type cells. These data suggest that there is some redundancy in the early steps of adipogenesis in vivo where alternative pathways operate to ensure expression of PPARγ and C/EBPα. Furthermore, it appears that C/EBPβ and C/EBPα, in addition to inducing expression of PPARγ and C/EBPα, provide other functions during terminal adipogenesis since their absence prevents terminal adipogenesis at a step downstream of PPARγ or C/EBPα. One possible function might include induction of programs responsible for production of PPARγ ligands (Hamm et al., 2001).

Identifying the factors that regulate C/EBPβ and C/EBPα expression as well as cooperate with these C/EBPs in an adipogenic-specific manner should provide additional insight into the mechanisms regulating the commitment of mesenchymal stem cells to the adipogenic lineage. Studies from Klemm and Lane provide convincing evidence that the cAMP regulatory element-binding protein, CREB, which is activated very early during adipogenesis in 3T3-L1 cells, participates in the induction of C/EBPβ expression (Zhang et al., 2004b). This observation is consistent with earlier studies showing a role for cAMP signaling in controlling C/EBPβ expression (Cao et al., 1991) and also explains the need for inducers of CAMP (isobutylmethylxanthine) in cocktails that initiate the adipogenic program. In contrast, induction of C/EBPα is facilitated by glucocorticoids and C/EBPβ (Cao et al., 1991).

**Other adipogenic factors**

Recent quantitative expression profiling utilizing both microarray and qPCR analysis of mRNAs expressed during the early phase of adipogenesis in vitro and in adipose tissue in vivo suggests that many additional transcription factors are potential components of this complex network of factors responsible for inducing adipogenic gene expression (Fu et al., 2005b; Soukas et al., 2001). Investigators have identified Krox20 as a factor that acts early in the adipogenic program and appears to contribute to induction of C/EBPβ expression. Krox20 (also known as early growth response gene 2, or Eg2) is a transcription factor that is induced immediately following exposure of cells to mitogens. Krox20 is activated early in the adipogenic program of 3T3-L1 cells and not only promotes expression of C/EBPβ but also cooperates with C/EBPβ to facilitate terminal adipogenesis (Chen et al., 2005).

The fact that these early events, including activation of CREB, Krox20, and C/EBPβ, precede induction of PPARγ and C/EBPα, transcription by 1 to 2 days suggests that additional processes are required in order to facilitate terminal adipogenesis. Lane and associates, in an attempt to explain this lag, suggested that C/EBPβ does not attain the capacity to bind to C/EBP response elements in the promoters of its target genes until several hours after its appearance in the nucleus because it is bound to satellite DNA (Tang and Lane, 1999). They proposed that its release from this compartment is facilitated by changes in chromatin structure that occur during clonal expansion and terminal adipogenesis. More recently, these investigators suggested that this lag in C/EBPβ activity also results from a delay in its phosphorylation by MAPKs and GSK3, which is required for its DNA-binding activity (Tang et al., 2005). Other studies have also identified an important site of phosphorylation within a regulatory domain of C/EBPβ, but, unlike the studies of Lane, these studies suggest that phosphorylation regulates C/EBPα expression (Park et al., 2004). More recent investigations suggest that the lag between the appearance of C/EBPβ and the expression of PPARγ results from the time required for synthesis of additional proteins that facilitate the activity of C/EBPβ. Specifically, transcription of the Kruppel-like factor KLF5 is activated by C/EBPβ and C/EBPα and, in concert with these C/EBPs, contributes to induction of PPARγ (Oishi et al., 2005). Neonatal heterozygous KLF5 knockout mice have a significant deficiency in adipose tissue formation (Oishi et al., 2005). Additionally, MEFs obtained from these KLF5−/− mice are compromised in their ability to undergo adipogenesis in culture. Studies also suggest a role for other members of the KLF family including KLF6 and KLF15 in promoting adipogenesis (Li et al., 2005; Mori et al., 2005).

It is likely that additional factors of parallel pathways are induced early and converge on PPARγ at a stage downstream of C/EBPβ and C/EBPα, such as the helix-loop-helix (HLH) transcription factor SREBP1c/ADD-1. A potential role for SREBP1c in regulating adipogenesis derives from studies showing that its expression is significantly enhanced in 3T3-L1 adipocytes in response to insulin (Kim et al., 1998a). Additionally, ectopic expression of a dominant-negative SREBP1c was shown to inhibit preadipocyte differentiation, while overexpression of this HLH protein significantly enhances the adipogenic activity of PPARγ (Kim and Spiegelman, 1996). Expression of SREBP1c alone, however, is only capable of inducing adipogenesis to a modest extent, and additional studies suggest that SREBP1c contributes to the production of PPARγ ligands, thereby facilitating the action of PPARγ (Kim et al., 1998b). There have been other investigations linking SREBP1c to the induction of PPARγ through SREBP binding sites within the ppara and γ3 promoters (Fajas et al., 1999). Support for an additional pathway regulating adipogenesis derives from recent investigations into the function of STAT5 proteins. STAT5A and STAT5B facilitate transmission of cytokine signaling to a host of target genes controlling many functions in several cell types. Ablation of these STATs in mice leads to a spectrum of pathological responses primarily associated with absence of growth hormone and prolactin signaling but also leads to a 5-fold reduction in adipose tissue mass compared to that of wild-type animals (Teglund et al., 1998). This phenotype could be due to the attenuation of proadipogenic prolactin signaling; however, recent studies suggest a direct role for STAT5 in adipogenesis. Specifically, ectopic expression of STAT5A in nonadipogenic fibroblasts induces preadipocyte differentiation, which includes activation of PPARγ activity as well as accumulation of multiple fat.
droplets (Floyd and Stephens, 2003). The mechanisms responsible for this activity of STAT5A, however, are not known since the direct target gene (or genes) has not been identified. There have been human genetic studies, however, supporting a role for STAT5 in regulating transcription from the pparγ3 promoter (Meirhaeghe et al., 2003).

An interesting series of investigations show that components of the molecular clock might also have a role in regulating both adipocyte formation and function. Specifically, MEFs lacking BMAL1 (brain and muscle ARNT-like protein 1), a transcription factor known to regulate circadian rhythm, fail to differentiate into adipocytes, and ectopic expression of BMAL1 in these cells restores adipogenesis (Shimba et al., 2005). Similarly, another component of the molecular clock, Rev-erbα, is induced by BMAL1 and PPARγ during adipogenesis in 3T3-L1 preadipocytes and facilitates expression of several adipogenic genes (Fontaine et al., 2003; Shimba et al., 2005). A model for the transcriptional cascade regulating adipogenesis is illustrated in Figure 1, including those factors that induce expression or activity of other adipogenic transcription factors.

**Role of clonal expansion and cell-cycle-related proteins in regulating adipogenesis**

It is generally thought that clonal expansion of a population of preadipocytes is a prerequisite for their subsequent differentiation into adipocytes. Adipogenesis is induced in a confluent population of the cells by exposure to insulin, inducers of cAMP signaling, and glucocorticoids in 10% FBS. This medium, rich in mitogens, induces the entire population of cells to reenter the cell cycle (G0 to G1) and undergo at least two rounds of cell division before proceeding into terminal adipogenesis. Inhibition of cell proliferation with drugs that block S phase prevents adipogenesis, and it has therefore been suggested that adipogenesis requires mitosis to reorganize chromatin to facilitate induction of the adipogenic genes (Tang et al., 2003). Alternatively, the necessity for the clonal expansion phase may be due to a requirement for components of the cell-cycle machinery in promoting adipogenic gene expression. As mentioned above, Krox20 is an early growth response gene that is induced as confluent preadipocytes reenter the cell cycle and also plays a direct role in inducing C/EBPβ and PPARγ2 expression. The most notable cell-cycle proteins that regulate the adipogenic program are the E2F family of transcription factors and associated pocket proteins.

**E2Fs, pocket proteins, and adipogenesis**

Studies by Auwerx and associates have provided evidence suggesting that the E2F family of transcription factors regulate adipocyte differentiation (Fajas et al., 2002b). The data show that E2F1-3 and E2F4 have opposing effects on differentiation, which appears to be due to their differential regulation of pparγ1 expression. In confluent preadipocytes, E2F4 represses PPARγ transcription through association with the pocket protein p130 and recruitment of the histone deacetylase HDAC3 to E2F response elements in the promoter of pparγ1. As preadipocytes progress through clonal expansion, the abundance of E2F4/p130 complexes subsides, while E2F1/Rb complexes appear. Additionally, the cyclin-dependent kinase inhibitor p27KIP is downregulated (Morrison and Farmer, 1999; Patel and Lane, 2000), thereby facilitating activation of cyclin D/Cdk4/6, which corresponds with phosphorylation of Rb, resulting in the release of E2F1 to induce transcription of pparγ1 (see Figure 2). These data demonstrating a function for E2Fs in adipogenesis correlate with a series of genetic studies performed in mice. E2F1+/− mice have a limited ability to accumulate adipose tissue in response to high-fat feeding, while E2F4−/−ES cells contribute more significantly to adipose tissue development than other tissues of chimeric mice. Consistent with the mouse models, E2F1+/− MEFs have a reduced capacity to differentiate into adipocytes, whereas E2F4-deficient MEFs and ES cells express an enhanced capacity for differentiation. Furthermore, the combined loss of the major E2F4-associated pocket proteins p107 and p130 leads to enhanced adipogenesis in corresponding

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**Figure 1.** Induction of adipogenesis by a cascade of transcription factors

Exposure of preadipocytes to a cocktail of adipogenic inducers comprised of insulin, glucocorticoids, agents that elevate cAMP (isobutylmethylxanthine), and fetal bovine serum activates expression of several transcription factors that converge on PPARγ. PPARγ then induces C/EBPα expression, and together, these factors oversee terminal adipogenesis.
MEFs (Classon et al., 2000), supporting the notion that E2F4/p107 or E2F4/p130 complexes and not E2F4 alone repress pparγ1 transcription. One would predict that a deficiency in Rb would enhance adipogenesis by facilitating E2F1 activity; however, Rb−/− MEFs interestingly have a reduced capacity for differentiation into white adipocytes (Classon et al., 2000). This is likely due to the requirement of Rb in facilitating cell-cycle exit as well as cooperation with C/EBPs to induce adipogenic gene expression (Chen et al., 1996). It appears, therefore, that the E2Fs and pocket proteins regulate two separate but parallel pathways that result in the activation of PPARγ1 and PPARγ2 expression (Figure 2). Specifically, factors such as Rb, which channel through C/EBPβ, lead to PPARγ2 production, whereas factors that promote E2F1 activity lead to PPARγ1 expression. Since PPARγ2 and not PPARγ1 is considered to be the predominant regulator of adipogenesis, factors such as E2F that converge on PPARγ1 need to have a means of enhancing PPARγ2 expression. This process could be facilitated through C/EBPα whereby PPARγ1 induces C/EBPα, which in turn induces PPARγ2 expression (Wu et al., 1999; Zuo et al., 2006). Such a process could explain the redundancy in mechanisms regulating PPARγ2 expression in mice deficient in C/EBPβ and C/EBPδ (Tanaka et al., 1997). In these mice, it is conceivable that signals in the developing adipose depot act on E2F to stimulate PPARγ1, which then induces C/EBPα followed by PPARγ2 without the need for expression of C/EBPβ or C/EBPδ.

Negative regulation of adipogenesis

The differentiation of mesenchymal stem cells along a particular lineage is regulated by both induction of various transcriptional activators and suppression of inhibitors. It is likely that the subtle balance in the activity of positive versus negative effectors determines whether adipogenesis proceeds within a particular population of progenitor cells. This concept is well illustrated by the studies of MacDougald and associates, who demonstrated that activation of the Wnt signaling pathway inhibits the differentiation of mesenchymal stem cells into adipocytes (Ross et al., 2000). Wnt signaling appears to favor differentiation of progenitor cells into bone or muscle, as opposed to adipocytes (Bennett et al., 2005). Wnts are a large family of extracellular effectors secreted by many different cell types and play a determining role during early development. The binding of various Wnts to corresponding Frizzled receptors and low-density lipoprotein receptor-related proteins (LRPs) activates signaling pathways that alter gene expression and cell function. The canonical Wnt pathway leads to mobilization of β-catenin into the nucleus, where it coactivates the TCF/LEF family of transcription factors. Exposure of preadipocytes to Wnts or ectopic expression of a constitutively active form of β-catenin inhibits adipogenesis by preventing induction of PPARγ and C/EBPα (Moldes et al., 2003; Ross et al., 2000). The precise mechanism involved is not known, but it likely involves expression of TCF/LEF target genes since expression of dominant-negative TCF (dnTCF) partially rescues the inhibitory effects of Wnt (Ross et al., 2000). Furthermore, expression of dnTCF causes spontaneous differentiation of preadipocytes, suggesting that the canonical Wnt signaling pathway acts in progenitor cells to suppress adipogenesis. An attractive candidate for a TCF-induced adipogenic inhibitor is cyclin D1 since its gene is a direct target of Wnt signaling, which has been shown to antagonize PPARγ activity (Fu et al., 2005b; Wang et al., 2003). It is also possible that β-catenin might contribute to the inhibition of PPARγ activity through mechanisms other than those involving TCF/LEF (Liu and Farmer, 2004; Liu et al., 2006), and it is worth noting that conditional deletion of β-catenin in the mesenchyme of the developing mouse results in a switch to adipogenesis in the myometrium (Arango et al., 2005).

Several studies have demonstrated that multiple effectors attenuate adipogenesis by compromising the activity of C/EBPβ. These observations not only identify the existence of negative regulators but also support a role for C/EBPβ in regulating preadipocyte differentiation. A series of these negative regulators,
including GATA2/3, ETO/MTG8, CHOP10, GILZ, and Delta-interacting protein A (DIPA), are expressed in preadipocytes, and their expression is downregulated during differentiation. Ectopic expression of each of these proteins in preadipocytes inhibits adipogenesis through antagonism of C/EBPβ activity and thereby prevents the induction of PPARγ and C/EBPα (Batchinarova et al., 1995; Bezy et al., 2005; Rochford et al., 2004; Shi et al., 2003; Tong et al., 2000, 2005). It is worth noting that the vitamin D receptor, which is induced early in adipogenesis (Fu et al., 2005b), blocks preadipocyte differentiation by downregulating C/EBPβ through mechanisms that possibly involve induction of ETO/MTG8 (Blumberg et al., 2006). Similarly, Hedgehog signaling, which is known to regulate vertebrate development, plays a conserved role in inhibiting fat formation, possibly by inducing expression of GATA2 (Suh et al., 2006). Additionally, Notch signaling plays an important role in early development, and the Notch target Hes-1 blocks adipogenesis by mechanisms that possibly involve recruitment of members of the Groucho/TLE family of corepressors (Ross et al., 2006; Ross et al., 2004). Other investigators have hypothesized that oxygen tension might control adipose tissue function by regulating adipogenesis (Swiersz et al., 2004). Specifically, Yun et al. (2002) have demonstrated that hypoxia inhibits preadipocyte differentiation through a mechanism that involves repression of pparγ expression by DEC1/Strata13. DEC1/Strata13 is a member of the Drosophila hairy/Enhancer of split transcription repressor family that is induced by hypoxia-inducible transcription factor 1α (HIF-1α). Strata13 is also induced by retinoic acid (RA) (Boudjelal et al., 1997) and, consequently, might also be the mediator by which RA inhibits adipogenesis (Schwarz et al., 1997).

As discussed above, insulin possesses significant proadipogenic activity in part by promoting expression of SREBP1c. Studies performed in animals as well as in cell culture demonstrate that insulin promotes adipogenesis by suppressing the inhibitory activity of the forkhead transcription factor FoxO1. Specifically, exposure of preadipocytes to insulin results in AKT-dependent phosphorylation of FoxO1, preventing its translocation into the nucleus and subsequent inhibition of adipogenic gene expression. To identify mechanisms responsible for this inhibitory activity, Accili and associates demonstrated that a constitutively active FoxO1, which is insensitive to AKT phosphorylation, inhibits the differentiation of ST3-F422A preadipocytes by arresting the cells in clonal expansion. This block in the adipogenic progression is likely due to a FoxO1-associated induction of the cyclin-dependent kinase inhibitor p21Cip (Nakae et al., 2003). In support of an inhibitory function for FoxO1 in adipose tissue, additional studies showed that FoxO1 haploinsufficiency (foxo1+/−) protects against diet-induced insulin resistance and diabetes possibly by preventing adipocyte hypertrophy (Nakae et al., 2003). It is interesting that two additional members of the forkhead family, FoxA2 and FoxC2, also attenuate adipogenesis upstream of PPARγ (Davis et al., 2004; Wolfrum et al., 2003).

It is important to mention that, while three members of the KLF family are proadipogenic (KLF5, KLF6, and KLF15), at least one KLF acts as a suppressor of adipogenesis. Specifically, KLF2/ lung Kruppel-like factor is abundantly expressed in adipose tissue in preadipocytes, and its expression is downregulated during adipogenesis (Banerjee et al., 2003; Wu et al., 2005). Ectopic expression of KLF2 in preadipocytes inhibits pparγ2 transcription, possibly by binding to KLF regulatory elements in the same region of pparγ2 that facilitates the proadipogenic activity of KLF5 (Banerjee et al., 2003; Oishi et al., 2005; Wu et al., 2005). The involvement of the different negative regulators in controlling adipogenesis is illustrated in Figure 3.

**Role of coregulators in controlling the adipogenic transcription factors**

All of the adipogenic transcription factors discussed above initiate their corresponding programs of gene expression by binding to response elements in target genes where they recruit appropriate coactivators following dissociation from corepressors. Most of these adipogenic coregulators are ubiquitously expressed and employed by other transcription factors in multiple cell types. Consequently, their selectivity in activating a specific gene is primarily defined by the interaction with the transcription factor that is docked on the response element within the promoter/enhancer of the target gene.
Coactivators

There is evidence suggesting that C/EBPβ can dock on the promoters of c/ebpα and pparγ prior to their activation during the early phase of adipogenesis (Salma et al., 2006). Adipogenic effectors then facilitate association of the chromatin remodeling complex SWI/SNF with C/EBPβ on the pparγ2 promoter (Salma et al., 2004). Glucocorticoid receptors (GRs) along with PPARγ are responsible for dislodging an mSin3a/HDAC-1 complex from C/EBPβ on the C/EBP response element in the c/ebpα promoter (Wiper-Bergeron et al., 2003; Zuo et al., 2006). Similarly, the adipogenic potential of C/EBPα depends on its interaction with SWI/SNF, which occurs through interaction with the transactivation element III (TEIII) domain in C/EBPα. This interaction mediates further association with TBP/TFIIB factors (Pedersen et al., 2001). C/EBPα can also associate with CBP/p300, but the precise role of this interaction during adipogenesis is not known (Erickson et al., 2001). PPARγ appears to be capable of interacting with several different coregulators, which explains how it functions to control expression of numerous gene programs in mature adipocytes. Notable among these coregulators is PPARγ coactivator 1α (PGC-1α), which coactivates a host of transcription factors in addition to PPARγ that collectively participate in energy balance (Lin et al., 2005). During development, PGC-1α and β regulate brown adipose formation by coactivating transcription factors including nuclear respiratory factor 1 (NRF-1) and PPARγ that regulate thermogenesis and mitochondrial biogenesis (Lin et al., 2005; Uldry et al., 2006). During adipogenesis, activation of most PPARγ target genes involves an elaborate process in which binding of PPARγ to corresponding ligands dislodges corepressor complexes (NCoR/SMRT with HDAC3) and recruitment of members of the p160 family of coactivators, usually TIF2 or SRC-1. Recent studies have shown that these p160 coregulators might also possess some nonredundant function since lack of TIF2 in mice decreases PPARγ activity in WAT and decreases fat accumulation. In BAT, the absence of TIF2 facilitates an interaction between SRC-1 and PGC-1α leading to an increase in thermogenic activity. Interestingly, TIF2−/− mice are protected against obesity and display enhanced adaptive thermogenesis, whereas SRC-1−/− mice are prone to obesity (Picard et al., 2002). The association of PPARγ with the p160 coregulators leads to further recruitment of histone acetyltransferases (HATs) that appropriately modify surrounding chromatin, allowing the transcriptional machinery access to the gene promoter. At present, most studies show that the p160 coactivators interact with the AF-2 domain of PPARγ in response to binding of appropriate ligands such as troglitazone. It is likely that other coactivators associate with the AF-1 domain at the N terminus of PPARγ. Investigations have shown that two homologous cofactors, p300 and CBP, bind to the N terminus of PPARγ2 in a ligand-independent manner, whereas binding to the C terminus is dependent on a ligand (Gelman et al., 1999). A possible role for CBP in regulating adipose tissue development has been supported by studies of CBP heterozygous mice, which show markedly reduced weight of adipose tissue but not of other tissues (Yamauchi et al., 2002).

PPARγ also communicates with the basal transcriptional machinery through its interaction with a large multicomponent Mediator complex that is required for adipogenesis. Specifically, PPARγ associates with the TRAP (thyroid hormone receptor-associated protein) coactivator-Mediator complex through binding to the TRAP220 subunit in a ligand-enhanced manner. MEFs lacking TRAP220 are resistant to PPARγ2-stimulated adipogenesis, but not to MyoD-stimulated myogenesis (Ge et al., 2002). These observations suggest that TRAP220 acts, via the Mediator complex, as a PPARγ2-selective coactivator; this interaction participates in commitment of mesenchymal cells along an adipogenic as opposed to myogenic lineage. This selectivity might also be facilitated by additional proteins that interact with the PPARγ-Mediator complex. Specifically, PPARγ-interacting protein (PRIP) can associate with CBP/p300 and TRAP130 of the Mediator complex. Supporting this notion, PRIP−/− MEFS are also resistant to PPARγ2-induced adipogenesis (Qi et al., 2003). In addition, a recent study identified a novel human TAF (TBP [TATA-binding protein]-associated factor), hTAFα43 (TAF8), which is induced and sequestered within TFIID complexes during adipogenesis in 3T3-L1 cells. Furthermore, ectopic expression of a dominant-negative TAF8 blocks 3T3-L1 preadipocyte differentiation (Guermah et al., 2003).

Corepressors

Both NCoR and SMRT appear to function as negative modulators of PPARγ activity during adipogenesis since RNAi knockdown of these factors in 3T3-L1 cells leads to increased expression of PPARγ target genes and increased production of lipid droplets (Yu et al., 2005). Studies also suggest that Rb functions in a fashion similar to these corepressors by facilitating the docking of HDAC3 on PPARγ-driven promoters (Fajas et al., 2002a). Interestingly, other studies suggest that Rb acts as a molecular switch promoting brown versus white adipocyte formation (Hansen et al., 2004a, 2004b). In contrast, the coactivator protein RIP140 appears to function in regulating development of adipose tissue favoring a white phenotype. Specifically, mice devoid of RIP140 are lean, show resistance to high-fat-diet-induced obesity, and have increased oxygen consumption (Leonardsson et al., 2004). It appears that this phenotype stems from the capacity of RIP140 to suppress transcription factors regulating oxidative metabolism and mitochondrial biogenesis, characteristics of brown fat cells (Christian et al., 2005; Leonardsson et al., 2004; Powelka et al., 2006).

As mentioned above, deacetylases that modify histones as well as regulate activity of transcription factors are critical components of various corepressor complexes. The HDACs participate in adipogenic regulation, illustrated by the suppression of C/EBPα and PPARγ by HDAC1 and HDAC3, respectively. Induction of adipogenesis includes dislodgement of these HDACs from their respective transcription factors by mechanisms that include their degradation in the 26S proteasome, and it is likely that the programmed turnover of these HDACs is an integral part of the adipogenic process (Yoo et al., 2006). Other deacetylases that target factors other than the histones might affect adipogenesis by altering the acetylation of coregulators of PPARγ. Specifically, SirT1, the mammalian ortholog of the yeast longevity gene srt1, attenuates adipogenesis by repressing PPARγ activity leading to fat mobilization in white adipocytes, which triggers lipolysis (Picard et al., 2004). SIRT1 is a NAD-dependent protein deacetylase capable of monitoring cellular oxidative state and altering the activity of nuclear regulators in response to metabolites and nutrients (Imai et al., 2000; Rodgers et al., 2005). Repression of PPARγ activity appears to involve docking of SIRT1 with NcoR and SMRT on the promoters of PPARγ target genes in adipocytes (Picard et al., 2004). The interplay between various coactivators and corepressors in determining the differentiation of white versus brown adipocytes is shown in Figure 4.
Concluding remarks
In conclusion, it is quite apparent that significant progress has been made during the last few years in identifying the transcriptional processes controlling the differentiation of preadipocytes into mature fat cells. The challenge for the future is to understand the mechanisms governing the commitment of mesenchymal stem cells to the adipogenic lineage. There are certainly some indications of possible players in this process; however, the adipocyte field lags far behind that of other developmental systems since it has been difficult to locate adipogenic progenitors during early development. Additionally, there is a dearth of information concerning the mechanisms that give rise to the various white fat depots. Recent studies have suggested that there are significant differences between subcutaneous and visceral depots, particularly with regard to their role in cardiovascular disease and diabetes. The reasons for these differences are essentially unknown. It is possible that each of the depots arises from different progenitors controlled by a separate set of transcriptional processes, resulting in distinct depot-specific adipocytes. Future research will no doubt address some of these questions by investigating the role of developmental cues shown to participate in the formation of other tissues. Finally, most of what we know has come from studies of rodents either in vivo or in cell culture. There are examples of adipose function that do not translate from the mouse to the human; consequently, attention needs to be given to understanding the transcriptional control of adipocyte formation and function in human adipose tissue.

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