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Insights into the Transcriptional Control of Adipocyte Differentiation

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Abstract The adipocyte is now known to play an active role in many physiological and pathological processes regarding energy metabolism. Consideration of adipose tissue as an endocrine organ that secretes a variety of unrelated bioactive molecules has broadened our appreciation of adipocyte function to exceed the once considered passive role in lipid metabolism. Growing interest in this tissue has lead to significant advances regarding the molecular basis for adipocyte differentiation. Several diverse families of transcription factors are currently under active investigation for their roles in mediating this complex process. Knowledge concerning the sequence of transcriptional events during adipogenesis and the interplay among adipogenic transcription factors provides a basis for understanding the physiological processes associated with adipose tissue as well as for the development of therapeutic intervention of adipocyte related diseases. J. Cell. Biochem. Suppls. 32/33:59–67, 1999. © 1999 Wiley-Liss, Inc.

Key words: adipocytes; transcription; energy metabolism

Energy balance is fundamentally important for human survival. Numerous processes have evolved to provide for the storage of energy during times of plenty and more importantly for the release of the energy stores during times of need. Central to this process is the adipocyte, with its enormous capacity to store and mobilize energy in the form of triglycerides that provide a source of free fatty acids for many vital functions including myocardial contraction. During most of human development, adipose tissue has played a physiological role in providing the energy needed to sustain life between infrequent periods of feeding. On an evolutionary scale, it has been a very short time during which the abundance of available nutrients has resulted in excessive accumulation of adipose tissue mass, a condition commonly known as obesity. The increasing incidence of obesity and associated, debilitating disorders including diabetes mellitus, cardiovascular disease, and cancer has generated an immediate need to better understand the etiology of obesity for the development of therapeutic intervention. Until recently, the adipocyte was thought to play a passive role in this process in much the same way that an oil can stores its contents. The recent discovery of leptin and its role in mediating satiety has given new incentive to adipose tissue research by demonstrating an active role for the adipocyte in energy metabolism [reviewed in Flier, 1998].

Emerging data regarding other important functions of adipose tissue (Fig. 1) have positioned the adipocyte near the center of mechanisms regulating energy metabolism as well as a plethora of unrelated processes that are mediated by its secretory products [reviewed in Gregoire et al., 1998]. In this regard, leptin has a wide spectrum of biological activities, independent of satiety, including effects on fertility, reproduction, and hematopoiesis. In addition to this hormone, adipose tissue secretes a variety of molecules, including peptides, cytokines, and complement factors in which their various functions are linked inseparably to the adipocyte as a source for their production. Adipocytes also store and release glycerol and free fatty acids that play a major function in hepatic and peripheral glucose metabolism. Regarding glucose homeostasis, adipose tissue along with heart and skeletal muscle are the only tissues known to

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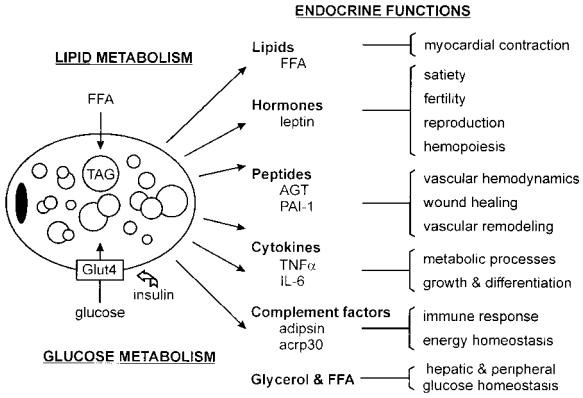

Fig. 1. Pleiotropic functions of the adipocyte. Adipocytes play major roles in lipid metabolism in the storage of free fatty acid (FFA) as triacylglycerol (TAG) and in glucose metabolism through expression of the insulin-dependent glucose transporter, Glut4. Endocrine functions include the secretion of angiotensinogen (AGT), plasminogen activator inhibitor type 1 (PAI-1), tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), adipsin, and adipocyte complement-related protein (acrp30). Potential functions associated with secretory products are illustrated.

express and regulate the insulin-dependent glucose transporter, Glut4, that facilitates the entry of glucose into these cells and out of the circulation postprandially.

Clearly, the adipocyte can no longer be considered as a cell whose only function is to passively store lipids. The emerging roles for adipose tissue in numerous physiological and pathological processes has resulted in a greater need to understand the molecular mechanisms regulating adipogenesis. This review attempts to refine the definition of an adipocyte and, thus, the functional processes to be included when considering adipocyte differentiation as an unified program. Moreover, we discuss future considerations regarding linear and parallel pathways of transcriptional events and the cooperation among adipogenic transcription factors that lead to adipogenesis.

DEFINING ADIPOCYTE DIFFERENTIATION

Considering the growing list of functions associated with adipose tissue, it has become important to redefine the biological characteristics that constitute a functionally mature adipocyte. While lipogenic and lipolytic processes have long been recognized, storage and release of triglycerides are no longer the only functions ascribed to this bioactive tissue. Therefore, molecular events comprising the "adipocyte differentiation program" should include the regulation of genes involved in all aspects of adipocyte biology, including those genes involved in glucose metabolism, as well as the many genes associated with the production and release of secretory products. Since lipogenicity is a characteristic of many tissues, transcriptional events that regulate only those aspects of adipocyte function should be regarded as part of a lipogenic versus an adipogenic program. Thus, future reports of experimental processes proposed to result in adipocyte differentiation should include more than the observation of lipid accumulation and the expression of a few genes associated only with lipid metabolism. Since few, if any, proteins are expressed exclusively in the adipocyte, the complete repertoire of proteins that are vital to adipocyte function, regardless of tissue specificity, should be considered as a whole with regard to adipocyte differentiation. The challenge of future explorations into adipogenesis will be to discover whether a master program regulates the process of adipocyte differentiation, in toto, through a sequence of transcriptional events arranged in series, or whether various functional processes are regulated by independent and/or interacting transcriptional events that occur along parallel pathways.

TRANSCRIPTIONAL CONTROL OF ADIPOGENESIS

Gain of function associated with adipocyte differentiation minimally includes (1) an increase in lipogenic capacity and the appearance of cytoplasmic lipid droplets, (2) acquisition of insulin sensitivity with regard to glucose uptake, and (3) the expression and secretion of numerous bioactive molecules. It is estimated that this structural and functional morphogenesis involves changes in the expression levels of approximately 300 proteins. The directional change of many of these proteins has been comprehensively reviewed [Cornelius et al., 1994]. Many of these changes occur at the level of gene expression through a series of molecular events involving several transcription factor families that exhibit diverse modes of activation and function. The following is intended to present a brief overview of these transcription factors and evidence supporting their role as important mediators of adipocyte differentiation. As the study of adipogenesis broadens to include newly discovered adipocyte functions, the list of important *trans*-acting factors mediating these processes will undoubtedly lengthen.

PPARs

The peroxisome proliferator-activated receptors (PPARs) are a subset of the nuclear hormone receptors whose transcriptional activities are modulated by ligand-receptor interactions [reviewed in Brun and Spiegelman, 1997]. The three known PPAR family members, PPARα, PPARγ, and PPARδ, bind similar peroxisome proliferator response elements but exhibit different transactivating functions that are mediated, in part, by tissue distribution, ligand specificity and coactivator recruitment. Through the use of different start sites and alternate splicing, the PPARγ gene gives rise to two isoforms: γ1 and γ2. Although each PPAR is expressed to some extent in multiple tissues, they exhibit markedly different levels of expression in a tissue-specific manner whereby the expression pattern appears to correlate with primary functions. PPARα is highly expressed in the liver and plays an important role in regulating the expression of enzymes in the β-oxidation pathway. PPARγ2 is highly enriched in adipose tissue and mediates gene expression regarding fatty acid metabolism. The notion that PPARγ plays a major role in regulating adipogenesis is supported by the fact that thiazolidinediones (TZDs), which are high-affinity, synthetic ligands for PPARγ, are potent inducers of adipocyte differentiation. Furthermore, ectopic expression of PPARγ in multiple non-progenitor cells lines under adipogenic conditions results in consistent and potent induction of adipocyte differentiation. While antisense and gene ablation studies have not been reported, it is well accepted that PPARγ and its obligate heterodimeric partner, retinoid X receptor α (RXRα), play a prominent role in regulating gene expression leading to adipogenesis.

C/EBPs

The CCAAT/enhancer-binding proteins (C/EBPs) belong to a large family of leucine zipper transcription factors that can form homodimers and heterodimers with each other and bind to the same C/EBP consensus sequence. Three of these family members, C/EBPα, C/EBPβ, and C/EBPδ, are expressed in both white and brown adipose tissue and have been extensively studied and reviewed for their roles in regulating adipogenesis [Lane et al., 1996; Darlington et al., 1998]. Ectopic expression of C/EBPα or C/EBPβ induces adipogenesis in nonprogenitor fibroblasts, whereas antisense expression of C/EBPα inhibits differentiation of cultured preadipocytes. Mice targeted for C/EBPα gene ablation die within 8 h postpartum due, in part, to hypoglycemia, since administration of glucose can rescue these animals for up to 40 h. Within this time frame, lipid droplets appeared in both white and brown adipose tissue of control animals. By contrast, C/EBPα-deficient mice are suppressed in their ability to develop brown adipocytes and completely devoid of characteristic white adipose tissue. Gene ablation studies that target both C/EBPβ and C/EBPδ also demonstrate a reduced propensity for adipogenesis with deficient animals developing markedly less adipose tissue compared to wildtype littermates. Collectively, these data demonstrate a prominent role for C/EBP family members during the development of adipocyte differentiation, both in vitro and in vivo.

ADD1/SREBP-1c

Sterol regulatory element binding proteins (SREBPs) are known to modulate transcription of numerous genes encoding proteins that function in both cholesterol and fatty acid metabolism [reviewed in Brown and Goldstein, 1997]. The SREBP family consists of three proteins, designated SREBP-1a, -1c, and -2, that are encoded by two independent genes. In humans and mice, SREBP-1a and SREBP-1c are produced from a single gene through the use of alternate transcription start sites. Adipocyte determination- and differentiation-dependent factor 1 (ADD1), cloned independently from a rat adipocyte cDNA library [Tontonoz et al., 1993], is homologous to human SREBP-1c. SREBPs are unique transcription factors in that they contain two transmembrane domains that anchor the protein to the endoplasmic reticulum. When cellular sterol levels are low, two proteolytic events result in the cleavage and release of the cytoplasmic N-terminal fragment that translocates to the nucleus, binds to the promoters of target genes, and regulates transcription. The N-terminal fragment of the SREBPs contains a basic-helix-loop-helix leucine zipper domain that exhibits dual specificity for classic E-box motifs as well as non-E-Box sterol regulatory elements. All three SREBPs are capable of activating similar gene expression, but with different efficiencies. Regulation of fatty acid biosynthesis is mediated primarily

by SREBP-1a and ADD1/SREBP-1c, whereas SREBP-2 regulates cholesterol metabolism. Adipose tissue, in vivo, predominantly expresses ADD1/SREBP-1c over other forms of SREBPs. Ectopic expression of a truncated form of ADD1/SREBP-1c, containing only the activated N-terminal portion of the protein, enhances adipocyte gene expression in nonprogenitor NIH-3T3 fibroblasts under adipogenic conditions. In addition, expression of a dominant negative form of this SREBP isoform represses 3T3-L1 preadipocyte differentiation [Kim and Spiegelman, 1996]. Ablation of the SREBP-1 gene (mice lacking both SREBP-1a and SREBP-1c) results in partial embryonic lethality [Shimano et al., 1997]. While surviving mice demonstrate elevated hepatic cholesterol biosynthesis, the mass of white adipose tissue in these mice is unaltered. Since white adipose tissue also expresses SREBP-2, redundancy of function has not been ruled out.

STATs

Signal transducers and activators of transcription (STATs) comprise a family of cytoplasmic proteins that are activated and mediate gene expression in response extracellular effectors that target receptors with intrinsic kinase activity or receptors to which Janus kinases (JAKs) are bound [reviewed in Darnell Jr, 1997]. Ligand-mediated dimerization of the receptor results in phosphorylation of the associated kinase, which subsequently phosphorylates the cytoplasmic tail of the receptor that serves as a docking site for STAT recruitment. The receptor-bound STAT is phosphorylated, then dimerizes with other STAT proteins and translocates to the nucleus to mediate specific gene expression. The seven known STAT family members are constitutively expressed in a variety of tissues in an inactive form until receptor mediated phosphorylation events occur. Interestingly, the expression of three members of this family, STAT1, STAT5A and STAT5B, is significantly upregulated during differentiation of cultured preadipocytes [Stephens et al., 1996]. While the function of STATs during adipocyte differentiation is unclear, gene ablation of STAT5A and STAT5B produces animals with markedly less white adipose tissue compared to wildtype littermates demonstrating a significant role for these proteins during adipogenesis [Teglund et al., 1998].

MEF2s

An important function of the adipocyte involves the uptake of glucose in response to circulating insulin. This process is mediated by trafficking of intracellular vesicles containing the glucose transporter protein, Glut4, to the membrane, where it functions to facilitate glucose transport into the adipocyte. Glut4 mRNA expression is largely restricted to white and brown adipose tissue as well as heart and skeletal muscle. While some tissue specificity is noted, changes in Glut4 gene expression are often paralleled in muscle and adipose tissue in response to physiological states of altered glucose homeostasis [reviewed in Charron et al., 1999]. These findings suggest that a common modality with regard to transcriptional regulation of Glut4 gene expression may exist between these diverse tissues. While Glut4 gene expression has been shown to be downstream of PPARγ during adipocyte differentiation [Wu et al., 1998], this transcription factor is not likely to be the regulator of Glut4 during myogenesis due to tissue specificity. Evidence suggests that a common mediator of Glut4 expression may be found in the myocyte enhancer factor 2 (MEF2) family of DNA binding proteins that belong to a larger family of MADS-box domain transcription factors. While this family of transcription factors, consisting of MEF2A, MEF2B, MEF2C, and MEF2D, has been studied largely in the context of myogenesis, MEF-2A is also abundantly expressed in white and brown adipose tissue. Promoter analysis using a series of Glut4 reporter genes in transgenic mice has revealed a conserved MEF2 binding domain that is necessary, but not sufficient, to support tissue-specific gene expression [Thai et al., 1998]. Nuclear extracts from differentiated adipocytes demonstrate MEF2A binding activity giving rise to the possibility that these proteins may act in a cooperative manner with other adipogenic transcription factors in regulating Glut4 gene expression and possibly insulin sensitivity during adipogenesis.

LINEAR AND PARALLEL PATHWAYS CONSTITUTING ADIPOGENESIS

Knowledge concerning the sequence of events mediating adipogenesis has been greatly enhanced by the development of cell lines (e.g., 3T3-L1, 3T3-F442A) that differentiate from determined, fibroblastic-like cells into function-

ally mature adipocytes resembling those found in white adipose tissue, in vivo. While brown adipocyte cell lines are beginning to emerge, equivalent data regarding the differentiation steps of brown adipose tissue are not yet available. Figure 2 summarizes the molecular process of adipocyte differentiation, focusing only on transcriptional events. Other processes involving clonal expansion, postmitotic growth arrest, and signaling molecules, that should be considered an integral part of adipocyte differentiation, have been extensively reviewed elsewhere [Cornelius et al., 1994; MacDougald and Lane, 1995; Gregoire et al., 1998]. Traveling from left to right across the schematic illustrates linear and parallel pathways of transcriptional events that occur as determined preadipocytes are induced to differentiate into functionally mature adipocytes. Many preadipocyte cell lines are "conditioned for differentiation" and begin to activate limited gene expression shortly after reaching confluence. Subsequent addition of exogenous mediators (e.g., insulin, glucocorticoids, and agents that lead to an increase in cAMP) initiates a cascade of transcriptional events that account collectively for the expression of most proteins mediating adipocyte function.

Immediately after exposure to exogenous mediators, the gene expression of C/EBPβ and C/EBPδ significantly and transiently increases (Fig. 2), marking an event that is likely to distinguish a preadipocyte from a nonadipogenic precursor cell [Cao et al., 1991]. The activity of these C/EBPs then mediates the expression of PPARγ [Wu et al., 1995; Clarke et al., 1997], which forms a functional heterodimer with RXRα. On the basis of a C/EBP consensus sequence in the C/EBPα promoter, it is likely that C/EBPβ and C/EBPδ also play a role in regulating the expression of C/EBPα [Christy et al., 1991; Lin et al., 1993]. Once activated, PPARγ and C/EBPα appear to cross-regulate each other, thus, maintaining their gene expression despite the ensuing decay of C/EBPβ and C/EBPδ [Schwarz et al., 1997; Shao and Lazar, 1997]. This notion is supported by the observation that induction of adipogenesis in C/EBPα-deficient fibroblasts by ectopic expression of PPARγ does not activate endogenous PPARγ gene expression [Wu et al., 1999] implicating an obligatory role for C/EBPs in activation and/or maintenance of PPARγ expression. PPARγ and C/EBPα then transactivate subsets of genes as a function of either *trans*-acting factor alone or requiring the cooperative efforts of both [reviewed in Gregoire et al., 1998]. Collectively, these events constitute a direct pathway linking exogenous effectors to adipocyte gene expression through a sequence of transcriptional steps that are aligned in series. In this review, this cascade is referred to as the C/EBP-PPAR linear pathway.

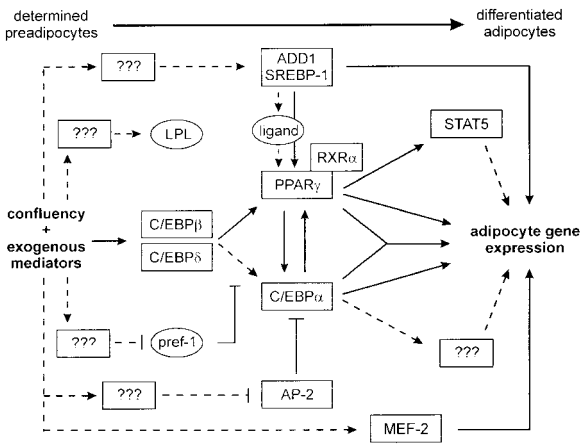

Fig. 2. Model illustrating linear and parallel pathways mediating adipocyte differentiation. Solid lines, direct or indirect transcriptional events; broken lines, interactions that are less well understood; square boxes; specific transcription factors; question marks, unknown factors.

A number of genes encoding for proteins mediating adipocyte function are known to contain active consensus sequences for PPARγ and/or C/EBPα. While this list is impressive, it represents only a small portion of the total number of genes that are regulated during adipogenesis. It is conceivable that these potent adipogenic transcription factors can modulate the expression of other genes, indirectly, through the activation of intermediary *trans*-acting factors. In this regard, recent evidence indicates that the differentiation-dependent induction of STAT1, STAT5A and STAT5B, are regulated downstream of PPARγ in the differentiation paradigm [Stephens et al., 1999] (Fig. 2). Although complete adipocyte differentiation requires the expression of STAT5, the precise function, indirectly linking the activity of PPARγ to adipocyte gene expression, remains to be determined. While future studies will undoubtedly identify other unknown transcription factors downstream of PPARγ and/or C/EBPα, activation of STAT expression represents the only known regulation of *trans*-acting factors by either of these adipogenic mediators.

Events upstream of C/EBPα and PPARγ, that mediate functional aspects of the adipocyte, provide examples of potential parallel pathways that may function in independent, synergistic, and supportive fashions to the transcriptional activity imparted by the linear C/EBP-PPAR pathway (Fig. 2). For example, lipoprotein lipase (LPL) is an enzyme secreted from the adipocyte and localizes to the endothelial surface of blood vessels where it hydrolyzes circulating triglycerides for transport and storage. LPL gene expression increases shortly after preadipocytes reach confluency [Amri et al., 1986]. Since this event occurs in the absence of exogenous mediators, it is likely that the early activation of LPL is independent of C/EBPβ and/or C/EBPδ activity. Early differentiation is also characterized by upregulation of ADD1/SREBP-1c gene expression [Ericsson et al., 1997]. This SREBP family member can synergize with the C/EBP-PPAR pathway by directly activating PPARγ gene expression [Fajas et al., 1999] and/or through the production of endogenous PPARγ ligands needed for transcriptional activity [Kim et al., 1999]. Furthermore, ADD1/SREBP-1c may synergise with C/EBPα and/or PPARγ in direct regulation of genes mediating fatty acid uptake, synthesis and desaturation. An example of a supportive parallel pathway is noted with the early activation of MEF2 binding activity during adipocyte differentiation (J.K. Hamm and S.R. Farmer; unpublished observation). Although MEF2 activity has been shown to be necessary for Glut4 gene expression, it is not sufficient. Therefore, it is conceivable that MEF2 may play a supportive role in the linear C/EBP-PPAR pathway in providing a necessary transcriptional event that modulates the activity of C/EBPα and/or PPARγ in regulating the expression of numerous genes that mediate the complex process of insulin-dependent glucose uptake. Early regulation of LPL, ADD1/SREBP-1c, and MEF2 during adipocyte differentiation appears to occur, at least in part, through mechanisms independent of C/EBPα and PPARγ, constituting potential parallel pathways important for adipocyte function. The mechanisms mediating these early events are unknown.

Parallel pathways are also represented in the form of gene repression. By way of illustration, undifferentiated preadipocytes express a number of inhibitory proteins that must be repressed or functionally inactivated to allow the differentiation process to occur (Fig. 2). One such protein, Pref-1, is an epidermal growth factor (EGF) repeat-containing transmembrane protein that inhibits adipocyte differentiation by an unknown mechanism that prevents the expression of both C/EBPα and PPARγ [Smas et al., 1997]. Likewise, C/EBPα gene expression is repressed by the transcription factor, AP-2α [Jiang et al., 1998]. The expression of both Pref-1 and AP-2α in preadipocytes decreases during early phases of differentiation permitting the activity of the linear C/EBP-PPAR pathway to promote adipogenesis. Inhibitory molecules may function, in vivo, to maintain the preadipocyte phenotype until environmental conditions are supportive for adipocyte differentiation. Similar paradigms are now considered dogma in cell proliferation pathways where numerous proteins serve checkpoint functions regulating commitment to cell cycle progression. While the mechanism regulating repression of AP-2α remains unclear, it now appears that Pref-1 is repressed by dexamethasone, presumably, through activation of the glucocorticoid receptor [Smas et al., 1999].

COOPERATION BETWEEN ADIPOGENIC TRANSCRIPTION FACTORS

The information presented above illustrates cooperation with regard to the interplay and cross-regulation between diverse families of transcription factors during adipogenesis. Analogously, regulation of gene expression encoding for proteins important for adipocyte function also appears to involve similar levels of cooperation between adipogenic transcription factors. A number of genes that contain consensus sequences for both PPARγ and C/EBPα can be modulated by either transcription factor alone, in vitro. The observation of dual binding sites leads to the possibility of synergy between PPARγ and C/EBPα with regard to regulating the expression of a single gene. Recent evidence also suggests that these transcription factors may cooperate in regulating the adipogenic program by activating specific gene expression, independently, whereby a variety of encoded proteins synergistically function to promote the process of adipogenesis. The potential for positive cooperation is best seen when expressing these transcription factors in NIH-3T3 fibroblasts. Under adipogenic conditions, the coexpression of PPARγ and C/EBPα results in a

marked increase in adipocyte conversion and related gene expression relative to expressing either factor alone (Fig. 3). Moreover, coexpression results in a state of PPARγ ligand independence that appears to be mediated, directly or indirectly, by C/EBPα [Tontonoz et al., 1994; Brun et al., 1996]. Although the precise mechanism for this synergy is unclear, it has been hypothesized that cooperation concerning genes containing dual binding sites may occur through synergistic recruitment of coactivators such as CBP/p300 that are known to form direct links to the basal transcriptional machinery. As for synergy resulting from independent gene expression, it has been proposed that C/EBPα may transactivate genes leading to the production of an endogenous ligand for PPARγ, thus resulting in the development of ligand independence. It is likely that this synergy occurs in 3T3-L1 preadipocytes that differentiate in the absence of exogenous ligands for PPARγ.

Positive cooperation relative to functional gene expression is also noted when expressing PPARγ in C/EBPα knockout mouse embryo fibroblasts [Wu et al., 1999] or in NIH-3T3 fibroblasts that are defective for C/EBPα expression [El-Jack et al., 1999]. Under potent adipogenic conditions, including PPARγ ligand supplement, fibroblasts in either case form characteristic lipid droplets and expressed many genes associated with adipocyte differentiation. However, these fibroblasts are not responsive to insulin regarding glucose uptake (Fig. 3). Rescue of this defect with coexpression of C/EBPα clearly demonstrates synergy among adipogenic transcription factors within a program of events involving many proteins necessary for the complex process of insulin sensitivity. Although the precise defect is unclear, positive

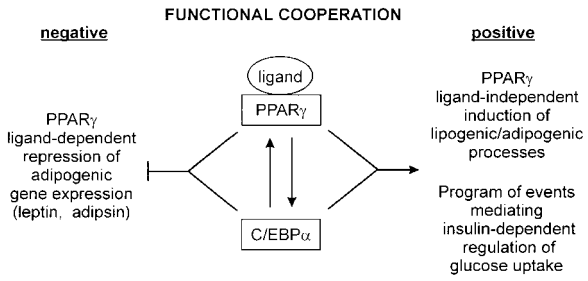

Fig. 3. Positive and negative cooperation between adipogenic transcription factors. Diagram illustrates examples of potential synergy (arrowhead) and functional antagonism (crossbar) between PPARγ and C/EBPα with regard to functional gene expression related to adipogenesis (see text for details).

cooperation between C/EBPα and PPARγ appears to be obligatory for this important aspect of adipocyte function.

Conversely, it now appears that there may be functional antagonism between C/EBPα and PPARγ under certain conditions. For example, treatment of rodents in vivo with potent PPARγ ligands, reduces leptin mRNA and serum protein levels (Fig. 3). This effect appears to be at the level of the adipocyte, where TZD activation of PPARγ inhibits the ability of C/EBPα to drive leptin gene expression [Hollenberg et al., 1997]. The mechanism for this negative cooperation between C/EBPα and PPARγ in regulating leptin expression is completely unknown. However, it may involve a process whereby PPARγ may serve as both activator and/or a repressor under certain circumstances. To this point, it has been observed that PPARγ ligands can suppress (e.g., adipsin) and enhance (e.g., aP2) adipocyte-specific gene expression under identical conditions during adipogenesis (R.F. Morrison and S.R. Farmer, unpublished observation). That this does not happen during differentiation of preadipocyte cell lines, in the absence of exogenous ligands, is an important paradox and may involve the nature of the ligand itself. For instance, certain synthetic ligands can bind PPARγ with high affinity, enhance activity under certain conditions and yet have the ability to completely inhibit adipocyte differentiation in culture [Oberfield et al., 1999].

These examples of positive and negative cooperation between PPARγ and C/EBPα emphasize the possibility that this type of interaction may occur between most, if not all, transcription factors that play a role during adipocyte differentiation. Identification of the physiological relevance and precise mechanisms for various cooperative interactions is central to the understanding of the molecular events mediating adipogenesis.

FUTURE DIRECTIONS

Future investigations into adipogenesis will be challenged to address the adipocyte as a pleiotropic cell with multiple functions and, therefore, multiple mediators of gene expression necessary for these functions. Transcriptional events regulating all aspects of lipid metabolism may not represent the same events necessary for the development of programs involving a multitude of proteins necessary for

insulin-dependent glucose uptake and the expression and regulated secretion of a vast array of unrelated bioactive molecules. It is conceivable that a genetic defect within the pathways that mediate adipogenesis could result in the development of a cell completely devoid of lipid droplets but that expresses and regulates Glut4 and the secretion of leptin, or any combination thereof. Thus, future analysis of gene ablation studies regarding the effect on adipose tissue should include functional aspects of the adipocyte that go beyond the acquisition of cytoplasmic lipid droplets. A cell defective in any one of these important functions is, by definition, only an adipocyte in part. Future consideration of the adipocyte as a pleiotropic cell will undoubtedly lead to the expansion of both linear and parallel aspects of transcriptional pathways as well as a greater understanding of the cooperation among adipogenic transcription factors necessary for complete adipogenesis.

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NOTE ADDED IN PROOF

Since the submission of this manuscript, three consecutive gene ablation studies were published demonstrating an obligatory role for PPARγ in adipocyte differentiation in vivo. Barak et al., 1999, Mol Cell 4:585–595; Kubota et al., 1999, Mol Cell 4:597–609; Rosen et al., 1999, Mol Cell 4:611–617.

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