Symposium: Adipocyte Function, Differentiation and Metabolism

Adipocyte Differentiation and Gene Expression\textsuperscript{1,2}

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ABSTRACT The major function of adipocytes is to store triacylglycerol in periods of energy excess and to mobilize this energy during times of deprivation. The short-term control of these lipogenic and lipolytic processes is carefully modulated by hormonal signals from the bloodstream, which provide an inventory of the body’s metabolic state. Long-term changes in fat storage needs are accomplished by altering both the size and number of fat cells within the body because terminally differentiated adipocytes cannot divide. Alterations in the number of fat cells within the body must be accomplished by the differentiation of preadipocytes, which act as the renewable source of adipocytes. Our understanding of the events that occur during preadipocyte differentiation has advanced considerably in the last few years and has relied mainly on the use of tissue culture models of adipogenesis. This article will discuss the various models used for studying the preadipocyte differentiation process, with the mouse 3T3-L1 cell culture line described in detail. We focus on those genetic events that link effectors to induction of adipocyte gene expression. J. Nutr. 130: 3122S-3126S, 2000.

KEY WORDS: • adipose tissue • differentiation • preadipocytes • transcription factors

The study of adipocyte development has become an area of intense research in recent years. First, adipocyte differentiation has many implications for human disease states. The primary health concerns related to adipocyte development stem from extreme aberrations in fat cell number. The largest class of health problems is caused by obesity, due in part to an overabundance of fat cells. Obesity is considered a major risk factor for noninsulin-dependent diabetes mellitus (NIDDM)\textsuperscript{4} (Moller and Flier 1991) and hypertension (Spiegelman et al. 1993). Obesity has also been linked to some types of cancers, immune dysfunction and, in a self-perpetuating manner, obesity (Spiegelman et al. 1993). The molecular link to cancer has not been strongly established but is believed to be related to an increase in free radical damage of DNA caused by an increase in fatty acid metabolism. A thorough understanding of the differentiation process would allow for manipulation of adipocyte cell number and control of certain diseases. The study of the molecular mechanisms of adipocyte development may suggest important patterns for comparison to other developmental systems and may provide an understanding of the physiology, in general, of late animal development. This article will discuss the various models used for studying the preadipocyte differentiation process, with the 3T3-L1 cell culture line described in detail. We focus on those genetic events that link exogenous effectors to induction of adipocyte gene expression in sequence. More detailed insight in transcriptional activation and repression of adipocyte genes and other aspects of adipocyte biology have been discussed in several excellent reviews (Cornelius et al. 1994, Cowherd et al. 1999, Gregoire et al. 1998, Lane et al. 1999, MacDougald and Lane 1995 Mandrup and Lane 1997, Miller and Ntambi 1998, Morrison and Farmer 1999, Vasseur-Cognet and Lane 1993).

Models of preadipocyte differentiation

The transition from egg to the determination and conversion of adipocyte precursor cells into mature adipocytes occurs in a series of stages as depicted in Figure 1. A single fertilized egg gives rise to nearly 200 different cell types that make up the multiple developmental lineages and multicellular organism. The full developmental program of preadipose tissue from fertilized egg is unknown. However, the pluripotent fibroblasts (stem cells) are known to have mesodermal origins (Cornelius et al. 1994) and can differentiate into committed preadipocytes, cartilage, bone or muscle tissue. In humans, preadipo-

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\textsuperscript{4} Abbreviations used: C/EBP, CCAAT/enhancer binding protein; DEX, dexamethasone; IGF-1, insulin-like growth factor 1; MDI, adipogenic cocktail containing MIX, DEX and insulin; MIX, methylisobutylxanthine; NIDDM, noninsulin-dependent diabetes mellitus; PPAR, peroxisome proliferator-activated receptor.
classes, i.e., pluripotent fibroblasts and unipotent preadipocytes. The remaining two thirds are a combination of small blood vessels, nerve tissue, fibroblasts and preadipocytes in various stages of development (Géloen et al. 1989). The distinction between preadipocytes and fibroblasts is difficult to make, and the inability to align preadipocytes at similar developmental stages confounds detailed in vivo studies. To some extent, preadipocyte primary culture has been used. However, the use of primary culture suffers major drawbacks. First, it is difficult to isolate preadipocytes from other fibroblast-like cells. Second, large amounts of fat tissue are required because preadipocytes constitute only a small percentage of total fat tissue. In addition, primary cultures have a limited life span in culture.

Preadipocyte differentiation has therefore been studied primarily by using in vitro models of adipogenesis; much of the knowledge of adipocyte differentiation has been based on the validity of these tissue culture models. There are advantages and disadvantages to using a cell line to study preadipocyte differentiation. A cell line derived from cloning is homogenous in population of cells that are all at the same stage of differentiation. This allows for a definitive response to treatments. In addition, these cells can be passaged indefinitely, which provides a consistent source of preadipocytes for study.

Preadipocyte precursor cell lines can be segregated into two classes, i.e., pluripotent fibroblasts and unipotent preadipocytes. The pluripotent fibroblasts (10T1/2, BALB/c 3T3, 1246, RCJ3.1 and CHEF/18 fibroblasts) have the ability to be converted into several cell types. 10T1/2 fibroblasts, which were derived from C3H mouse embryos (Reznikoff et al. 1973), can be converted to preadipose, premuscle and precartilage tissue upon treatment with 5-azacytidine, an inhibitor of DNA methylation (Taylor and Jones 1979). These multipotent fibroblasts act as good models for understanding the events responsible for cellular determination of the separate cell fates. Such work led to the discovery of MyoD as a critical regulator of muscle cell determination (Edmondson and Olson 1993).

The second class of culture cells, the unipotent preadipocytes (3T3-L1, 3T3-F422A, 1246, Ob1771, TA1 and 30A3), have undergone determination and can either remain as preadipocytes or undergo conversion to adipocyte tissue. They are ideal for studying the molecular events responsible for the conversion of preadipocytes into adipocytes. The 3T3-L1 and 3T3-F422A culture lines, derived from disaggregated Swiss 3T3 mouse embryos (Green and Kehinde 1974), are the most widely used culture models. TA1 (Chapman et al. 1984) and 30A5 preadipocytes (Konieczny and Emerson 1984), obtained from 5-azacytidine treatment of 10T1/2 fibroblasts, have also been used. Ob1771 preadipocytes, obtained from genetically obese adult mice, are used to investigate aspects of differentiation resulting from genetic obesity (Negrel et al. 1978). The 1256 cell line has been used to study the effects of fatty acids on adipocyte differentiation (Serrero et al. 1992). There is some variation in the differentiation requirements of each cell line. It is believed that these differences represent variations in the developmental stage at which cells were arrested when derived (Cornelius et al. 1994, Smith and Sul 1995). The identification of specific developmental markers will allow (and has to some extent) for the alignment of the developmental programs of the various cell lines.

Progression of differentiation in 3T3-L1 preadipocytes

The 3T3-L1 cell line is one of the most well-characterized and reliable models for studying the conversion of preadipocytes into adipocytes. When injected into mice, 3T3-L1 preadipocytes differentiate and form fat pads that are indistinguishable from normal adipose tissue (Green and Kehinde 1979). In culture, differentiated 3T3-L1 preadipocytes possess most of the ultrastructural characteristics of adipocytes from animal tissue (Novikoff et al. 1980). The formation and appearance of developing fat droplets also mimic live adipose tissue (Green and Kehinde 1974).

Confluent 3T3-L1 preadipocytes can be differentiated synchronously by a defined adipogenic cocktail. Maximal differentiation is achieved upon treatment with the combination of insulin, a glucocorticoid, an agent that elevates intracellular cAMP levels, and fetal bovine serum (Student et al. 1980). Insulin is known to act through the insulin-like growth factor 1 (IGF-1) receptor. IGF-1 can be substituted for insulin in the adipogenic cocktail (Smith et al. 1988). Dexamethasone (DEX), a synthetic glucocorticoid agonist, is traditionally used to stimulate the glucocorticoid receptor pathway. Methylisobutylxanthine (MIX), a cAMP-phosphodiesterase inhibitor, is traditionally used to stimulate the cAMP-dependent protein kinase pathway. This adipogenic cocktail, containing MIX, DEX and insulin, is commonly abbreviated MDI.

Approximately 24 h after induction by MDI, differenti-
ating preadipocytes undergo a postconfluent mitosis and subsequent growth arrest (Bernlohr et al. 1985). The cells undergo at least one round of DNA replication and cell division. By day 2 of differentiation, the cells complete the postconfluent mitosis and enter into an unusual growth arrest called G apoptotic (Scott et al. 1982). The mitosis is believed necessary to unwind DNA, allowing transcription factors access to regulatory response elements present in genes involved in modulating the mature adipocyte phenotype (Cornelius et al. 1994). After the growth arrest, cells are committed to becoming adipocytes. The growth arrest is required for subsequent differentiation. Growth-arrested cells begin to express late markers of differentiation at day 3. These late markers consist of lipogenic and lipolytic enzymes, as well as other proteins responsible for modulating the mature adipocyte phenotype. The cells then round up, accumulate fat droplets and become terminally differentiated adipocytes by day 5–7.

**Gene expression during 3T3-L1 preadipocyte differentiation**

The events depicted in Figure 2 constitute a direct pathway linking exogenous effectors to adipocyte gene expression through a sequence of transcriptional steps that are aligned in series. At confluence, 3T3-L1 preadipocytes express very early markers of adipocyte differentiation. The cell-cell contact induces the expression of lipoprotein lipase and type VI collagen genes (Cornelius et al. 1988, Dani et al. 1990). Within 1 h after the addition of MDI, the expression of c-fos, c-jun, junB, c-myc and CCAAT/enhancer binding proteins (C/EBP) β and δ is observed (Cornelius et al. 1994). Fos and jun proteins have not been implicated directly in any differentiation-specific events. On the basis of their roles in other systems, they are believed to have mitogenic properties. c-myc has been shown to initiate mitogenesis in differentiating preadipocytes (Cornelius et al. 1994). Enforced expression of the c-myc oncogene was shown to block cell differentiation, which was reversible upon high expression of c-myc antisense RNA (Freytag 1988). This suggests that c-myc expression may be a branch point at which the growth arrested cells are either directed toward cell cycle reentry or committed to terminal differentiation. The expression of c-fos, c-myc and c-jun is transient, dissipating by 2–6 h after treatment with MDI. C/EBPβ and C/EBPδ are the first transcription factors induced after exposure of the preadipocytes to the differentiation cocktail; they are therefore postulated to be involved in directing the differentiation process. In accord with this notion, expression of C/EBPβ or C/EBPδ under the control of exogenous promoters of differentiation induces and accelerates adipogenesis in response to hormonal inducers. C/EBPβ is responsive primarily to DEX, whereas C/EBPδ is responsive primarily to MIX. After removal of MIX and DEX from the culture medium, expression of C/EBPδ dissipates over the subsequent 48 h, whereas the decline of C/EBPβ is more gradual such that by day 8, its expression is ~0% of maximal levels (Erickson et al. 2000, Lane et al. 1999). The activity of C/EBPβ and δ is thought to mediate the expression of peroxisome proliferator-activated receptor γ (PPARγ) (Clarke et al. 1997, Wu et al. 1995), which is transcriptionally induced during the 2 d after induction of differentiation and is maximum by day 3–4. It also appears that C/EBPβ and δ mediate the expression of C/EBPα, which increases from undetectable levels in preadipocytes to detectable levels 2 d after MDI stimulation and to full expression ~5 d after initiation of the differentiation program (Christy et al. 1991, Lin and Lane 1994). Once
activated, PPARγ and C/EBPα cross-regulate each other to maintain their gene expression despite a reduction in the expression of C/EBPβ and δ (Shao and Lazar 1997). PPARγ and C/EBPα alone or in cooperation with each other induce the transcription of many adipocyte genes encoding proteins and enzymes involved in creating and maintaining the adipocyte phenotype (Gregoire et al. 1998).

C/EBPs and PPARγ are being studied in great detail with the hope of identifying an adipocyte-specific master switch for terminal differentiation. Although both factors are critical for the late stages of differentiation in a cooperative and synergistic manner, neither factor is expressed at high levels in preadipocytes and therefore cannot be involved in early development. Furthermore, unlike coexpression of these two factors, ectopic expression of either transcription factor does not promote differentiation to the same extent as the full differentiation initiated by the hormonal MDI cocktail (Brun et al. 1996, Hu et al. 1995, Tontonoz et al. 1994).

The antimitotic activity of C/EBPα prevents postconfluent mitosis when added to preadipocytes (Umeck et al. 1991). This prevents the cells from progressing through intermediate differentiation. Forced expression of PPARγ in preadipocytes does not cause differentiation without the addition of an exogenous ligand (Tontonoz et al. 1994). This indicates that an endogenous PPARγ ligand is not present in 3T3-L1 preadipocytes. The endogenous ligand must be produced in response to MDI during the course of early differentiation. The upregulation of ADD1/SREBP-1c gene expression that occurs during preadipocyte differentiation (Ericsson et al. 1997) is thought to lead to the production of endogenous PPARγ ligands required for transcriptional activity (Kim et al. 1999). These endogenous ligands believed to be fatty acid–like molecules, have not been identified and are now the focus of several investigations.

Summary

The major role of adipose tissue is to store ingested energy in the form of triacylglycerides during periods of energy excess; thus, this energy can be made available during periods of energy deprivation. Through studies on adipocyte differentiation, the adipocyte is now known to play an active role in many physiologic and pathologic processes regarding energy metabolism. The discovery of leptin and other secretory molecules in adipose tissue puts the adipocyte at the center of mechanisms of energy metabolism regulation as well as other unrelated processes. The growing interest in this tissue has led to significant advances regarding the molecular basis for adipocyte differentiation. These advances are contributing to a change in conventional opinion, which has regarded adipose tissue only as a dormant tissue playing a passive role in lipid metabolism.

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LITERATURE CITED


