Molecular mechanisms of adipocyte differentiation

E.D. Rosen

Dana-Farber Cancer Institute
Boston, MA USA.

Within the past decade, our scientific notions of adipose tissue have changed dramatically, from the simplistic idea that fat serves only to store excess energy to the realization that adipose tissue is a central regulator of metabolic physiology. We know that too much fat leads to a variety of metabolic derangements, including insulin resistance and type 2 diabetes, while too little fat, as in the lipodystrophic conditions, is equally pathological. Adipocytes store energy in the form of triglyceride; they release this energy in a regulated way as the body demands it, such as during fasting. The functions of fat go far beyond energy storage, however, and there has been increasing recognition of fat tissue as an endocrine organ as well. This began with the discovery of leptin in the mid 1990's and continues through the present, as more secreted factors are being discovered in adipose tissue which affect a bewildering array of biologically important pathways ranging from metabolism, blood pressure control, coagulation, and immunity. The process by which fat cells develop is called adipogenesis. Adipogenesis is believed to begin with pluripotent fibroblastic stem cells; these cells have the ability to differentiate into muscle, cartilage, and bone as well as adipocytes if stimulated with the appropriate inducers [19, 25]. In response to as yet poorly characterized cues, these stem cells become committed to the adipocytic lineage. These cues might be hormones and/or they might be based in the extracellular matrix of a developing fat pad. Regardless, this process is called “determination” and it marks the loss of multipotency. Cells that have been determined can only become adipocytes, even though they may look identical to their precursor cells at the microscopic level.

Mammals actually have two distinct types of adipocytes, which make up brown adipose tissue (BAT) and white adipose tissue (WAT). WAT is the type of fat that most of us think of when we think of adipose tissue. It is the classic tissue of energy storage that marbles our steaks and accumulates around our mid-sections as we age. Brown fat cells have smaller collections of lipid and are very rich in mitochondria [16]. They serve primarily to generate heat through the actions of an uncoupling protein (UCP-1), which dissipates the proton gradient established across the inner mitochondrial membrane during respiration without generating ATP. Small animals like rodents have a large collection of brown fat located in a discrete pad between the scapulae. Humans, on the other hand, have small deposits of brown fat as infants in the thorax and around the kidneys. This disappears as we age, until one can only find occasional brown fat cells scattered among the white adipocytes in our other depots as adults. We know very little about how BAT develops; it may undergo adipogenesis in the same overall way as WAT, with extra factors being turned on to induce BAT-specific proteins like UCP-1. Alternatively, BAT could develop along entirely unique pathways. Finally, it is not known if BAT and WAT can “trans-differentiate” into one another.

Fat cells have also served as a well-studied model of cellular differentiation. In part, this is due to the importance of this tissue in physiology and pathology, as has just been mentioned, but progress has been aided by the availability of immortalized cell lines that recapitulate the process of adipogenesis in vitro. These cell lines, most notably the 3T3-L1 and 3T3-F442A systems, have provided an invaluable reagent for investigators studying the mechanism of adipogenesis [9, 10]. With few exceptions, the phenotype of the cultured adipocytes matches that of endogenous adipocytes in vivo remarkably well.

These in vitro models are induced to differentiate in the presence of a hormonal cocktail; in the case of 3T3-L1 cells this would include a glucocorticoid, insulin, and a cAMP analog or phosphodiesterase inhibitor. When treated with such a mixture, these cells undergo a stereotypical procession of events that includes morphological changes such as “rounding up” of the spindly preadipocytes, gene expression changes, accumulation of lipid, and the development of insulin sensitivity.

Early on, it was noted that members of the CAAT/enhancer binding protein (C/EBP) family of transcription factors were induced during the differentiation of fat cells in vitro [2, 32]. Specifically, C/EBPβ and C/EBPδ were turned on very early after the addition of the hor-
monal cocktail, reached a peak within the first few days, and then returned down toward basal levels as the process continued. C/EBPα, on the other hand, is induced shortly after the appearance of C/EBPβ and δ, and remains elevated for the life of the cell. In gain-of-function experiments, the ectopic expression of any of these proteins will induce the full fat cell phenotype, suggesting the existence of a transcriptional cascade with C/EBPβ and δ turning on expression of C/EBPα, which induces the fat cell phenotype.

Embryonic fibroblasts lacking either C/EBPβ or δ show slight reductions in adipogenic potential, but cells lacking both C/EBPβ and δ are severely impeded from developing as adipocytes [24]. The results from mice lacking these factors are more ambiguous, although they still generally support a role in fat cell development. Mice lacking either C/EBPβ or δ have normal WAT, although their BAT shows reductions in lipid accumulation and UCP-1 expression. Mice that lack both C/EBPβ and δ, however, have a more dramatic phenotype. Approximately 85% of these animals die in the perinatal period of unknown causes; the remaining 15% have sharply reduced BAT and smaller decreases in WAT [24]. Interestingly, the reduction in BAT appears to be due to reduced lipid accumulation, while the reduction in WAT is reported to involve decreased cell number, with normal size, morphology and gene expression profiles in those white adipocytes that do differentiate.

The involvement of C/EBPα in adipogenesis is supported by more definitive data. Overexpression of C/EBPα in 3T3-L1 preadipocytes induces their differentiation into mature fat [7, 15], and the expression of C/EBPα antisense RNA in these cells blocks this process [14]. Animals that carry a homozygous deletion of the C/EBPα gene have dramatically reduced fat accumulation in WAT and BAT pads [29]. However, these mice succumb to hypoglycemia within the first week of life as a result of a failure to activate gluconeogenesis in the liver, and their reduced adiposity needs to be considered in light of their severe metabolic derangement. More recently, one group has replaced the C/EBPα gene with C/EBPβ [3]. This rescues the hepatic phenotype, but still results in greatly diminished BAT accumulation. Some adipocyte markers, such as adipin and leptin, are dramatically down-regulated in these animals as well.

Another important transcription factor in adipogenesis is the nuclear hormone receptor PPARγ. PPARγ was discovered to be a trans-acting factor involved in the expression of the fat cell-selective fatty acid binding protein ap2 [27, 28]. It was soon recognized that PPARγ levels were induced during adipogenesis in the same time course as C/EBPα, and that like C/EBPα, ectopic expression of PPARγ in preadipocytes is sufficient to force differentiation of these cells [27, 28]. Like most nuclear hormone receptors, PPARγ is believed to be a ligand-activated transcription factor. The identity of the bona fide endogenous ligand for PPARγ remains unknown, but several synthetic agonists have been developed. These drugs include the thiazolidinedione (TZD) class of anti-diabetic agents used clinically as insulin sensitizers [13]. The addition of these drugs to preadipocytes is sufficient to hasten their development into mature adipocytes.

Obtaining loss-of-function data for PPARγ has been more difficult. Targeted ablation of the PPARγ locus in mice leads to early lethality at embryonic day 9.5 to 10.5 [1, 12]. This is because of a failure of normal placentation, and occurs before the development of adipocytes in mice. Several strategies have been utilized to bypass this obstacle. One approach was to create chimeric embryos using a combination of wild-type tetraploid cells that allow rescue of the placental defect and PPARγ null cells that are the sole contributor to the embryo proper. Using this approach, one homozygous mutant mouse developed to term, though it died shortly after birth [1]. Nevertheless, it was clear that this neonate lacked significant brown fat depots. No conclusion was drawn regarding WAT, as this tissue develops postnatally in mice, and this animal died prior to the time it would have formed. Another approach was to create chimeric mice derived from both wild-type ES cells and cells with a homozygous deletion of PPARγ. This strategy allows one to measure the contribution of PPARγ null cells to adult tissues in healthy animals. By showing exclusion of null cells from WAT, but not several other tissues, PPARγ was demonstrated to be required for adipogenesis in vivo [21]. It was also shown that PPARγ was required for the in vitro differentiation of adipose cells from ES cells [21] or from embryonic fibroblasts [12]. Interestingly, animals heterozygous for PPARγ exhibit resistance to diet-induced obesity, although this results at least in part from elevated serum leptin levels and decreased food intake in these mice [12].

Cumulative consideration of the experiments just described have led to a model for a transcriptional cascade in adipogenesis involving the sequential activation of C/EBPs and PPARγ. In this model, one major function of C/EBPβ and δ is to induce the expression of PPARγ. As stated earlier, the endogenous expression of C/EBPβ and δ precedes that of PPARγ, and ectopic expression of the former leads to induction of the latter [30]. This induction is likely to be a direct transcriptional effect through C/EBP binding sites in the PPARγ promoter [6]. In the absence of PPARγ, C/EBPα levels are greatly reduced [12, 20]. Interestingly, fibroblasts made from C/EBPα (-/-) embryos have reduced levels of PPARγ and do not readily form fat when exposed to hormonal in-
dicing agents in culture [31]. When C/EBPα is added back to these cells with a retroviral vector, the expression of PPARγ (and the ability to differentiate) is restored. This reveals a positive feedback loop within the cascade, in which there is mutually reinforcing expression of PPARγ and C/EBPα; this feature ensures that, once initiated, the cascade will maintain the expression of these critical factors and therefore the terminally differentiated state.

This opens a question about whether the actions of C/EBPs and PPARγ represent parallel, reinforcing pathway of adipogenesis, or whether there is really one factor that drives adipogenesis, with the other factors serving primarily to “fine-tune” the process. It is already known that PPARγ can stimulate most, but not all, aspects of adipogenesis in C/EBPα deficient cells [31]. Fat cells lacking C/EBPα accumulate lipid and express most adipogenic markers, but they have poor insulin sensitivity. This is a result of diminished levels of insulin receptor and one of its primary substrates (IRS-1), as well as an uncharacterized postreceptor defect in insulin signaling. We have recently performed the converse experiments by generating a fibroblastic cell line that lacks PPARγ. The addition of C/EBPα to these cells at high levels does not restore their ability to differentiate into adipocytes (Rosen and Spiegelman, unpublished data). This is in stark distinction to the results already described, in which PPARγ almost fully restores adipogenesis to C/EBPα null cells. This suggests that there is one dominant pathway to adipogenesis that leads through PPARγ. The role of C/EBPα appears to be more ancillary, consisting primarily in the regulation of insulin sensitivity, expression of a few key target genes like leptin and adipsin, and of course, in maintaining PPARγ levels in mature adipocytes.

There are still many aspects of adipogenesis that remain mysterious. For example, very little is known about the molecular events that promote determination of mesenchymal stem cells into preadipocytes. Recent discoveries have focused attention on the wnt/β-catenin pathway, which may play a role in helping multipotent cells decide whether or not to enter the adipocytic lineage [22]. Members of the GATA family of transcription factors have also been shown to regulate entry of preadipocytes into the differentiation process [26]. Another area of interest is in understanding transcriptional elements downstream of C/EBPα and PPARγ. It is clear that many fat cell genes are direct targets of these proteins, but other markers appear to rely on intermediary factors that have yet to be elucidated. The search for such factors is ongoing in our laboratory.

Adipogenesis occurs in both the prenatal and postnatal states. While there is an older literature that suggested that people are born with all the adipocytes they would ever have, there is now convincing evidence that adipogenesis occurs throughout the lifetime of an organism. This new adipocyte development occurs both as a consequence of normal cell turnover, and due to the requirement for more fat mass upon significant calorie storage and weight gain [20]. Indeed, while fat cell size can vary with the amount of lipid stored, there is a physical limit to how large these cells can become. On the other hand, people and other animals will continue to store fat as long as energy intake exceeds nutritional requirements, thus providing a theoretical requirement for de novo differentiation of adipocytes in obesity. Direct evidence has come from studies of rats fed a high calorie diet, where 3H-thymidine incorporation into new fat cells occurs throughout adulthood [17]. Whether this is true for humans is unknown, but it is clear that preadipocytes purified from adult human fat pads can be induced to differentiate in vitro, although this capacity diminishes as age increases [4, 5, 11]. This argues for a role for both adipocyte hypertrophy and hyperplasia in human obesity.

If it is true that adipogenesis plays a role in human obesity, then it would seem logical to identify adipogenesis as a target in anti-obesity therapy. There is at least one important caveat to this strategy, however. There are several human and animal models of lipodystrophy, in which the development of fat cells is retarded [8, 18, 23]. Lipodystrophy is associated with severe metabolic derangements, ranging from hyperlipidemia and fatty liver to insulin resistance and frank type 2 diabetes. The lesson seems to be that fat cells serve an important purpose in storing the excess energy that we consume. In the absence of fat cells, this energy, in the form of carbohydrates and lipids, spills into the bloodstream and accumulates in tissues where it ought not to be. There are therapeutic possibilities to consider, however, if we could induce the formation of brown fat. By reprogramming our adipose tissue to burn calories rather than storing them, we might reduce obesity without exposing patients to the adverse consequences of lipodystrophy.

Future developments in this area are sure to come at a rapid pace. Specific areas that need to be addressed include the mechanism by which precursor cells become determined to the adipocytic lineage, and the identification of downstream factors that induce fat cell-specific target genes. Additionally, a better understanding of brown adipogenesis and the development of specific white adipocyte depots is lacking. Finally, these findings need to be applied to the clinical setting, to help with the treatment of such fat cell-related disorders as obesity, lipodystrophy, and liposarcoma.
REFERENCES


15. Lin FT and Lane MD. CCAAT/enhancer binding protein alpha is sufficient to initiate the 3T3-L1 adipocyte differentiation program. Proc Natl Acad Sci USA 1994 ; 91 : 8757-61.


