The Molecular Control of Adipogenesis, with Special Reference to Lymphatic Pathology

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ABSTRACT: Adipogenesis is the process by which mature fat cells are formed from pre-adipocytes. Adipogenesis has come under increasing scrutiny not only because the availability of reliable *in vitro* models makes it an attractive choice for developmental studies, but also because adipocytes are increasingly recognized as major players in a variety of physiological and pathophysiological states, such as obesity and type 2 diabetes. Adipocytes develop from mesenchymal stem cell precursors that are characterized by multipotency. Under the influence of various cues, these cells become committed to the adipocyte lineage. Further hormonal stimulation recruits these pre-adipocytes to accumulate lipid, express fat-specific markers, and become sensitive to the metabolic effects of insulin. A complex transcriptional cascade regulates this process, involving several distinct classes of transcription factor. In particular, the role of the nuclear hormone receptor PPARγ will be discussed, along with bZip family members C/EBPα, C/EBPβ, and C/EBPδ. The relationship of adipose depots to the lymphatic system will also be discussed.

KEYWORDS: adipogenesis; PPARγ; C/EBP; lymphedema; lymph nodes

WHY STUDY ADIPOGENESIS?

In the not-so-distant past, few tissues were felt to be as unworthy of study as adipose tissue. Fat has been routinely ignored and discarded by anatomists and physiologists, while the general public has invested adipocytes with more emotional baggage than any other non-neoplastic cell type. In the last decade or so, the tide has begun to turn in favor of adipose tissue as a subject of scientific inquiry. The reasons for this are threefold. First, the development of immortal preadipocyte cell lines by Green and colleagues in the 1970s has

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made tractable the study of adipocyte development and physiology.[1,2] There are few other developmental processes that can be reliably studied in a nearly synchronous population of cells *in vitro*. Compared to many other cellular lineages, fat cell differentiation *in vitro* is rather authentic, recapitulating most of the key features of adipogenesis *in vivo*. This includes morphological changes associated with lipid accumulation, cessation of cell growth, expression of many lipogenic enzymes, and establishment of sensitivity to most or all of the key hormones that impact on this cell type. The second reason for the recent surge in popularity of adipocytes is the recognition that these cells are far more than passive depots for energy storage. Fat is now known to secrete a wide variety of proteins that influence energy homeostasis (such as leptin and adiponectin),[3–5] coagulation (such as PAI-1),[6,7] blood pressure (such as angiotensinogen),[8] and immune function (such as TNF-α, IL-6, adipsin).[9,10] These findings firmly establish fat as an endocrine organ to be reckoned with. Finally, the dramatic rise in the incidence of obesity and type 2 diabetes worldwide has also focused attention on the biology of the adipocyte.[11]

HOW DO ADIPOCYTES DEVELOP?

Adipose development is unusual in that it occurs *de novo* in multiple, anatomically distinct sites. Generally speaking, most adipose tissues form at sites rich in loose connective tissues, such as the subcutaneous layers between the muscle and dermis. However, fat deposits form in many locations, such as around the heart, kidneys, and other internal organs. Older studies have described subcutaneous fat development in some morphological detail. In the absence of a clear molecular marker for pre-adipocytes, the earliest event associated with adipogenesis is a proliferating network of capillaries in an otherwise undistinguished region of loose connective tissue. These "primitive organs," as they have been called, develop into *bona fide* adipose tissue.[12] These observations also point out a potentially important relationship between blood vessel development and adipogenesis. It is not clear, however, whether adipogenesis can induce angiogenesis, vice versa, or both.

Adipocytes develop from multipotent mesenchymal stem cells that can also give rise to muscle, bone, or cartilage[13,14] (FIG. 1). In the developing fat pad, these cells become committed to the adipocytic lineage under the influence of "cues" that remain undiscovered. These cues might be hormonal interactions, or the result of cell–cell or cell–matrix interactions, for example. This process, called determination, results in a cell with fibroblastic morphology called a pre-adipocyte. Unfortunately, there are no known expression markers that absolutely identify a cell as a pre-adipocyte. Because of the complexity inherent in these systems, almost all work on adipogenesis has

utilized predetermined clonal cell lines, such as 3T3-L1 and 3T3-F442A. Upon reaching confluence, exposure to a regimen including dexamethasone, methylisobutylxanthine (a phosphodiesterase inhibitor), and insulin (DMI) leads to differentiation of these cells.

Adipogenesis occurs in both the prenatal and postnatal states in humans; in rodents, most fat cell development occurs postnatally. While older literature suggested that people are born with all the adipocytes they will possess in life, there is now convincing evidence that adipogenesis occurs throughout the lifetime of an organism. This adipogenesis occurs both as a consequence of normal cell turnover, and as a consequence of the requirement for additional fat mass that arises with significant calorie storage and weight gain.[15] 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, humans and other animals will continue to gain fat as long as energy intake exceeds nutritional requirements, demonstrating a theoretical requirement for *de novo* differentiation of adipocytes. More convincing evidence has come from studies of rats fed a high-calorie diet, where [^{3}H]thymidine incorporation into new fat cells occurs throughout adulthood.[16] Whether this is true for humans is unknown, but it is clear that pre-adipocytes purified from the fat pads of even elderly people can be induced to differentiate *in vitro*.[17–19] This observation argues for a role for both adipocyte hypertrophy *and* hyperplasia in human obesity.

HORMONAL AND ENVIRONMENTAL ASPECTS OF ADIPOGENESIS

The literature is rife with papers identifying extracellular and intracellular signals that control pre-adipocyte growth and terminal differentiation. The first agent noted to affect this process was insulin, the classic hormone of the fed state. Insulin increases the percentage of cells that differentiate and also increases the amount of lipid that each fat cell contains.[20] Recent data have shown that neutralization of insulin *in vivo* stimulates the apoptosis of fat cells,[21] dramatically confirming the importance of this hormone in adipocyte biology. Glucocorticoids have also been used for many years to induce optimal differentiation of cultured pre-adipose cell lines and primary adipocytes. It is not as clear, however, if glucocorticoids are required for efficient adipogenesis *in vivo*; since patients with Cushing's syndrome (elevated circulating glucocorticoids, usually from a pituitary or adrenal source) show visceral obesity but wasting of subcutaneous fat.[22] The effects of other hormones are also not completely understood. Growth hormone, for example, can clearly induce adipogenesis in a variety of cultured pre-adipose cell lines, but primary pre-adipocytes do not show this effect.[23–25] In fact, differentiation of

these cells appears to be inhibited by growth hormone. This is consistent with the observation that humans with growth hormone deficiency have normal fat stores, and can, in fact, be obese. Thyroid hormone,[26] retinoic acid,[27] and various prostaglandins[28] are some of the wide variety of other hormones that have been shown to affect adipogenesis *in vitro*, but for which there is scant evidence to support such a role *in vivo*.

A variety of cytokines have been found to suppress fat cell differentiation. TNF-α, IL-1, and many other pro-inflammatory cytokines have this effect on most cultured pre-adipocyte lines, and in fact can "de-differentiate" already mature fat cells.[29,30] Indeed, the suppression of adipocyte lipoprotein lipase was used to purify "cachectin," which was then found to be identical to TNF-α[31] The precise molecular basis for these cytokine effects is still not known, though suppression of expression of several key transcriptional regulators of adipogenesis has been observed. In addition, several growth factors are rather potent inhibitors of adipogenesis, including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF).[32] Much of this inhibition is likely to be through activation of mitogen-activated protein (MAP) kinases (see below).

As might be expected from the wide array of hormones, cytokines, and growth factors that can affect adipogenesis, several different signal transduction pathways have been implicated in the process as well. There has been quite a bit of interest in the MAP kinase family of intracellular protein kinases. As just described, many growth factors that exert their influence through tyrosine kinase receptors inhibit fat cell differentiation. It is now clear that much of this inhibition occurs through activation of the "classic" MAP kinases, Erk 1 and 2. These kinases directly phosphorylate PPARγ (see subsequent text), and inhibit its adipogenic activity.[33] The JNK MAP kinase can also phosphorylate PPARγ at this inhibitory site.[34] In contrast, p38 MAP kinase has been associated with an activation of adipogenesis. Chemical p38 inhibitors and a dominant negative allele of p38 decrease fat cell differentiation, and overexpression of constitutively active MKK6, an activator of p38, enhance the process.[35,36] The relevant targets of p38 are unknown at this time, but speculation centers on C/EBPβ, an early actor in the adipogenic transcriptional cascade. Another kinase, Akt (also known as PKB) has been shown to be a downstream effector of certain of the metabolic effects of insulin. Interestingly, expression of a constitutively active form of Akt causes spontaneous differentiation of 3T3-L1 cells.[37] The experimental manipulation of cAMP, various G proteins, and protein kinase C isoforms have also been shown to affect adipogenesis *in vitro*.[38] A more thorough knowledge of the critical extracellular signals involved in fat cell development would better enable us to place these findings in their appropriate context.

Another environmental condition that affects adipogenesis is hypoxia, which inhibits the process. This effect seems to be mediated through

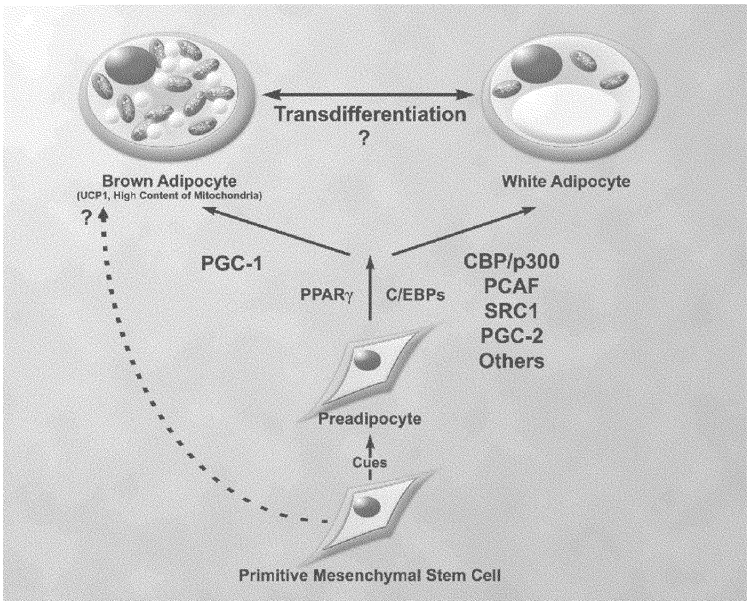

FIGURE 1. Adipogenesis proceeds from mesenchymal stem cells in two major steps. First, the multipotent stem cells become committed to the adipocytic lineage in a process called determination. Next, the committed pre-adipocytes fully differentiate through the concerted actions of a host of transcriptional regulators (see text for details). It is not known whether brown and white adipocytes use similar pathways for adipogenesis. There is some evidence that these two cell types can "*trans*-differentiate" into one another. Alternatively, brown adipocytes may develop along an independent pathway.

hypoxia-inducible factor 1α (HIF-1α), which represses transcriptional induction of PPARγ via an intermediate factor, called DEC1/Stra13.[39]

TRANSCRIPTIONAL CONTROL OF ADIPOSE DIFFERENTIATION

Studies in the pre-adipose cell lines 3T3-L1 and 3T3-F442A have yielded enormous insight into the transcriptional cascade that operates during adipose conversion (FIG. 2). Several groups have published data on a handful of transcription factors that are intimately involved in the transition from pre-adipocyte to adipocyte. We know, for example, that members of the CCAAT/enhancer binding protein (C/EBP) family are important in this process. C/EBPβ and C/EBPδ are expressed early in adipogenesis *in vitro*, reaching maximum levels within the first 2 days after differentiation is induced.[40, 41]

These factors in turn induce C/EBPα and the nuclear hormone receptor peroxisome proliferator-activated receptor γ (PPARγ), whose levels peak after a few days of differentiation and remain elevated throughout the life of the adipocyte. The ectopic addition of either C/EBPα or C/EBPβ is sufficient to drive the complete program of adipogenesis in pre-adipocyte cell culture models; C/EBPδ is not sufficient per se, but does accelerate differentiation induced by C/EBPβ[42] *In vivo,* the total loss of C/EBPα is associated with neonatal lethality resulting from hepatic and other defects[43]; until recently; this has hampered a careful study of the effect of loss of C/EBPα on postnatal events such as fat cell development. New studies have now been performed with alternative strategies that rescue the hepatic phenotype; a strong case has been made that C/EBPα is required for the normal development of white adipose tissue (WAT), but not for brown adipose tissue (BAT).[44,45] Interestingly, combined loss of C/EBPβ and C/EBPδ has the converse effect—modest reductions in WAT with more dramatic defects in BAT.[46]

A role for PPARγ in adipogenesis was first suggested by analysis of the aP2 (the adipocyte-specific fatty-acid-binding protein) 5′-flanking region. Elements were identified in this promoter region that were shown to be both necessary and sufficient to direct gene expression from a minimal promoter specifically to fat cells in culture and in transgenic mice.[47,48] Cloning of the cognate *trans*-acting factor identified it as a heterodimer consisting of two nuclear receptors, PPARγ and RXRα (retinoid X receptor-α) [49] PPARγ exists as two isoforms created by alternative splicing at the 5′ end of a single gene; PPARγ2 contains 30 amino acids more at the N-terminus than does PPARγ1. While many tissues express a low level of the PPARγ1 protein, fat expresses high levels of PPARγ2 (and also PPARγ1).

As a member of the nuclear hormone receptor superfamily, PPARγ is activated through the binding of ligands to a distinct carboxyl-terminal ligand-binding domain. It is now appreciated that the synthetic thiazolidinedione (TZD) class of anti-diabetic drugs function as ligands for PPARγ[50] These molecules, which have affinity (K_D) in the 50–700 nM range, were originally developed through medicinal chemistry approaches, with no insight into their mechanism of action. While these compounds are very effective at promoting adipogenesis in culture and *in vivo*, it is not known whether this effect is related to the anti-diabetic actions of these drugs. The endogenous PPARγ ligand is not known. Certain fatty acids can bind PPARγ with low affinity, but most investigators feel that these compounds are not present at physiological concentrations in nuclei *in vivo*. An unusual prostanoid, 15-deoxy-$\Delta^{12,14}$-PGJ2 (prostaglandin J2), has also been identified as a ligand for PPARγ[51, 52] but it is not known if this compound even exists in nature. This prostanoid has also been shown to activate other, unrelated signal transduction pathways in cells (such as NF-κB), making it difficult to ascribe its effects as belonging to PPARγ.[53]

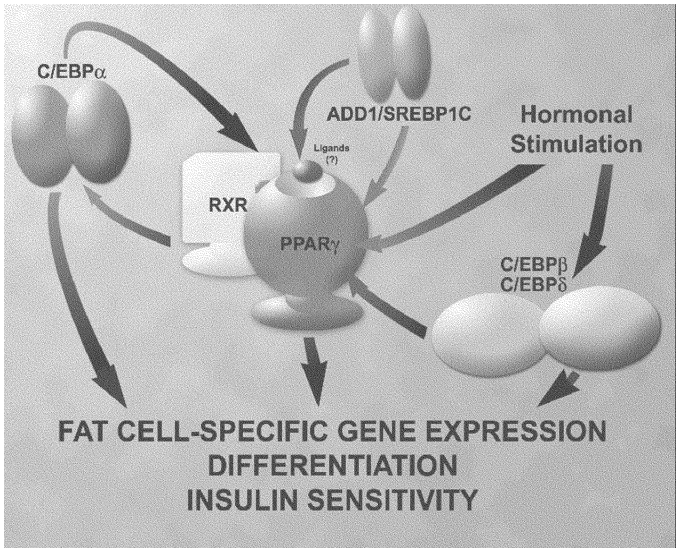

FIGURE 2. Transcriptional cascade in adipogenesis. Under the influence of unknown cues, C/EBPβ and C/EBPδ rise early during the conversion of pre-adipocytes to adipocytes. These factors induce the expression of C/EBPα and PPARγ, each of which maintains the expression of the other. Together these factors (and ADD1/SREBP1c) induce terminal gene expression and the final differentiated phenotype.

PPARγ plays a crucial role in the function of many fat-cell selective genes. PPARγ binding is absolutely required for the function of the fat-selective enhancers for the *FABP4* (aP2) and *PEPCK* genes in cultured fat cells.[54] This analysis of PEPCK has recently been extended *in vivo*, where the expression of the gene in fat was shown to be dependent on PPARγ binding, while expression in other tissues was not.[55] In addition to directly binding to and regulating fat cell-selective genes, PPARγ plays a dominant role in the regulation of differentiation per se. This was shown first in "gain of function" experiments that expressed PPARγ in non-adipogenic, fibroblastic cells (NIH-3T3) using retroviral vectors.[56] While ligands selective for PPARγ were not known at this time, application of the pan-PPAR ligand ETYA (5,8,11,14-eicosatetraynoic acid) activated PPARγ and caused a powerful differentiation response. The use of high-affinity TZD ligands has greatly improved these experiments. PPARγ-mediated differentiation included lipid accumulation and the expression of many endogenous genes characteristic of this cell type. The ability of PPARγ to promote adipogenesis is not limited to fibroblastic cells. Myoblastic cell lines can also be converted to adipocytes, particularly when the cells co-express both C/EBPα and PPAR.[57]

Loss-of-function studies with PPARγ have been more difficult to perform, because mice with targeted ablation of PPARγ die *in utero* at embryonic day 9.5–10.[58,59] This occurs because of a failure of placentation, precluding useful analysis of the role played by this factor in adipogenesis, most of which occurs postnatally in rodents. Our group has circumvented this problem by using chimeric lineage analysis to demonstrate that PPAR is required for adipogenesis *in vivo*.[60] Another group reported the same results using tetraploid rescue of the placental defect in PPARγ knockout mice.[59] These findings were extended by demonstrating that PPARγ null ES cells or primary embryonic fibroblasts were incapable of undergoing adipogenesis *in vitro*.[58,60]

The synthesis of these and other studies has led to the following model: C/EBPβ and C/EBPδ are activated by unknown cues in the committed pre-adipocyte to induce PPARγ and C/EBPα. These two factors then induce each other's expression in a positive feedback loop that maintains the differentiated state. We and others have shown that cells lacking C/EBPα can in fact be differentiated to adipocytes as long as PPARγ is added ectopically (although they lack normal insulin sensitivity).[61,62] This raises the question of whether C/EBPα and PPARγ represent redundant pathways in adipogenesis or whether the role of C/EBPα is primarily directed at inducing and maintaining PPARγ levels. We now have data from PPARγ null fibroblasts that shows that C/EBPα *cannot* rescue the adipogenic phenotype in these cells.[63] The role of C/EBPα in adipogenesis may in fact be more ancillary than had been previously thought; at least one major function of C/EBPα is to induce and maintain appropriate levels of PPARγ

In addition to this cross-regulation, it is clear that these proteins can act synergistically to activate differentiation and differentiation-linked gene expression. The molecular basis for this synergy is not known, but is worth noting that many fat cell genes have binding sites for both C/EBP and PPARγ/RXR. Possibilities for this synergy include regulation of co-activators involved in adipogenesis, and/or the possibility that C/EBPα is involved in regulatory genes that produce PPARγ ligands.

Another transcription factor that promotes adipogenesis is adipocyte determination and differentiation factor-1 (ADD1), also known as sterol regulatory element binding protein-1c (SREBP1c). ADD1/SREBP1c is induced in differentiating 3T3-L1 cells at around the same time as PPARγ and C/EBPα.[64] A constitutively active form of ADD1/SREBP1c enhances 3T3-L1 adipogenesis, while a dominant negative form represses this process.[65] There is evidence that ADD1/SREBP1c activates the PPARγ promoter,[66] and may also contribute to the generation of a PPARγ ligand.[67] Beyond these effects, ADD1/SREBP1c mediates some of the transcriptional effects of insulin in adipose tissue, particularly on lipogenic genes such as fatty acid synthase, acetyl-CoA carboxylase, and stearoyl-CoA desaturase.[68]

There have been reports of several other transcription factors that are linked to adipogenesis. Though none of these have been shown to affect adipogenesis as profoundly as PPARγ and the C/EBPs, it is quite possible that some of these will be important modulators of fat cell development and function. PPARδ in particular has been studied in this light, with conflicting results. Some workers have shown that NIH-3T3 fibroblasts stably expressing PPARδ are unable to undergo differentiation,[69] while others have shown that they can as long as a cAMP-inducing agent is added to the differentiation cocktail.[70] Similarly, claims have been made that some pre-adipocyte lines (such as Ob1771 and 3T3-F442A) can be induced to differentiate more completely if PPARδ is forcibly expressed or if a PPARδ ligand like 2-bromopalmitate is added.[71,72] Another group showed that 3T3-L1 preadipocytes, on the other hand, were only modestly affected by the addition of a more specific PPARδ ligand.[70] Loss-of-function studies with PPARδ have not shed as much light as might be hoped. Two different groups have generated PPARδ null mice; most PPARδ –/– embryos die mid-gestation, but a few survive to term and show reduced adiposity.[73, 74] Of note, mice with PPARδ targeted only in adipocytes show no decrease in adiposity, indicating that an extra-adipose site must be involved in the decreased body mass seen in the global knockout.[73] Other factors have also been discovered, including the forkhead factor foxc2 which is expressed in an adipose-selective manner and which promotes adipocyte-specific (especially BAT-specific) gene expression in a transgenic model.[75]

Finally, a few transcription factors have been shown to inhibit adipogenesis. These include GATA2 and GATA3, which are expressed in preadipocytes.[76] These factors work at least in part by repressing the PPARγ2 promoter. Reductions in GATA2 and GATA3 levels are required for adipogenesis to proceed *in vitro*. DFosB is a naturally occurring variant of FosB that appears to repress adipogenesis in marrow stromal cells while promoting the formation of osteoblasts.[77]

WHAT IS THE RELATIONSHIP OF ADIPOGENESIS TO LYMPHATIC PATHOLOGY?

Adipose tissue is found in discrete depots scattered through the body. Some of these depots are more metabolically active than others, and tend to expand or shrink in proportion to nutritional status. Other depots, such as the structural fat found in the heel and fingerpads, are quite metabolically inert, and barely change in size even in the leanest animals. Another place where fat can always be found, regardless of nutritional status, is around lymph nodes. These perinodal adipocytes typically do not respond to fasting, but are fully capable of activating lipolysis when stimulated by local lymph nodes or

co-cultured lymphoid cells.[78,79] There are interesting studies to demonstrate that adipocytes that are physically juxtaposed to lymphatic tissue are exquisitely sensitive to the lipolytic effects of lymphoid-derived cytokines like TNF-α and interleukin-6, while other adipocytes from distal sites within the same depot do not respond to these stimuli to the same magnitude.[80] There also appear to be qualitative differences in the fatty acid composition of resident triglycerides in perinodal adipocytes relative to fat cells elsewhere.[81] These observations have led to the hypothesis that adipose tissue forms an important energy depot for local lymphoid metabolism, and that these stores are important enough that they are spared by more mundane stimuli, such as fasting. Consistent with this notion is the fact that chronic inflammatory states such as Crohn's disease can lead to redistribution of fat.[82]

Another pathological state involving the lymphatic system is lymphedema, characterized by the accumulation of interstitial lymph in a defined region of the body, often as a result of interruption of lymphatic drainage. Chronic lymphedema leads to a variety of pathological changes in the affected region, including overgrowth of connective tissue and adipose tissue.[83] This exuberant growth of adipose tissue has led some to question whether lymph possesses an adipogenic activity, although there has been remarkably little work done to address this possibility. One study showed that cultured rabbit pre-adipocytes could be induced to differentiate more completely if lymph was included in the medium.[84] The stimulatory effect could be fully replicated by adding chylomicrons to the medium in place of whole lymph. On the one hand, this observation might suggest that the phenomenon is created by pre-existing, small adipocytes that take up and incorporate the triglycerides in the chylomicrons. The effect would thus be on lipogenesis rather than on adipogenesis per se. One should not, however, discount the possibility that a *bona fide* adipogenic stimulus is present in the chylomicron fraction of lymph. An example of this might be a lipophilic ligand for PPARγ, which could be enriched in the very low-density lipoprotein (VLDL).

CONCLUSIONS

Despite its complexity, adipogenesis has proven to be a developmental system tractable to study, because of the availability of cultured cell lines that faithfully mimic fat cell development *in vivo*. Such studies have allowed the identification of several critical transcription factors that promote the differentiated state. These factors include PPARγ, ADD1/SREBP1c, and members of the C/EBP family. Despite these advances, many aspects of adipogenesis remain mysterious, including the steps that lead to commitment of the multipotent mesenchymal stem cell to the adipocytic lineage, as well as the control of terminal gene expression in mature fat cells. Additionally, several obser-

vations indicate the presence of cross-talk between adipocytes and the lymphatic system. Unraveling these mysteries will certainly lead to insights that could have benefits in conditions ranging from obesity and type 2 diabetes to lymphedema.

REFERENCES

1. GREEN, H. & O. KEHINDE. 1975. An established preadipose cell line and its differentiation in culture. II. Factors affecting the adipose conversion. Cell **5:** 19–27.
2. GREEN, H. & O. KEHINDE. 1976. Spontaneous heritable changes leading to increased adipose conversion in 3T3 cells. Cell **7:** 105–113.
3. SCHERER, P.E., S. WILLIAMS, M. FOGLIANO, *et al.* 1995. A novel serum protein similar to C1q, produced exclusively in adipocytes. J. Biol. Chem. **270:** 26746–22749.
4. HU, E., P. LIANG & B.M. SPIEGELMAN. 1996. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J. Biol. Chem. **271:** 10697–10703.
5. ZHANG, Y., R. PROENCA, M. MAFFEI, *et al.* 1994. Positional cloning of the mouse obese gene and its human homologue. Nature **372:** 425–432.
6. LUNDGREN, C.H., S.L. BROWN, T.K. NORDT, *et al.* 1996. Elaboration of type-1 plasminogen activator inhibitor from adipocytes: a potential pathogenetic link between obesity and cardiovascular disease. Circulation **93:** 106–110.
7. SAMAD, F., K. YAMAMOTO & D.J. LOSKUTOFF. 1996. Distribution and regulation of plasminogen activator inhibitor-1 in murine adipose tissue in vivo: induction by tumor necrosis factor-alpha and lipopolysaccharide. J. Clin. Invest. **97:** 37–46.
8. SAYE, J.A., L.A. CASSIS, T.W. STURGILL, *et al.* 1989. Angiotensinogen gene expression in 3T3-L1 cells. Am. J. Physiol. **256:** C448–C451.
9. COOK, K.S., H.Y. MIN, D. JOHNSON, *et al.* 1987. Adipsin: a circulating serine protease homolog secreted by adipose tissue and sciatic nerve. Science **237:** 402–405.
10. HOTAMISLIGIL, G.S., N.S. SHARGILL & B.M. SPIEGELMAN. 1993. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science **259:** 87–91.
11. MOKDAD, A.H., B.A. BOWMAN, E.S. FORD, *et al.* 2001. The continuing epidemics of obesity and diabetes in the United States. J. Am. Med. Assoc. **286:** 1195–1200.
12. WASSERMAN, F. 1926. The fat organs of man: development, structure, and systematic place of the so-called adipose tissue. Z. Zellforsch. Mikroskop. Anat. Abt. Histochem. **3:** 325.
13. TAYLOR, S.M. & P.A. JONES. 1979. Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. Cell **17:** 771–779.
14. PITTENGER, M.F., A.M. MACKAY, S.C. BECK, *et al.* 1999. Multilineage potential of adult human mesenchymal stem cells. Science **284:** 143–147.
15. PRINS, J.B. & S. O'RAHILLY. 1997. Regulation of adipose cell number in man. Clin. Sci. (London) **92:** 3–11.

16. MILLER, W.H., JR., I.M. FAUST & J. HIRSCH. 1984. Demonstration of de novo production of adipocytes in adult rats by biochemical and radioautographic techniques. J. Lipid Res. **25:** 336–347.
17. KIRKLAND, J.L., C.H. HOLLENBERG & W.S. GILLON. 1990. Age, anatomic site, and the replication and differentiation of adipocyte precursors. Am. J. Physiol. **258:** C206–C210.
18. ENTENMANN, G. & H. HAUNER. 1996. Relationship between replication and differentiation in cultured human adipocyte precursor cells. Am. J. Physiol. **270:** C1011–C1106.
19. DESLEX, S., R. NEGREL, C. VANNIER, *et al.* 1987. Differentiation of human adipocyte precursors in a chemically defined serum-free medium. Int. J. Obes. **11:** 19–27.
20. GIRARD, J., D. PERDEREAU, F. FOUFELLE, *et al.* 1994. Regulation of lipogenic enzyme gene expression by nutrients and hormones. FASEB J. **8:** 36–42.
21. KIESS, W. & B. GALLAHER. 1998. Hormonal control of programmed cell death/apoptosis. Eur. J. Endocrinol. **138:** 482–491.
22. NEWELL-PRICE, J., P. TRAINER, M. BESSER, *et al.* 1998. The diagnosis and differential diagnosis of Cushing's syndrome and pseudo-Cushing's states. Endocr. Rev. **19:** 647–672.
23. CATALIOTO, R.M., D. GAILLARD, G. AILHAUD, *et al.* 1992. Terminal differentiation of mouse preadipocyte cells: the mitogenic-adipogenic role of growth hormone is mediated by the protein kinase C signalling pathway. Growth Factors **6:** 255–264.
24. HAUSMAN, G.J. & R.J. MARTIN. 1989. The influence of human growth hormone on preadipocyte development in serum-supplemented and serum-free cultures of stromal-vascular cells from pig adipose tissue. Domest. Anim. Endocrinol. **6:** 331–337.
25. WABITSCH, M., H. HAUNER, E. HEINZE, *et al.* 1995. The role of growth hormone/insulin-like growth factors in adipocyte differentiation. Metabolism **44:** 45–49.
26. GHARBI-CHIHI, J., P. GRIMALDI, J. TORRESANI, *et al.* 1981. Triiodothyronine and adipose conversion of OB17 preadipocytes: binding to high-affinity sites and effects on fatty acid synthetizing and esterifying enzymes. J. Recept. Res. **2:** 153–173.
27. SAFONOVA, I., C. DARIMONT, E.Z. AMRI, *et al.* 1994. Retinoids are positive effectors of adipose cell differentiation. Mol. Cell. Endocrinol. **104:** 201–211.
28. REGINATO, M.J., S.L. KRAKOW, S.T. BAILEY, *et al.* 1998. Prostaglandins promote and block adipogenesis through opposing effects on peroxisome proliferator-activated receptor gamma. J. Biol. Chem. **273:** 1855–1858.
29. PETRUSCHKE, T. & H. HAUNER. 1993. Tumor necrosis factor-alpha prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells. J. Clin. Endocrinol. Metab. **76:** 742–747.
30. OHSUMI, J., S. SAKAKIBARA, J. YAMAGUCHI, *et al.* 1994. Troglitazone prevents the inhibitory effects of inflammatory cytokines on insulin-induced adipocyte differentiation in 3T3-L1 cells. Endocrinology **135:** 2279–2282.
31. FRIED, S.K. & R. ZECHNER. 1989. Cachectin/tumor necrosis factor decreases human adipose tissue lipoprotein lipase mRNA levels, synthesis, and activity. J. Lipid Res. **30:** 1917–1923.
32. HAUNER, H., K. ROHRIG & T. PETRUSCHKE. 1995. Effects of epidermal growth factor (EGF), platelet-derived growth factor (PDGF) and fibroblast growth

factor (FGF) on human adipocyte development and function. Eur. J. Clin. Invest. **25:** 90–96.
33. HU, E., J.B. KIM, P. SARRAF, *et al.* 1996. Inhibition of adipogenesis through MAP kinase-mediated phosphorylation of PPAR-gamma. Science **274:** 2100–2103.
34. CAMP, H.S., S.R. TAFURI & T. LEFF. 1999. c-Jun N-terminal kinase phosphorylates peroxisome proliferator-activated receptor-gamma1 and negatively regulates its transcriptional activity. Endocrinology **140:** 392–397.
35. ENGELMAN, J.A., A.H. BERG, R.Y. LEWIS, *et al.* 1999. Constitutively active mitogen-activated protein kinase kinase 6 (MKK6) or salicylate induces spontaneous 3T3-L1 adipogenesis. J. Biol. Chem. **274:** 35630–35638.
36. ENGELMAN, J.A., M.P. LISANTI & P.E. SCHERER. 1998. Specific inhibitors of p38 mitogen-activated protein kinase block 3T3-L1 adipogenesis. J. Biol. Chem. **273:** 32111–32120.
37. MAGUN, R., B.M. BURGERING, P.J. COFFER, *et al.* 1996. Expression of a constitutively activated form of protein kinase B (c-Akt) in 3T3-L1 preadipose cells causes spontaneous differentiation. Endocrinology **137:** 3590–3593.
38. ROSEN, E.D. & B.M. SPIEGELMAN. 2000. Molecular regulation of adipogenesis. Annu. Rev. Cell Dev. Biol. **16:** 145–171.
39. YUN, Z., H.L. MAECKER, R.S. JOHNSON, *et al.* 2002. Inhibition of PPAR-gamma2 gene expression by the HIF-1-regulated gene DEC1/Stra13: a mechanism for regulation of adipogenesis by hypoxia. Dev. Cell **2:** 331–341.
40. YEH, W.C., Z. CAO, M. CLASSON, *et al.* 1995. Cascade regulation of terminal adipocyte differentiation by three members of the C/EBP family of leucine zipper proteins. Genes Dev. **9:** 168–181.
41. CAO, Z., R.M. UMEK & S.L. MCKNIGHT. 1991. Regulated expression of three C/EBP isoforms during adipose conversion of 3T3-L1 cells. Genes Dev. **5:** 1538–1552.
42. WU, Z., Y. XIE, N.L. BUCHER, *et al.* 1995. Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR-gamma and stimulates adipogenesis. Genes Dev. **9:** 2350–2363.
43. WANG, N.D., M.J. FINEGOLD, A. BRADLEY, *et al.* 1995. Impaired energy homeostasis in C/EBP-alpha knockout mice. Science **269:** 1108–1112.
44. CHEN, S.S., J.F.CHEN, P.F. JOHNSON, *et al.* 2000. C/EBP-beta, when expressed from the C/EBP-alpha gene locus, can functionally replace C/EBP-alpha in liver but not in adipose tissue. Mol. Cell. Biol. **20:** 7292–7299.
45. LINHART, H.G., K. ISHIMURA-OKA, F. DEMAYO, *et al.* 2001. C/EBP-alpha is required for differentiation of white, but not brown, adipose tissue. Proc. Natl. Acad. Sci. USA **98:** 12532–12537.
46. TANAKA, T., N. YOSHIDA, T. KISHIMOTO, *et al.* 1997. Defective adipocyte differentiation in mice lacking the C/EBP-beta and/or C/EBP-delta gene. EMBO J. **16:** 7432–7443.
47. GRAVES, R.A., P. TONTONOZ, K.A. PLATT, *et al.* 1992. Identification of a fat cell enhancer: analysis of requirements for adipose tissue-specific gene expression. J. Cell. Biochem. **49:** 219–224.
48. GRAVES, R.A., P. TONTONOZ, S.R. ROSS, *et al.* 1991. Identification of a potent adipocyte-specific enhancer: involvement of an NF-1-like factor. Genes Dev. **5:** 428–437.
49. TONTONOZ, P., E. HU, R.A. GRAVES, *et al.* 1994. mPPAR-gamma 2: tissue-specific regulator of an adipocyte enhancer. Genes Dev. **8:** 1224–1234.

50. LEHMANN, J.M., L.B. MOORE, T.A. SMITH-OLIVER, *et al.* 1995. An antidiabetic thiazolidinedione is a high-affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR-gamma). J. Biol. Chem. **270:** 12953–12956.
51. FORMAN, B.M., P. TONTONOZ, J. CHEN, *et al.* 1995. 15-Deoxy-Delta-12,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR-gamma. Cell **83:** 803–812.
52. KLIEWER, S.A., J.M. LENHARD, T.M. WILLSON, *et al.* 1995. A prostaglandin J2 metabolite binds peroxisome proliferator-activated receptor gamma and promotes adipocyte differentiation. Cell **83:** 813–819.
53. CASTRILLO, A., M.J. DIAZ-GUERRA, S. HORTELANO, *et al.* 2000. Inhibition of I-kappa-B kinase and I-kappa-B phosphorylation by 15-deoxy-Delta(12,14)-prostaglandin J(2) in activated murine macrophages. Mol. Cell. Biol. **20:** 1692–1698.
54. TONTONOZ, P., E.HU, J. DEVINE, *et al.* 1995. PPAR-gamma2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. Mol. Cell. Biol. **15:** 351–357.
55. DEVINE, J.H., D.W. EUBANK, D.E. CLOUTHIER, *et al.* 1999. Adipose expression of the phosphoenolpyruvate carboxykinase promoter requires peroxisome proliferator-activated receptor gamma and 9-cis-retinoic acid receptor binding to an adipocyte-specific enhancer in vivo. J. Biol. Chem. **274:** 13604–13612.
56. TONTONOZ, P., E. HU & B.M. SPIEGELMAN. 1994. Stimulation of adipogenesis in fibroblasts by PPAR-gamma2, a lipid-activated transcription factor. Cell **79:** 1147–1156.
57. HU, E., P. TONTONOZ & B.M. SPIEGELMAN. 1995. Transdifferentiation of myoblasts by the adipogenic transcription factors PPAR-gamma and C/EBP-alpha. Proc. Natl. Acad. Sci. USA **92:** 9856–9860.
58. KUBOTA, N., Y. TERAUCHI, H. MIKI, *et al.* 1999. PPAR-gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol. Cell **4:** 597–609.
59. BARAK, Y., M.C. NELSON, E.S. ONG, *et al.* 1999. PPAR-gamma is required for placental, cardiac, and adipose tissue development. Mol. Cell **4:** 585–595.
60. ROSEN, E.D., P. SARRAF, A.E. TROY, *et al.* 1999. PPAR-gamma is required for the differentiation of adipose tissue in vivo and in vitro. Mol. Cell **4:** 611–617.
61. WU, Z., E.D. ROSEN, R. BRUN, *et al.* 1999. Cross-regulation of C/EBP-alpha and PPAR-gamma controls the transcriptional pathway of adipogenesis and insulin sensitivity. Mol. Cell **3:** 151–158.
62. EL-JACK, A.K., J.K. HAMM, P.F. PILCH, *et al.* 1999. Reconstitution of insulin-sensitive glucose transport in fibroblasts requires expression of both PPAR-gamma and C/EBP-alpha. J. Biol. Chem. **274:** 7946–7951.
63. ROSEN, E.D., C.H. HSU, X. WANG, *et al.* 2002. C/EBP-alpha induces adipogenesis through PPAR-gamma: a unified pathway. Genes Dev. **16:** 22–26.
64. TONTONOZ, P., J.B. KIM, R.A. GRAVES, *et al.* 1993. ADD1: a novel helix-loop-helix transcription factor associated with adipocyte determination and differentiation. Mol. Cell. Biol. **13:** 4753–4739.
65. KIM, J.B. & B.M. SPIEGELMAN. 1996. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes Dev. **10:** 1096–1107.
66. FAJAS, L., K. SCHOONJANS, L. GELMAN, *et al.* 1999. Regulation of peroxisome proliferator-activated receptor gamma expression by adipocyte differentiation

and determination factor 1/sterol regulatory element binding protein 1: implications for adipocyte differentiation and metabolism. Mol. Cell. Biol. **19:** 5495–5503.

67. KIM, J.B., H.M. WRIGHT, M. WRIGHT, *et al.* 1998. ADD1/SREBP1 activates PPAR-gamma through the production of endogenous ligand. Proc. Natl. Acad. Sci. USA **95:** 4333–4337.
68. KIM, J.B., P. SARRAF, M. WRIGHT, *et al.* 1998. Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. J. Clin. Invest. **101:** 1–9.
69. BRUN, R.P., P. TONTONOZ, B.M. FORMAN, *et al.* 1996. Differential activation of adipogenesis by multiple PPAR isoforms. Genes Dev. **10:** 974–984.
70. HANSEN, J.B., H. ZHANG, T.H. RASMUSSEN, *et al.* 2001. Peroxisome proliferator-activated receptor delta (PPAR-delta)-mediated regulation of preadipocyte proliferation and gene expression is dependent on cAMP signaling. J. Biol. Chem. **276:** 3175–3182.
71. BASTIE, C., D. HOLST, D. GAILLARD, *et al.* 1999. Expression of peroxisome proliferator-activated receptor PPAR-delta promotes induction of PPAR-gamma and adipocyte differentiation in 3T3C2 fibroblasts. J. Biol. Chem. **274:** 21920–21925.
72. BASTIE, C., S. LUQUET, D. HOLST, *et al.* 2000. Alterations of peroxisome proliferator-activated receptor delta activity affect fatty acid-controlled adipose differentiation. J. Biol. Chem. **275:** 38768–38773.
73. BARAK, Y., D. LIAO, W. HE, *et al.* 2002. Effects of peroxisome proliferator-activated receptor delta on placentation, adiposity, and colorectal cancer. Proc. Natl. Acad. Sci. USA **99:** 303–308.
74. PETERS, J.M., S.S. LEE, W. LI, *et al.* 2000. Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor beta(delta). Mol. Cell. Biol. **20:** 5119–5128.
75. CEDERBERG, A., L.M. GRONNING, B. AHREN, *et al.* 2001. FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. Cell **106:** 563–573.
76. TONG, Q., G. DALGIN, H. XU, *et al.* 2000. Function of GATA transcription factors in preadipocyte-adipocyte transition. Science **290:** 134–138.
77. SABATAKOS, G., N.A. SIMS, J. CHEN, *et al.* 2000. Overexpression of Delta-FosB transcription factor(s) increases bone formation and inhibits adipogenesis. Nat. Med. **6:** 985–990.
78. POND, C.M. & C.A. MATTACKS. 1998. In vivo evidence for the involvement of the adipose tissue surrounding lymph nodes in immune responses. Immunol. Lett. **63:** 159–167.
79. POND, C.M. & C.A. MATTACKS. 2002. The activation of the adipose tissue associated with lymph nodes during the early stages of an immune response. Cytokine **17:** 131–139.
80. MATTACKS, C.A. & C.M. POND. 1999. Interactions of noradrenalin and tumour necrosis factor-alpha, interleukin 4 and interleukin 6 in the control of lipolysis from adipocytes around lymph nodes. Cytokine **11:** 334–346.
81. MATTACKS, C.A. & C.M. POND. 1997. The effects of feeding suet-enriched chow on site-specific differences in the composition of triacylglycerol fatty acids in adipose tissue and its interactions in vitro with lymphoid cells. Br. J. Nutr. **77:** 621–643.

82. DESREUMAUX, P., O. ERNST, K. GEBOES, *et al.* 1999. Inflammatory alterations in mesenteric adipose tissue in Crohn's disease. Gastroenterology **117:** 73–81.
83. ROCKSON, S.G. 2001. Lymphedema. Am. J. Med. **110:** 288–295.
84. NOUGUES, J., Y. REYNE & J.P. DULOR. 1988. Differentiation of rabbit adipocyte precursors in primary culture. Int. J. Obes. **12:** 321–333.