Obesity, defined as an increase in adipose tissue mass, is the most prevalent nutritional disorder in industrialized countries and is a growing problem in developing countries. An increase in adipose tissue mass can be the result of the production of new fat cells through the process of adipogenesis and/or the deposition of increased amounts of cytoplasmic triglyceride per cell. Although much has been learned about the differentiation of adipocytes in vitro, less is known about the molecular basis for the mechanisms regulating adipogenesis in vivo. Here oligonucleotide microarrays have been used to compare the patterns of gene expression in preadipocytes and adipocytes in vitro and in vivo. These data indicate that the cellular programs associated with adipocyte differentiation are considerably more complex than previously appreciated and that a greater number of heretofore uncharacterized gene regulatory events are activated during this process in vitro. In addition, gene expression changes associated with adipocyte development in vivo and in vitro, while overlapping, are in some respects quite different. These data further suggest that one or more transcriptional programs are activated exclusively in vivo to generate the full adipocyte phenotype. This gene expression survey now sets the stage for further studies to dissect the molecular differences between in vivo and in vitro adipocytes.

Adipogenesis has been studied extensively in vitro using a number of preadipocyte cell lines including 3T3-L1 cells (1). When cultured in defined media, 3T3-L1 cells deposit triglyceride in cytoplasmic lipid droplets and express genes that are also expressed in adipocytes in vivo (2–8). Elegant studies of this process have led to the identification of several key regulatory genes that are necessary and/or sufficient for the transition of preadipocytes into adipocytes in vitro including C/EBPα, β, and δ and peroxisome proliferator-activated receptor (PPARγ) (9). Studies of these transcription factors have suggested that adipogenesis is the result of a temporally ordered pattern of 3–5 distinct phases of gene expression (summarized in the above reviews). Indeed several of the more than 100 molecules that have been identified as differentially expressed during the transition of preadipocytes to adipocytes in culture have been shown to have binding sites for the transcription factors PPARγ and C/EBPα in their promoters (9, 10).

Although cell culture models of adipocytes faithfully express many genes that are markers of adipocytes in vivo, the events that trigger this transformation in vivo are not as well understood. Knockout studies of C/EBPα, C/EBPβ, C/EBPδ, and PPARγ have confirmed that these molecules are necessary in vivo for adipogenesis, but it is not known with certainty whether these factors are sufficient (11–15). Thus it is unclear whether the high level of expression of these factors evident in in vitro adipocytes can recapitulate the gene expression profile of adipocytes in vivo. It has already been shown that some adipocyte-derived molecules such as leptin are expressed at lower levels in cultured adipocytes (16) and that in vivo levels of ob mRNA are restored in fat pads derived from subcutaneously implanted 3T3-F442A preadipocytes (17).

To characterize the regulation of gene expression during adipogenesis in vivo and in vitro further, the abundance of 11,000 genes and expressed sequence tags was measured at 10 different time points during in vitro 3T3-L1 adipocyte differentiation using oligonucleotide microarrays (Affymetrix, Santa Clara, CA). The abundance of the same 11,000 genes was also measured in adipocytes and stromal cells (including preadipocytes) isolated from wild-type and ob/ob white adipose tissue. Independent analyses of these data indicated that a more complex program of gene expression than was known previously is activated during adipocyte differentiation in vitro and in vivo. Comparative analysis of the in vitro and in vivo expression data revealed that although some genes are expressed at in vivo levels in fully differentiated 3T3-L1 cells, most notably target genes for C/EBPα and PPARγ, large clusters of genes are expressed at much higher levels or even exclusively in vivo. Conversely, a large group of genes is expressed in vitro that is not or is poorly expressed in vivo in adipocytes or preadipocytes. Although these data represent a descriptive survey of gene expression profiles of adipogenesis in vivo and in vitro, together they indicate that adipogenesis is likely to be more complex than previously appreciated and that specific transcriptional programs that generate the full adipocyte phenotype are not activated in cultured adipocytes but are activated by signals present exclusively in vivo.

**EXPERIMENTAL PROCEDURES**

3T3-L1 Cell Culture—3T3-L1 cells (ATCC, Manassas, VA) were maintained in subconfluent cultures in Dulbecco's modified Eagle's
medium supplemented with 33 μM biotin, 17 μM calcium pantotenate, 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 0.25 μg/ml amphotericin B (Life Technologies, Inc.). For differentiation, 3 x 10^6 cells were seeded per 100-mm plate, allowed to reach 100% confluence, and induced 1 day post-confluence with the same medium supplemented with 170 nM insulin, 2 μM triiodothyronine, 250 nM dexamethasone, and 500 μM isobutylmethylxanthine for 2 days (Life Technologies, Inc.). After induction, the cells were fed every 2 days with maintenance medium supplemented with 170 nM insulin and 2 nM T3. At indicated time points (Fig. 1), the medium was drained, the cells were lysed by the addition of Trizol reagent (Life Technologies, Inc.), and total RNA was isolated as per protocol. Northern blotting was conducted as described previously (18). For histology, cells were grown and differentiated as described above on lab-tek slides (Nunc, Naperville, IL), fixed (2% formaldehyde/0.2% glutaraldehyde in PBS for 15 min.), rinsed in PBS, stained with oil-red O (0.7% in 60% isopropanol for 10 min), and counter-stained with hematoxylin (40% in water for 5 min).

Adipose Tissue Fractionation—Adipose tissue was divided into stromal and adipocyte fractions as described previously (19). Briefly, freshly excised peri-uterine fat pads from 8-week-old female C57Bl/6J, wild-type, or ob/ob mice were rinsed in PBS, minced, and digested for 45 min–1 h at 37°C in Krebs-Ringer bicarbonate (pH 7.4) with 4% bovine serum albumin and 1.5 mg/ml type I collagenase (Worthington, Freehold, NJ). The digested tissue was filtered through a 250-μm nylon mesh to remove undigested tissue and centrifuged at 500 x g for 5 min. The floating adipocyte fraction was removed, washed in buffer, and recentrifuged to isolate free adipocytes. The stromal-vascular pellet was resuspended in erythrocyte lysis buffer (154 mM NH4Cl, 10 mM KHCO3, and 1 mM EDTA), filtered through a 28-μm nylon mesh to remove endothelial cells, and pelleted at 500 x g for 5 min. Total RNA from the adipocyte and stromal fractions was isolated with Trizol reagent from five different ob/ob and wild-type preparations from which equal amounts were pooled for microarray analysis. Adequate separation of adipocyte and stromal fractions was confirmed by Northern blotting for the adipocyte markers aP2 and PPARγ (data not shown).

Affymetrix Oligonucleotide Microarray Analysis and k-means Clustering—Samples were prepared for murine 11k microarrays from 10 μg of total RNA as outlined in the Affymetrix technical bulletin and as described previously (20). Hybridization and analysis were carried out using Affymetrix hybridization, washing, scanning, and GeneChip 3.3 analysis stations as described in the Affymetrix technical manual. For 3T3-L1 analysis clustering, genes were included if greater than 3-fold and more than 500 average difference change units (abundance measurement) were detected as described in the Affymetrix technical manual. For 12 clusters of genes including cell cycle progression, cell adhesion, cell differentiation, and signaling molecules.

RESULTS

3T3-L1 Adipocyte Differentiation Occurs Through Multiple, Overlapping, Coordinated Phases of Gene Expression

The initial analysis of the expression data from the oligonucleotide arrays indicated that the abundance of 1259 genes changed 3-fold or more during the course of the differentiation response. These data corroborated many previously reported patterns of gene expression including changes of PPRE1, AEBP1, C/EBPβ, C/EBPδ, C/EBPα, aP2, adipin, Acrp30/Adiponectin, lipoprotein lipase, hormone-sensitive lipase, stearoyl-CoA desaturase 1, α2 type VI collagen RNAs, and others (see below).

These data were analyzed using a modified k-means clustering algorithm with a dot product metric, which groups genes based on the similarity of their patterns of gene expression (20). Cluster analysis indicated that the 1259 differentially expressed genes can be grouped most parsimoniously into 27 temporarily distinct patterns, each containing between 16 and 118 genes (Fig. 2), suggesting that the regulation of adipogenesis may be considerably more complex than previously appreciated. These clusters include ~1000 genes not identified previously as being differentially regulated during adipocyte differentiation and more than 100 known transcription factors and signaling molecules.

15 distinct clusters of RNAs increased in abundance during adipogenesis in vitro (Fig. 2, left). 12 clusters of genes decreased during adipogenesis (Fig. 2, right). Because of space restrictions, only a subset of the genes identified by this analysis are shown. All of the data are available online (arrays. rockefeller.edu/obesity/adipocyte).

Patterns of Gene Expression in Adipocyte Differentiation Invoke Multiple Additional Transcriptional Mechanisms

Induced Genes—Microarray analysis indicated that the transcription factors SREBP-1 (Fig. 2, A and C), C/EBPα (Fig. 2C), and PPARγ2 (Fig. 2E) were up-regulated dramatically, each with different kinetics, over the course of adipocyte differentiation. Many genes that are markers of the differentiated adipocyte increased in parallel with these factors. These included many known gene targets of these factors including the SREBP-1 and C/EBPα target genes fatty acid synthase (Fig. 2C), stearoyl-CoA desaturase-1 (Fig. 2C), stearoyl-CoA desaturase-2 (Fig. 2D), the PPARγ and C/EBPα target gene aP2 (Fig. 2E), and the highly adipocyte-enriched genes glycerophosphate dehydrogenase (Fig. 2E), adipin (Fig. 2D), and Acrp30/AdipoQ/adiponectin (21) (Fig. 2D).

However, many genes that mark the differentiated adipocyte were expressed with different kinetics than SREBP-1, C/EBPα, and PPARγ. For example, phosphoeneolpyruvate carboxykinase, a glyceroenolic enzyme that has been shown to have a PPARγ binding site in its promoter (22), is first expressed in adipocytes at 7 days and continues to increase in abundance until 28 days (Fig. 2D). The β3-adrenergic receptor, cytochrome c oxidase VIII, glucose-6-phosphate isomerase, phosphofructokinase I, and insulin-like growth factor II demonstrate a similar pattern of expression and are also in this cluster. These patterns differed from the expression profile of the aforementioned transcriptional factors. These observations suggest that other regulatory factors also play a role in adipogenesis.

Repressed Genes—12 clusters of genes including cell cycle genes (Fig. 2Q and R), cytoskeletal genes (Fig. 2Q and T), splicing factors and protein turnover genes (Fig. 2U), and members of other cell types including myelocytes and lymphocytes (Fig. 2S) were down-regulated during differentiation. These clusters decrease with varying kinetics beginning as early as confluence (Fig. 2, P, R, W, X, and AA), after the addition of growth factors (Fig. 2Q), or later in the process of adipocyte maturation (Fig. 2, S, T, U, V, Y, and Z). Although several clusters contained transcription factors known to be
repressed during adipogenesis including COUP-TF1 (Fig. 2Q) and AEBP1 (Fig. 2Y), little is known about the transcriptional mechanisms responsible for regulating these clusters of genes.

**Transcription Factors and Signaling Molecules Regulated During Adipogenesis**—More than 100 known additional transcription factors, transcriptional coactivators, and signaling molecules were regulated during adipogenesis. These factors were either stably induced or repressed during differentiation (Fig. 3, A, C, and D) or transiently regulated (Fig. 3, B and E). In addition, in all clusters where a known adipogenic transcription factor was present, additional transcription factors were coexpressed. Coordinate with a 71-fold increase in C/EBPα and a 20.6-fold increase in SREBP-1, the transcription factors X-box-binding protein (up 6.4-fold), estrogen receptor-related (up 4.2-fold), and Ig/enhancer binding protein (up 5.2-fold) were up-regulated (Figs. 3A and 2C). The 18.1-fold induction of PPARγ was associated with a 15.3-fold up-regulation of the transcriptional corepressor RIP140 and a 14.6- and 3.3-fold up-regulation of the transcription factors STAT-1 and iron response element binding protein, respectively (Figs. 3A and 2E). In addition to these factors, which show similar profiles to C/EBPα, SREBP-1, and PPARγ, other transcription factors were identified, which show different transcriptional profiles including Mxi-1, Zic3, and the albumin D-box-binding protein.

The glucocorticoid-induced leucine zipper (up 42.7-fold), N10 nuclear hormone receptor (up 11.8-fold), and Wnt-4 signaling molecule (up 34.8-fold) were induced to a greater extent than even C/EBPβ (up 3.6-fold) and C/EBPδ (up 3.9-fold) (Fig. 3B), which have been invoked previously as responsible for mediating the early phases of adipogenesis (23). All of these factors are transiently up-regulated after the addition of growth factors to stimulate adipose conversion of confluent adipocytes. Thyroid hormone receptor c-erbA-α2 increased 13.8-fold prior to the addition of growth factors and remained elevated throughout differentiation. Together, these results suggest the possible involvement of a large cluster of regulatory molecules and signaling pathways during differentiation.

**Gene expression analyses indicated that a large group of DNA binding inhibitors (Id genes) and high mobility group proteins (HMG genes) are down-regulated with distinct kinetic profiles during differentiation (Fig. 3C) These gene regulatory changes are accompanied by the transient or sustained down-regulation of fos/jun family members, homeobox, forkhead, and other transcription factors (Fig. 3, D and E). The role these complex patterns play in generation of the differentiated adipocyte remains to be determined.**

**Gene Expression in Adipocytes and Preadipocytes in Vivo**

The phenotype of preadipocytes and adipocytes in vitro and in vivo was compared by scoring the abundance of the same 11,000 genes in RNA from the adipocyte and stromal (preadipocyte) fractions of C57Bl/6 wild-type and ob/ob mice. A total of 1435 genes represented on the array were at least 5-fold enriched in adipocytes or preadipocytes as compared with other...
Global Expression Profiles of Adipogenesis

Arvind V. J. Prasad, Ali Eckmann, Leonard Thiele, Sten G. H. Svanberg, and Arne Klemm

34170

Left dance during 3T3-L1 differentiation into adipocytes. Experiments are ordered along the x axis, and genes are ordered along the y axis. The clusters are labeled A-AA, and boundaries between clusters are indicated by the alternating red and blue colorbar (far left). Fold change relative to preconfluent 3T3-L1 cells is shown colorimetrically as indicated at the bottom left. Right, the normalized mean expression level is shown for each cluster of genes in graphical form. 15 clusters of genes increased in abundance (left column), and 12 clusters decreased in abundance during the course of differentiation (right column).

tissues including liver, brain, skeletal muscle, and exocrine pancreas (see “Experimental Procedures”). k-means clustering of the absolute expression levels of these genes in adipocytes and preadipocytes in vivo and in vitro identified 18 distinct groupings of genes with significant expression levels. These included six clusters of genes that were enriched in adipocytes both in vivo and in vitro, five clusters of genes that were enriched in preadipocytes in vivo and in vitro, four clusters of genes that were specifically expressed in vivo, and three clusters that were expressed specifically in 3T3-L1 cells (Fig. 4).

Genes Expressed in Vivo and in Vitro—This analysis confirmed that for many genes, differentiated 3T3-L1 cells accurately recapitulate the in vivo patterns of gene expression observed in the transition of preadipocytes to adipocytes (Fig. 4A). Thus, many genes that are not expressed (or expressed at a low level) in preadipocytes were highly expressed in mature 3T3-L1 adipocytes, wild-type adipocytes in vivo, and ob/ob adipocytes in vivo (Table I). These six clusters of adipocyte-enriched genes varied principally in their absolute level of expression and were further divided into distinct subgroups by the k-means algorithm. The most notable members of this group include C/EBPa, PPARγ2, SREBP-1 (nonspecific SREBP-1a/1c probe set), and RXRa. Many other adipocyte-specific and adipocyte-enriched genes are present in these groups including genes necessary to synthesize fatty acids from acetyl-CoA, the GLUT-4 glucose transporter, aP2, ACRP30/AdipoQ/adiponectin, and the β3-adrenergic receptor (Table I).

A second group of five clusters were highly expressed in preadipocytes in vitro and in vivo and decreased in abundance during adipocyte conversion (Fig. 4B). These clusters include the transcriptional repressor AEBP1, which is negatively regulated during adipogenesis and in preadipocytes serves to negatively regulate the aP2 AE-1 enhancer (24). The transcription factors Prx2 homeobox, junB, nuclear LIM interactor, Kruppel-like factor, forkhead box F2, E2a, and C/EBPα are among others highly expressed in preadipocytes (prior to the accumulation of cytoplasmic lipid) and down-regulated during differentiation (Table I).

Genes Expressed at High Levels in Vivo and Absent or Lower Levels in Vitro—68 genes that were highly expressed in adipocytes in vivo were expressed at an average of 20-fold lower levels in differentiated 3T3-L1 adipocytes (Fig. 4C, Vivo1). This cluster of genes indicates that adipocytes in vitro do not express the fully differentiated in vivo phenotype. This group of in vivo enriched genes included some genes encoding metabolic enzymes such as ATP-citrate lyase (10.4-fold lower in 3T3-L1 day-28 adipocytes than wild-type adipocytes), phosphoenolpyruvate carboxykinase (>200-fold lower on day 7 and 1.9-fold lower on day 28), acetyl-CoA synthetase (3.1-fold lower on day 28), and leptin mRNA (63.4-fold lower on day 28). The high molecular weight growth hormone receptor and the thyrotropin receptor are 5.5- and 6.1-fold more highly expressed in vivo. Finally, the transcription factor skeletal muscle LIM protein FHL1 is 18.4-fold more highly expressed in wild-type adipocytes relative to 3T3-L1 day-28 adipocytes, which show only background hybridization levels. These results indicate that the absolute levels of expression of these genes are lower in adipocytes in vitro, and although some are induced during differentiation, additional signals seem necessary to direct high levels of expression typical of in vivo adipocytes. Further studies of the regulation of these and the other genes in this cluster should reveal whether a common regulatory mechanism underlies their high level of gene expression in vivo.

The expression analyses also revealed inconsistencies between adipogenesis in vitro and in vivo. aP2 has been considered to be a marker of the mature adipocyte in vitro because of...
its dramatic induction during adipogenesis. The expression data revealed that aP2, although induced 2.7-fold in wild-type mature adipocytes in vivo, was still expressed at high levels (~80% of GAPDH expression) in preadipocytes in vivo. This observation was verified independently by the Northern analysis of RNA from the adipocyte and stromal fractions of white adipose tissue with a probe specific for the aP2 mRNA (data not shown). In contrast, PPARγ2 and C/EBPα, which are thought to be responsible for the high level expression of aP2 in vitro, are absent in preadipocytes in vivo and 40.9- and 31.3-fold induced in vivo in adipocytes relative to background hybridization intensities present in preadipocytes, respectively. This apparent difference (i.e. why a target gene of PPARγ2 and C/EBPα is expressed in preadipocytes while the factors themselves are absent in vivo) with the 3T3-L1 system remains to be reconciled.

293 genes expressed in the stromal (preadipocyte) fraction in vivo were not expressed or were expressed at very low levels (Fig. 4C, Vivo2–4) in both differentiated or undifferentiated 3T3-L1 cells. Many molecules that have immune function were present in these clusters. Although some of these results could denote the presence of cell types other than bona fide preadipocytes in this cell fraction, many of these molecules including TNFα, macrophage inflammatory protein-2, IL-1β, IL-6, JE, KC, and C10-like chemokines were also expressed at ~3–10-fold lower levels in adipocytes, suggesting that these immunologic cell markers were expressed in cells committed to the adipocyte lineage (Fig. 4C, Vivo2, and Table I). These data are consistent with previous data from wild-type and ob/ob mice (20).

**Genes Enriched in Vitro**—Finally, several groups of genes that were expressed poorly in vivo but highly enriched in 3T3-L1 cells were evident (Fig. 4C, Vitro1–3, and Table I). These clusters of genes include PREF1, a marker of 3T3-L1 preadipocytes that is down-regulated during differentiation, but that has not been shown to be expressed in any cell type in adipose tissue in vivo. These clusters of genes indicate additional differences between 3T3-L1 cells and preadipocytes and adipocytes in vivo.

**DISCUSSION**

Oligonucleotide microarrays have been used to compare the patterns of gene expression in preadipocytes and adipocytes in vitro and in vivo. These analyses indicate that the gene expression changes associated with adipocyte development in vivo and in vitro, although overlapping, are in many respects quite different. Specifically, large groups of genes have been identified that are expressed at high levels in vivo and are not or poorly expressed in 3T3-L1 cells. Additional noncell autonomous factors may be necessary to achieve maximal levels of expression of these genes in vivo. Therefore, this study invokes additional in vivo specific transcriptional programs as being required for the development of the fully differentiated phenotype of adipocytes in vitro.

The microarray data also indicated that the cellular programs associated with adipocyte differentiation are considerably more complex than previously appreciated and that a number of previously uncharacterized gene regulatory events are likely to be activated during this process in vitro. Cluster analysis of the 1249 genes that change in abundance during the course of 3T3-L1 differentiation into adipocytes indicated that the temporal pattern of gene expression can be described by at least 27 distinct phases. These data emphasize the heretofore unappreciated complexity of the transcriptional programs activated during adipogenesis and suggest the possibility that a larger number of genes than previously appreciated play a role in this process. The complexity of these events was underestimated in a recent report in which the use of global expression profiling identified genes differentially expressed between confluent 3T3-L1 preadipocytes and day-6 differentiated 3T3-L1 adipocytes (25). However, as indicated by the current analysis, many clusters of genes that are transiently repressed or induced during differentiation show equivalent expression levels in confluent preadipocytes and day-7 adipocytes and would have been missed by the previous report.

The expression data generated from oligonucleotide microarrays verified the gene expression changes of many genes during adipocyte differentiation in 3T3-L1 cells including the tran-
phase that consists of 1–2 rounds of cell division prior to terminal differentiation. Prior to the addition of adipogenic factors, cells are growth-arrested at confluence, indicated in this analysis by the potent repression of this large cluster of cell cycle genes (such as CDC25, centromere protein A, cyclin A, cyclin B, cyclin B1, cyclin B2, cytosolic thymidine kinase, topoisomerase IIa, DNA ligase, DNA Polδ catalytic subunit, CDC2, CDC20, CDK regulatory subunits 1 and 2, centromere protein A, inner centromere protein, mitotic centromere-associated kinesin, p34, CDC46, CDC47, ribonucleotide reductase M1, histone H2A.1, etc.) at the confluent and 6-h time points. However, after the addition of adipocyte-inducing factors that serves to induce cell division, this entire cluster of genes returns to preconfluent (dividing cell) expression levels at 12–48 h. After this time, at which cells are known to have entered terminal differentiation, this entire cluster of genes is repressed permanently and dramatically. These expression profiles provide new insight into the mRNA changes necessary for this phase of adipocyte clonal expansion. Although the ability of the adipogenic transcription factor PPARγ to induce cell cycle arrest through the cyclin-dependent kinase inhibitors p18 and p21 has been demonstrated recently (28), the specific and additional events that lead to the regulation of this and other coordinated phases of gene repression remain to be explained.

This unbiased approach toward expression characterization further implicated a number of previously unappreciated regulatory molecules as playing a role in the development of the adipocyte phenotype in vitro. Regulation of RNA and protein levels of the known adipogenic transcription factors, e.g. C/EBPα, C/EBPβ, C/EBPδ, PPARγ, and SREBP-1, is not sufficient to generate the level of complexity seen during 3T3-L1 differentiation. For example, the kinetics of different known targets of these genes can in some cases be markedly different, e.g. PPARγ targets aP2 and phosphoenolpyruvate carboxykinase. This observation suggests that many other genes either modulate the behavior of these known factors or act independently to direct adipose gene expression. This study identifies a large number of such candidate regulatory molecules including transcription factors, transcriptional coactivators, and signaling molecules. These data can now be analyzed further to test whether some of these factors can account for the regulation evident in the clusters, the genes of which were expressed with different kinetics from the clusters containing of PPARγ, C/EBPα, SREBP-1, and their target genes.

The relevance of these findings to adipogenesis in vivo was evaluated further in a formal comparison of the expression profile of in vitro preadipocytes and adipocytes to their in vivo counterparts. These data indicate that although adipocytes in vitro express many of the same genes as well as morphologic and metabolic characteristics of in vivo adipocytes, the expression profile of preadipocytes and adipocytes in vitro are different from those of the stromal and adipocyte fractions of white adipose tissue in vivo. The key lipogenic enzymes ATP-citrate lyase and phosphoenolpyruvate carboxykinase are expressed at 20–200-fold lower levels (at day 7) or 2–10-fold lower levels (at day 28) in vitro relative to in vivo adipocytes, suggesting at least one possible explanation for the lower total accumulation of triglyceride in cultured adipocytes relative to in vivo adipocytes. These results suggest that other heretofore unknown factors are necessary for the development of the fully differentiated adipocyte in vivo. The data also suggest that preadipocytes in vitro exhibit a novel phenotype that is not entirely mimicked by preadipocytes in vitro. These cells express a number of genes with immune functions, suggesting an even broader array of roles for the in vivo preadipocyte than previously appreciated.

**Global Expression Profiles of Adipogenesis**

**FIG. 4.** Comparison of preadipocyte and adipocyte gene expression levels in vivo and in vitro. 1435 genes that were enriched in preadipocytes or adipocytes were grouped by k-means clustering using a dot product metric according to their absolute level of expression in 10 3T3-L1 time points and in isolated adipocytes and stromal cells from wild-type and ob/ob white adipose tissue. A, six clusters of genes labeled Adip1–Adip6 were enriched in adipocytes in vitro and in vivo and were expressed at quantitatively similar levels in those two states. B, five clusters of genes labeled Preadip1–Preadip5 were more highly expressed in preadipocytes than cells that had accumulated cytoplasmic lipid both in vitro and in vivo. These clusters of genes provide novel markers for this unique population of preadipocyte cells. C, four clusters of genes labeled Vivo1–Vivo4 were expressed at high levels in vivo and were undetectable or expressed at much lower levels in vitro. One of these clusters, Vivo1, is enriched specifically in adipocytes in vivo and is lowly or not expressed in vitro. In cluster Vivo2, many genes were expressed in cells of the preadipocyte/adipocyte lineage that were not expressed in vitro. In the clusters Vivo3 and Vivo4, genes were uniquely expressed in the stromal fraction isolated from wild-type and ob/ob adipose tissue. These two clusters characterize the population of preadipocytes that includes preadipocytes and possibly other cell types responsible for supporting the fully differentiated adipocyte phenotype. Three clusters of genes labeled Vitro1–Vitro3 were more highly expressed in vitro and expressed at low or undetectable levels in vivo.

**Description factors C/EBPα, PPARγ2, SREBP-1, C/EBPβ, C/EBPδ, CHOP-10, AEBP1, COUP-TF (4, 26, 27), and others (see arrays.rockefeller.edu/obesity/adipocyte for the complete list of differentially expressed molecules).**

A large group of genes was repressed during in vitro adipogenesis. One particular phase of interest is a large group of cell cycle-related genes (Fig. 2R). 3T3-L1 cells, after the addition of growth factors at confluence, go through a clonal expansion...
Global Expression Profiles of Adipogenesis

| Genes from adipocyte-specific (common to in vivo and in vitro), preadipocyte-specific (common to in vitro and in vivo), in vivo specific, and in vitro specific clusters are shown along with the cluster they were present in from Fig. 4. Transcription factors are shown in bold type. Complete membership of these clusters is available online at arrays.rockefeller.edu/obesity/adipocyte.

| In vivo and in vitro adipocyte enriched genes | In vivo and in vitro preadipocyte enriched genes | In vivo enriched genes | In vitro enriched genes |
|-----------------------------------------------|-----------------------------------------------|-----------------------|------------------------|
| **Gene**                                      | **Cluster**                                   | **Gene**              | **Cluster**            |
| ACRP30/AdipoQ/Adiponectin                    | Adip1                                         | HMGI-Y                | Preadip1               |
| Adipin                                       | Adip1                                         | IFNg                  | Preadip1               |
| Alddehyde DH                                 | Adip1                                         | Nuclear LIM           | Preadip1               |
| Angiotensinogen                              | Adip1                                         | Prx2 Homeobox         | Preadip1               |
| Ap2                                          | Adip1                                         | TSC-3                 | Preadip1               |
| Fat Specific Protein 27                      | Adip1                                         | α-Amylase             | Preadip2               |
| Glycero-phosphate DH                         | Adip1                                         | a-B2 Crystallin       | Preadip2               |
| Haptoglobin                                  | Adip1                                         | AEBP1                 | Preadip2               |
| Hormone Sensitive Lipase                     | Adip1                                         | Id related            | Preadip2               |
| LAP1 Transketolase                           | Adip1                                         | junB                  | Preadip2               |
| Long Chain Fatty Acyl-CoA Synthetase          | Adip1                                         | Kruppel-like factor   | Preadip2               |
| Transferrin                                  | Adip1                                         | Mscf                  | Preadip2               |
| Acyl-CoA BP                                  | Adip2                                         | Pai-1                 | Preadip2               |
| Angiotensinogen                              | Adip2                                         | Spi2                  | Preadip2               |
| ApoC1                                        | Adip2                                         | FGF Receptor          | Preadip3               |
| β3 Adrenoreceptor                            | Adip2/3                                       | Forkhead Box P2       | Preadip3               |
| C/EBPα                                       | Adip2                                         | Histone Deacetylase   | Preadip3               |
| CPT II                                       | Adip2                                         | LCAT                  | Preadip2               |
| Citrate Transporter                          | Adip2/3                                       | Phospho-activated     | Preadip3               |
| GLUT-4                                       | Adip2                                         | C/EBPδ                | Preadip4               |
| Insulin Activated AA                         | Adip2/3                                       | McsF                  | Preadip2               |
| Malate DH                                    | Adip2                                         | C/EBPδ                | Preadip4               |
| Malic Enzyme                                 | Adip2                                         | C/EBPδ                | Preadip4               |
| MEST                                         | Adip2                                         | C/EBPδ                | Preadip4               |
| Monoglyceride Lipase                         | Adip2                                         | 204 interferon        | Preadip4               |
| p18                                          | Adip2                                         | ApoD                  | Preiadp6               |
| p19                                          | Adip2                                         | fos-related antigen   | Preadip5               |
| PPARγ                                        | Adip2                                         | HES-1 HLH             | Preadip5               |
| Spc14                                        | Adip2                                         | Mbh-1                 | Preadip5               |
| SREBP-1                                      | Adip2                                         | MkR3 Zn Finger        | Preadip5               |
| Glucose-6-P DH                               | Adip3                                         | OSF-2                 | Preadip5               |
| Histone H1                                   | Adip3                                         | P58 Secreted          | Preadip5               |
| TOR/RORγ                                    | Adip3                                         | Pdgfa Receptor        | Preadip5               |
| VLDL Receptor                                | Adip3                                         | Prostaglandin         | Preadip5               |
| FPP Synthetase                               | Adip3                                         | dEF1                  | Preadip5               |
| 3-Phosphoglycerate DH                        | Adip4                                         | UCP-2                 | Preadip5               |
| Fatty Acid Synthetase                        | Adip4                                         | VCAM-1                | Preadip5               |
| Low MW GH Receptor                           | Adip4                                         | PFK-1                 | Adip4                  |
| Mall Lipid Binding                           | Adip4                                         | Pyruvate Carboxylase  | Adip4                  |
| RXXα                                         | Adip4                                         | Transaldolase         | Adip4                  |
| Triosephosphate Isomerase                    | Adip4                                         | VEGF                  | Adip4                  |
| VEGF                                         | Adip4                                         | VEGF-b                | Adip4                  |
| SCD2                                         | Adip5                                         | Lactate DH            | Adip5                  |
| αEnolase                                     | Adip5/6                                       | Acyl-CoA DH           | Adip5                  |
| PHAS II                                      | Adip5                                         | Adducine              | Adip5                  |
| Cyclin D                                     | Adip5                                         | Cyclin E              | Adip4                  |
| 3-hydroxyacyl-CoA DH                         | Adip6                                         | Adipocyte p27         | Adip6                  |
| Adipocyte p27                                | Adip6                                         | CHOP-10               | Adip6                  |
| Cytosolic Malate DH                          | Adip6                                         | Dihydroxipamide DH    | Adip6                  |
| p21/Waf1                                     | Adip6                                         | Aldolase A            | Adip6                  |

**TABLE I**

**Genes responsive to adipocyte differentiation in vivo and in vitro**

In vivo and in vitro and preadipocyte-gene expression analysis revealed 34173 genes that were enriched in adipocytes and/or preadipocytes (Table 1). These genes were classified into three categories: those enriched in adipocytes (Adip), those enriched in preadipocytes (Preadip), and those enriched in both cell types (Adip/Preadip). The table also includes a column for in vivo enriched genes and in vitro enriched genes. The genes are listed in alphabetical order by their corresponding Cluster ID, and the gene names are indicated in bold for transcription factors. The clusters are represented by different colors and geometrical shapes.

**Notes:**
- The table includes a column for in vivo enriched genes and in vitro enriched genes.
- The genes are listed in alphabetical order by their corresponding Cluster ID.
- The gene names are indicated in bold for transcription factors.
- The clusters are represented by different colors and geometrical shapes.

**References:**
- Gene expression data is available online at arrays.rockefeller.edu/obesity/adipocyte.
- The full membership of these clusters can be accessed through the website.
- The table provides an overview of the genes enriched in adipocytes and preadipocytes during adipogenesis.

**Acknowledgments:**
- The authors thank [acknowledgments text].
These in vivo expression data provide a new framework in which the functional significance of observed changes in various in vitro models of adipogenesis can be evaluated. Finally, these data suggest the importance of evaluating cell culture models for studying in vivo phenomena using microarrays.

Acknowledgments—We thank Drs. A. Viale and P. Cohen for discussions and critical reading of the manuscript. Thanks to Drs. J. Montez, M. Stoffel, and P. Cohen for Affymetrix data for selection of adipocyte-enriched genes. We also thank G. Fergus, R. Diskin, and Lillian Bloch at Affymetrix.

REFERENCES
1. Green, H., and Meuth, M. (1974) Cell 3, 127–133
2. Spiegelman, B. M., Choy, L., Hotamisligil, G. S., Graves, R. A., and Tontonoz, P. (1993) J. Biol. Chem. 268, 6823–6826
3. Cornelius, P., MacDougald, O. A., and Lane, M. D. (1994) Annu. Rev. Nutr. 14, 99–129
4. MacDougald, O. A., and Lane, M. D. (1995) Science 269, 1108–1112
5. Tanaka, T., Yoshida, N., Kishimoto, T., and Akira, S. (1997) EMBO J. 16, 7432–7443
6. Rosen, E. D., Sarraf, P., Troy, A. E., Bradwin, G., Moore, K., Milestone, D. S., Spiegelman, B. M., and Mortensen, R. M. (1999) Mol. Cell 4, 611–617
7. Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., Kodir, A., and Evans, R. M. (1999) Mol. Cell 4, 685–695
8. Mandrup, S., and Lane, M. D. (1997) J. Biol. Chem. 272, 5367–5370
9. He, G. P., Muise, A., Li, A. W., and Ro, H. S. (1995) Nature 378, 92–96
10. Soukas, A., Cohen, P., Socci, N. D., and Friedman, J. M. (2000) Genes Dev. 14, 963–980
11. Scherer, P. E., Williams, S., Baldini, G., and Lodish, H. F. (1995) J. Biol. Chem. 270, 26746–26749
12. Lane, M. D., Tang, Q. Q., and Jiang, M. S. (1999) Biochem. Biophys. Res. Commun. 266, 677–683
13. Morrison, R. P., and Farmer, S. R. (1999) J. Biol. Chem. 274, 17088–17097
Distinct Transcriptional Profiles of Adipogenesis in Vivo and in Vitro
Alexander Soukas, Nicholas D. Socci, Barbara D. Saatkamp, Silvia Novelli and Jeffrey M. Friedman

J. Biol. Chem. 2001, 276:34167-34174.
doi: 10.1074/jbc.M104421200 originally published online July 9, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M104421200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 27 references, 15 of which can be accessed free at http://www.jbc.org/content/276/36/34167.full.html#ref-list-1