# Acquisition of the adipocyte phenotype biology essay

Science, Biology

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\n[/toc]\n \nWAT expansion takes place rapidly after birth as a result of increased fat cell size as well as an increase in fat cell number. Even at the adult stage, the potential to generate new fat cells persists. It has been demonstrated that fat cell number can increase when rats are fed a high-carbohydrate or high-fat diet (67, 68, 176). Increase in fat cell number is also observed in severe human obesity. However, the relative contribution of fat cell size and fat cell number to human adipose tissue growth on nutritional stimulation remains to be clarified. Moreover, fat cell precursors isolated from adult WAT of various species, including humans, can be differentiated in vitro into mature adipocytes (21, 58, 98, 104, 160, 213, 264). The potential to acquire new fat cells from fat cell precursors throughout the life span is now undisputed. The committed preadipocyte maintains the capacity for growth but has to with draw from the cell cycle before adipose conversion. During adipocyte differentiation, acquisition of the adipocyte phenotype is characterized by chronological changes in the expression of numerous genes. This is reflected by the appearance of early, intermediate, and late mRNA/protein markers and triglyceride accumulation. These changes take place primarily at the transcriptional level, although posttranscriptional regulation occurs for some adipocyte genes (180, 295).

## 3. 1. Growth Arrest

In preadipose cell lines as well as in primary preadipocytes, growth arrest and not cell confluence or cell-cell contact appears to be required for adipocyte differentiation. Although confluence leads to growth arrest, cell-cell contact is not a prerequisite for adipocyte conversion. Primary rat preadipocytes plated at low density in serum free medium can also differentiate in the absence of cell-cell contact (277). C/EBP-α and PPAR-γ transcription factors have been shown to transactivate adipocyte specific genes. Both C/EBP-α and PPAR-γ also appear to be involved in the growth arrest that is required for adipocyte differentiation. McKnight and coworkers (276) have demonstrated the antimitotic activity of C/EBP-α through the use of a C/EBP-α-estrogen receptor fusion protein. Activation by estrogen treatment results in cessation of cell growth as assessed by cell number and DNA synthesis (276). In PPAR-γ-expressing cells, cell cycle withdrawal is accompanied by a decrease in the DNA binding. Therefore, C/EBP-α and PPAR-γ may act cooperatively to bring growth arrest (6). Although C/EBP-α and PPAR-γ expression increases dramatically during adipocyte differentiation, the low level of these factors expressed in preadipocytes may be sufficient to mediate growth arrest that precedes differentiation.

## 3. 2. Clonal Expansion

After growth arrest at confluence, preadipocytes must receive an appropriate combination of mitogenic and adipogenic signals to continue through subsequent differentiation steps. Studies on preadipose cell lines have shown that growth arrested cells undergo at least one round of DNA

replication and cell doubling. This has been proposed to lead to the clonal amplification of committed cells (196). However, primary preadipocytes derived from human adipose tissue do not require cell division to enter the differentiation process (63). In these cells, inhibition of mitosis with cytosine arabinoside does not impair adipocyte development, indicating that clonal amplification of committed cells is not a critical step. These cells may have already undergone potential critical cell divisions in vivo and may therefore correspond to a later stage of adipocyte development. Similarly, another group of growth arrest-specific (gas) genes shows a distinct expression pattern during clonal expansion. Gas6 appears to be preferentially expressed during clonal expansion of post confluent preadipocytes, whereas gas1and gas3 are expressed in serum-starved preadipocytes (244). Combined, these observations suggest differential regulation of the cell cycle in preconfluent proliferation versus postconfluent hormonally stimulated clonal expansion.

## 3. 3. Early Changes in Gene Expression

Growth arrest and clonal expansion are accompanied by complex changes in the pattern of gene expression that can differ with the cell culture models and the specific differentiation protocols employed. Expression of lipoprotein lipase (LPL) mRNA has often been cited as an early sign of adipocyte differentiation (3, 47, 98, 123, 166). LPL is secreted by mature adipocytes and plays a central role in controlling lipid accumulation (48, 86). However, LPL expression occurs spontaneously at confluence and is independent of the addition of agents required for adipocyte differentiation (8, 9, 277). This suggests that LPL expression may reflect the growth-arrest stage rather than

being an early differentiation step. It is also synthesized and secreted by other mesenchymal cell types including cardiac muscle cells and macrophages (49, 267). Because LPL expression is not adipocyte specific and it is independent of the additional agents required for adipocyte differentiation, classification of LPL as an early marker of adipocyte differentiation remains somewhat questionable. The early expression of C/EBP and PPAR is logical given their subsequent involvement in terminal differentiation by transactivation of adipocyte-specific genes. PPAR-γ is largely adipocyte specific and is expressed at low but detectable levels in preadipocytes. Its expression rapidly increases after hormonal induction of differentiation. A transient increase in the expression of C/EBP-β and C/EBP-δ isoforms precedes the increase in PPAR-γ expression (27, 167, 299). The subsequent decrease of C/EBP-β and C/EBP-δ in early to intermediary stages of differentiation is concomitant with the induction of C/EBP-α mRNA. This increase in C/EBP-α expression occurs slightly before the expression of adipocyte-specific genes (27, 153, 167). During adipocyte differentiation, cells convert from a fibroblastic to a spherical shape, and dramatic changes occur in cell morphology, cytoskeletal components, and the level and type of extracellular matrix (ECM) components. It is likely that these changes could influence the expression and action of PPARs and/or C/EBPs during adipocyte differentiation. Decrease in actin and tubulin expression is an early event in adipocyte differentiation that precedes overt changes in morphology and the expression of adipocyte-specific genes (255). These changes in cell shape reflect a distinct process in differentiation and are not the result of accumulated lipid stores. A switch in collagen gene expression is also an

early event of adipocyte differentiation. The relative concentrations of fibroblast-expressed type I and type III procollagen mRNA decline by 80–90%, while secretion of type IV collagen and entactin/nidogen increases (13, 290). The amount of pericellular fibronectin, as well as cellular synthesis of fibronectin, decreases by four- to five folds during differentiation (10). Preadipocyte factor-1 (pref-1), a preadipocyte protein with epidermal growth factor (EGF)-like repeats, has been hypothesized to be involved in maintaining the preadipose phenotype (246–248). A dramatic decrease in pref-1 expression accompanies adipocyte differentiation; it is abundant in preadipocytes and is not detectable in mature fat cells. It is the only known gene whose expression is completely downregulated during adipocyte differentiation