Review

Positive and negative regulators of adipocyte differentiation

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Introduction

Adipocytes are highly specialized cells that play a crucial role in energy balance. They provide the ability to synthesize and store fat during times of positive energy balance in preparation for periods of nutritional deprivation. However, obesity that results from excess accumulation of adipose tissue has become a major health problem in Western society. Adipose tissue development may occur not only during the embryonic stage but also in the adult, where an increase in fat cell number has been documented.[1-4] The development of adipose tissue involves the commitment of multipotential mesenchymal stem cells to preadipocytes, and their subsequent differentiation to mature adipocytes with a distinct pattern of tissue-specific expression of adipocyte genes.

Preadipocyte differentiation in culture

The establishment of clonal adipogenic cell lines, such as 3T3-L1, 3T3-F442A, ob17, and TA1 cells,[5-9] have made possible the study of the molecular mechanisms underlying adipose tissue development. These cells exhibit the properties of fibroblasts during growth. At confluence and under appropriate culture conditions, cells withdraw from the cell cycle and spontaneously differentiate into adipocytes. After treatment of the cells with various hormones and agents, differentiation, which is defined as the acquisition of the biochemical profile and rounded lipid-filled morphology of typical mature adipocytes, occurs within 7–10 days. Studies utilizing preadipocyte cell lines have demonstrated that adipocyte conversion is accompanied by significant alterations in the level of over 100 proteins.[10,11] Cells express adipocyte-specific genes, including those encoding lipogenic and lipolytic enzymes, as well as those involved in nutrient transport and hormone responsiveness.[12-25]

Alterations of structural and extracellular matrix components occur during adipocyte differentiation.[26-32] These changes are probably necessary for cellular reorganization and may also provide a permissive environment for the expression of adipocyte genes. mRNA levels for the cytoskeletal proteins actin and tubulin decrease preceding the expression of adipocyte-specific enzymes.[26] Extracellular matrix proteins have been shown to influence adipose conversion. Differentiation of 3T3 cells is inhibited if the cells are plated on fibronectin-coated dishes.[27] Detachment or reduction of the interaction of cells with fibronectin may be a precondition for differentiation, and fibronectin may interfere with the cytoskeletal and morphological changes necessary for the adipose conversion of 3T3-F442A cells. A switch in collagen gene expression occurs with a decrease in type I and type III procollagen synthesis. Synthesis of type IV collagen, which may function as an adaptor in cell adhesion, increases.[28] Entactin synthesis increases and an unusual laminin complex that does not contain the typical A subunit is produced during the adipose conversion of 3T3-L1 cells.[31] These changes may be related to the loose interaction of basement membranes with adipose cells that has been observed in fat tissue. In addition, versican-like chondroitan sulfate proteoglycans, which are both cell-associated and secreted into the medium, have been reported to increase.[32] These proteoglycans may permit cells to move and thereby allow the changes in cell shape that occur during adipocyte differentiation.

Transcriptional activation of adipocyte genes

Most recent studies focus on the transcriptional activation of genes that encode proteins required for adipocyte function, although stabilization of mRNAs is an additional mechanism used during adipose conversion.[33,34] The promoters of several adipocyte genes, such as aFABP, adipsin, and stearoyl CoA desaturase, which are expressed in a differentiation-dependent manner, are currently being dissected.[35-44] Putative *cis*-acting DNA sequences and the factors that participate in

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the transcriptional activation of these genes are being pursued. It is not known if common *cis*-regulatory sequences are responsible for the differentiation-dependent expression of adipocyte genes. C/EBP, a bZIP family transcription factor first purified from liver nuclear extracts, has recently been implicated in the differentiation-dependent expression of some adipocyte-specific genes.[45-49] C/EBP sites are present in the promoter regions of several adipocyte genes including adipocyte fatty acid binding protein (aP2), stearoyl CoA desaturase, insulin-responsive glucose transporter (GLUT4), and FSP27. C/EBP trans-activates these genes in adipocytes. Studies on aP2 gene activation during adipose conversion suggest that C/EBP may also function by displacement of a factor that binds to a negative element.[46,50] Premature expression of C/EBP as an estrogen receptor fusion protein causes early expression of aFABP compared with its normal induction pattern during adipocyte differentiation.[51] Antisense C/EBP inhibits adipocyte gene expression and triacylglycerol accumulation. These defects are rescued when the cells are transfected with sense C/EBP.[52,53] Moreover, C/EBP is transcriptionally activated early in the adipocyte differentiation process.[48,54,55] C/EBP, however, is expressed in several tissues (liver, fat, intestine) and also transactivates liver-specific genes, including albumin and transthyretin.[56,57] Moreover, its premature expression does not in itself induce terminal differentiation of preadipocytes. Only after the appropriate hormonal treatment can C/EBP accelerate the differentiation process by activating adipocyte-specific genes resulting in lipid accumulation. C/EBP may function by suppressing the cell division of preadipocytes that occurs before entry into the quiescent differentiated state. An additional complication is that studies in transgenic mice indicate that the proximal promoter of aFABP contains the C/EBP site, but is not sufficient to direct expression in adipose tissue. Rather, a yet unknown but differentiation-dependent factor that binds to a strong enhancer at -5.2 kb upstream of the gene appears to be involved in aFABP expression in adipose tissue.[40,41]

Determination of stem cells to adipocyte lineage

The aspects of adipocyte differentiation thus far discussed pertain to the activation of cell-type specific genes during the differentiation process. However, these studies do not provide clues as to how cells make the initial choice of preadipocyte fate. Little is known about the mechanisms or the molecules that control conversion of embryonic cells into determined stem cells capable of adipocyte differentiation. Master regulatory genes that govern cell-fate decisions by controlling a battery of other genes during development have been well illustrated in a variety of systems. In addition, the concept that one or more genes are capable of specifying cell lineage has been demonstrated by treatment of 10T1/2 fibroblasts with 5-azacytidine.[58,59] The resultant demethylation of one or more specific loci generates colonies of myotubes, adipocytes, and chondrocytes. Recent work has determined the details of the changes that occur during commitment and differentiation of the muscle cell lineage. MyoD belongs to a family of myogenic genes, which are expressed only in myoblasts and skeletal muscle tissue, and includes MyoD, myogenin, myf-5, and mrf-4-herculin.[60] Each member of the MyoD family has a distinct temporal expression pattern during embryogenesis. It has been suggested that these proteins may function in a cascade of regulatory events.[61] Gene-targeting experiments indicate possible redundancy of function in that null mutation of MyoD or myf-5 did not cause abnormal muscle formation.[62,63] In vitro transfection of 10T1/2 fibroblasts with MyoD gives rise to stable myogenic cells capable of undergoing muscle differentiation. Moreover, MyoD expression activates muscle-specific genes and is sufficient for the onset of the myogenic program in fibroblasts and several other cell types. Studies have characterized the domains of MyoD involved in transcriptional control, DNA-binding, and its interaction with other proteins. MyoD can also interact with Jun, which acts as a repressor for MyoD function, and this may explain the diversion of myoblasts from growth to differentiation. Moreover, expression of MyoD may be regulated by factors that communicate environmental signals. For example, protein kinase C phosphorylates myogenin, leading to decreased DNA-binding activity.[64] The identical site is phosphorylated by FGF, which is known to inhibit myogenesis. Insulin-like growth factor-I (IGF-I) stimulates myogenesis of L6 cells, probably by induction of myogenin, another member of the myogenic gene family.[65]

The most simple hypothesis would be that analogous regulatory protein(s) function in the adipocyte lineage. Chen at al. reported that 3T3-C2 cells, a 3T3 subline with a negligible adipose conversion rate, became capable of differentiating into adipocytes when transfected with DNA from 3T3-F442A preadipocytes.[66] Human adipose tissue DNA also proved to be effective. The putative gene(s) responsible for this conversion have not been identified. A major step in the understanding of adipose tissue development would be to clone and characterize the key regulatory molecule(s) that function in either a positive or negative manner in the adipocyte lineage. Once the identities of the key regulators are known, studies could examine the molecular actions of factors that communicate signals from neighboring cells or the environment to influence nuclear events during adipose tissue development.

Factors that stimulate adipocyte differentiation

The process of cell determination and differentiation is controlled by communication between individual cells or between cells and the extracellular microenvironment. Molecules that mediate this communication include the classic diffusible growth factors that act via specific receptors to transduce external signals through a cascade of intracellular events. The variability that is routinely observed in the degree of adipocyte differentiation, even following clonal selection, suggests that the signal to differentiate may depend on as yet unidentified local environmental cues. The precise combination of

hormones and growth/differentiation factors required for the initiation of and progression through the adipogenic program is not fully understood. Most studies on the role of adipogenic and anti-adipogenic factors employ one of the above-mentioned preadipocyte cell lines. Because these cell lines are already committed to the adipogenic lineage, such studies address only the process of differentiation. Cells are usually maintained in medium supplemented with fetal calf serum during growth. Identification of factors that affect adipocyte differentiation involves the addition of various hormones and factors and the subsequent determination of the extent of differentiation. This is judged by staining for accumulated lipid, measuring activities of lipogenic enzymes such as glycerol-3-phosphate dehydrogenase, or more recently, by the expression of adipocyte-specific genes. Several laboratories have developed chemically defined media that allow adipose conversion.[67,68] There are conflicting reports, however, on the stimulatory and inhibitory factors involved in adipogenesis. This is probably due to variation in culture conditions and differences among the type of preadipocyte cell lines employed, each of which may represent different developmental stages in the adipocyte lineage.

Green et al.,[67-71] who originally established the 3T3 preadipocyte cell lines, showed that confluent 3T3-F442A cells undergo spontaneous adipose conversion when maintained in calf serum. Growth hormone present in the serum was proposed to account for the triggering of 3T3-F442A differentiation. These investigators also reported that IGF-I supports the selective limited multiplication of young, growth hormone-induced cells and leads to the clonal expansion of adipocytes. On the other hand, insulin, which is routinely added to differentiation medium, is considered to only modulate the process of adipogenesis by increasing triacylglycerol synthesis.[72] In agreement with these observations, adipose conversion in serum-free media requires growth hormone, even in the presence of IGF-I.[73] Moreover, protein kinase C activators, such as prostaglandin $F2_{\alpha}$, phorbol esters, and diacylglycerol, are able to mimic the growth hormone effect, indicating involvement of the protein kinase C signalling pathway.[74]

Rubin et al., on the other hand, observed that 3T3-L1 cells undergo very little spontaneous differentiation in media that contain fetal calf serum alone, but necessitates treatment with dexamethasone and methylisobutylxanthine to trigger differentiation.[24,75] These investigators also reported that IGF-I is an essential factor for adipocyte differentiation of 3T3-L1 cells.[76] Media used to demonstrate the IGF-I requirement in this experiment contained fetal calf serum that had been depleted of growth hormone, insulin, and IGF-I by charcoal and ion-exchange resin treatment. It is not known, however, what other factors normally present in fetal calf serum were also depleted. Studies employing serum-free defined media showed that adipose conversion of 3T3-L1 cells is dependent on IGF-I in combination with EGF, a condition that brings about post-confluent mitoses.[67] Adipose conversion of 3T3-L1 cells in serum-free media is also dependent on the presence of both corticosterone and methylisobutylxanthine, as is true for serum-containing media. Methylisobutylxanthine may promote adipocyte conversion by inhibiting cAMP phosphodiesterase activity because it can be replaced by forskolin or cAMP analogues. This suggests that elevated intracellular cAMP concentration may be involved in the adipocyte differentiation process. Ailhaud et al. have described arachidonic acid as an adipogenic factor for ob17 cells in serum-free defined media.[77,78] The arachidonic acid metabolite, prostacyclin, increases in cAMP synthesis. There is also an increase in intracellular Ca^{2+} concentration. cAMP may trigger adipose conversion by directly activating adipocyte genes. The AP-1 site present at the proximal promoter of the aFABP gene may be responsible for the activation of this promoter by cAMP in confluent preadipocytes.[35] However, AP-1 sites have not been found in promoters of other adipocyte genes. A clear understanding of the intracellular action of either growth hormone or IGF-I in adipocyte differentiation is not yet available at the molecular level. Although the structure and ligand binding properties of both receptors are known, the precise signal transduction pathways are not. Ras proteins may mediate IGF-I signalling in 3T3-L1 adipocyte differentiation. Transfection of ras oncogenes causes differentiation of 3T3-L1 cells to adipocytes in the absence of externally added IGF-I or pharmacological concentrations of insulin.[79] Moreover, transfection of a dominant inhibitory ras mutant results in the inhibition of differentiation.

Although glucocorticoids are routinely added to differentiation media, there are conflicting reports on their role in preadipocyte differentiation, however, their contribution to the development of adiposity has been demonstrated in animal experiments.[80] In both 3T3-L1 and TA1 cells dexamethasone stimulates adipose conversion as described above.[24,81] On the other hand, in 3T3-F442A cells, glucocorticoids inhibit adipocyte differentiation as reflected by an increase in glycerol-3-phosphate dehydrogenase activity and triacylglycerol accumulation.[82] Experiments have not examined the molecular events responsible for the effects of glucocorticoid on adipocyte differentiation. As indicated by the generally accepted mechanism of steroid hormone action, glucocorticoids may directly modulate transcription of regulatory genes or adipocyte-specific genes via the glucocorticoid response element. Ailhaud et al.,[77] however, suggest a more indirect effect of glucocorticoids. Corticosterone may increase arachidonic acid metabolism and thus synthesis of prostacyclin to increase cAMP production.[77]

Factors that inhibit adipocyte differentiation

In addition to the adipogenic factors described in the previous section, serum also contains factors that suppress preadipocyte differentiation. Growth factors such as fibroblast growth factor (FGF) and platelet-derived growth factor (PDGF) are inhibitory for preadipocyte differentiation in fetal calf serum containing media.[83,84]

As is the case for the function of other growth factors on development and differentiation, the signal transduction pathways for these growth factors in adipose conversion are not clear. Nonetheless, the inhibitory effect of these agents on preadipocyte differentiation, as in other cell types, may be independent of their mitogenic properties. In TA1 cells, the phorbol ester 12-0-tetradecanoyl-phorbol-13-acetate (TPA) mimics the inhibition of adipocyte differentiation elicited by bFGF, and indicates that protein kinase C activation mediates the inhibitory effect.[85] FGF also extinguished adipocyte-specific gene expression when added to adipocytes. However, this effect was observed even in TPA-pretreated cells and indicates maintenance of adipocyte differentiation in a protein kinase C-independent manner. The adipogenic effect of growth hormone on ob17 cells in serum-free media has been reported to occur via the activation of protein kinase C.[74] TGF-β blocked adipogenesis when 3T3-L1 or TA1 cells were exposed to this growth factor during the initial stages of differentiation.[86,87] 3T3 adipose conversion, however, was not affected when TGF-β was added during later stages of differentiation.[86] TGF-β did not affect cell proliferation. Rather, its inhibitory effect on adipocyte differentiation has been attributed to an increase in synthesis of extracellular matrix proteins such as fibronectin and collagen.

Ringold et al. reported that tumor necrosis factor-α (TNF-α), a cytokine secreted by macrophages that causes cachexia in animals, inhibits conversion of TA1 cells to adipocytes.[84] TNF-α treatment of differentiated TA1 adipocytes not only caused cells to suppress expression of adipocyte genes to their predifferentiated levels, but required that cells be retreated with the differentiation-accelerating agents dexamethasone and indomethacin for expression of the adipocyte phenotype. These investigators therefore suggest that TNF-α may not simply bring about a lipolytic state but may actually cause reversion to the preadipocyte state. At a minimum, because it downregulates C/EBP expression, the expression of those adipose-specific genes that contain C/EBP binding sites in their promoters can be suppressed by TNF-α.[49,88] The question of whether adipose differentiation is a reversible process is intriguing. It may be that maintenance of the differentiated state requires continuous control by both positive and negative regulators.

Function of a unique EGF-like protein in adipocyte differentiation

It has recently been shown that a novel preadipocyte protein containing EGF-repeats inhibits adipocyte differentiation.[89] This molecule is a member of the EGF-like family of proteins. These membrane-bound or secreted proteins act on cell growth and differentiation in an astonishing array of biological settings. The distinguishing feature of these proteins is the presence of at least one EGF-like repeat, a 35–40 amino acid motif characterized by the conserved spacing of six cysteine residues that form three disulfide bonds.[90] This motif was originally described for EGF. Interestingly, Serrero et al. reported that EGF inhibits differentiation of adipocytes both in rat primary precursor and in teratoma-derived adipogenic 1246 cells.[91] This inhibitory effect on differentiation was not linked to the proliferative effect of EGF. These investigators also demonstrated an inhibitory effect of EGF on adipose tissue development in the newborn rat.[92] In addition to EGF, other growth factor members of this family, such as transforming growth factor-α (TGF-α), amphiregulin, and heparin binding epidermal growth factor (HB-EGF), all bind and act through the EGF receptor.[93,94] Several multi-domain ECM proteins and cell adhesion molecules (CAMs) with demonstrated roles in cell guidance and development contain EGF-like motifs.[95] These include the extracellular matrix (ECM) molecules laminin, versican, and tenascin and the selectin family of adhesion molecules. The importance of this motif is further demonstrated by its presence across many species, notably in the Drosophila proteins notch, with 36 such repeats, and delta, with 9 repeats.[96,97] The notch and delta genes belong to the group of neurogenic genes that mediate the decision between the neural and epidermal fate in cells of the neurogenic ectoderm. Lin-12 and glp-1 also encode proteins with multiple EGF-like repeats that control distinct decisions between cell fates in the nematode C. elegans.[98,99] The universality of this motif suggests that similar mechanisms may also control cell fate determination in vertebrates.

Preadipocyte factor-1 (pref-1) was cloned from a 3T3-L1 preadipocyte library and is highly expressed in 3T3-L1 preadipocytes at confluence.[89] Sequence analysis shows that pref-1 contains a transmembrane domain and six EGF-like repeats (*Figure 1*). None of the EGF-like repeats in pref-1 maintain the exact spacing of mature EGF, nor contain those residues shown to be critical for binding to the EGF receptor.[100] Overall, the spacing of cysteines and the conservation of other residues is most similar to that of the Drosophila protein delta, a molecule known to be involved in cell fate determination via cell-cell interaction. Pref-1 mRNA is expressed at high levels in confluent 3T3-L1 preadipocytes and decreases during differentiation. It is not detected at all in adipocytes. Western analysis of membrane fractions prepared from preadipocytes and differentiated adipocytes indicates that pref-1 protein levels decrease during adipose conversion. Interestingly, Northern analysis detects pref-1 mRNA only in the adrenal gland, and not in liver, adipose tissue, lung, brain, muscle, or in other adult tissues. The absence of pref-1 mRNA in mature 3T3-L1 adipocytes is in accord with the fact that it is not detected in adult adipose tissue. Pref-1 is also expressed during mouse embryogenesis. The mRNA is first detected at 8.5 days of gestation and its level increases through 18.5 days. A developmental role for pref-1 is suggested by the presence of pref-1 mRNA during embryogenesis and its very restricted expression in adult tissues.

The abolition of pref-1 mRNA and protein expression during adipocyte differentiation indicates that it is regulated during adipogenesis. A possible role for pref-1 in the control of adipocyte differentiation is indi-

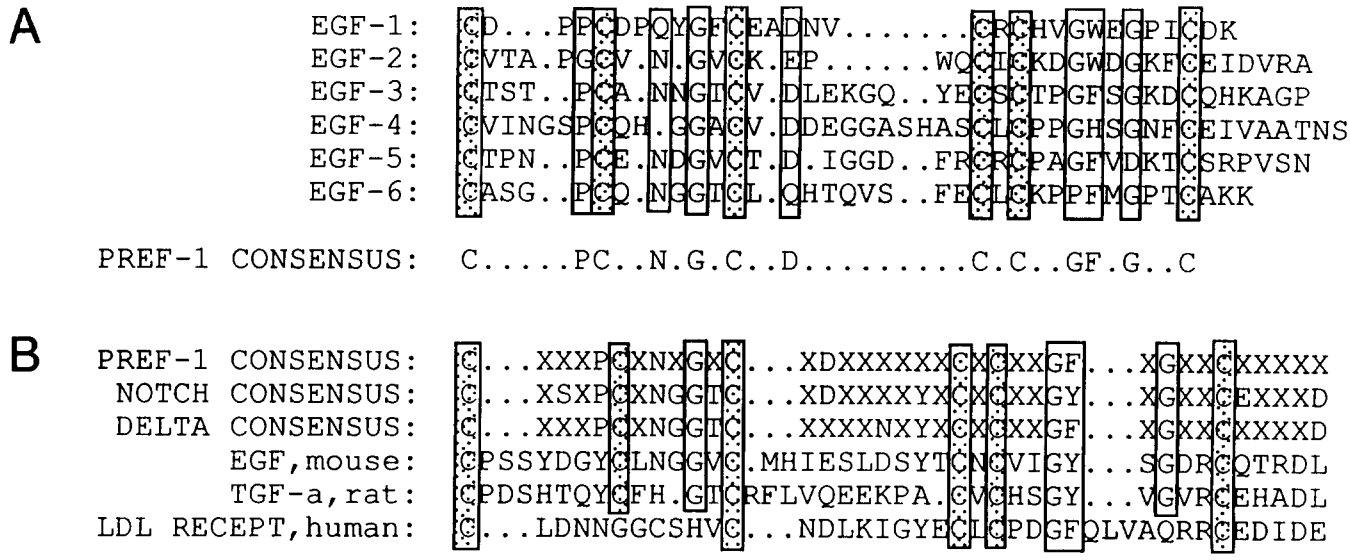

Figure 1 Sequence alignment of EGF-life domains of pref-1. A. The six EGF-like repeats of pref-1 are shown with the first cysteine residue of each of the six repeats on the left. The six conserved cysteines characteristic of this protein family are boxed and shaded. Other amino acids conserved in this motif are boxed. B. Alignment of the pref-1 consensus EGF-like repeat with consensus sequences of the Drosophila homeotic proteins Notch[97] and Delta[96]; of the EGF-like growth factors, EGF, mouse,[101] and TGF-a, rat[102]; and of the LDL receptor.[103]

cated by the down-regulation of pref-1 mRNA by serum, an essential factor for adipose conversion, and by the higher levels of pref-1 mRNA in nondifferentiating 3T3-C2 cells than in differentiation-competent 3T3-L1 cells (*Figure 2*). The role of pref-1 in adipocyte differentiation was tested by stable transfection of 3T3-L1 cells. Cells transfected with the correct orientation of the pref-1 open reading frame (ORF) express higher levels of pref-1 mRNA than their reverse orientation counterparts or nontransfected controls, as shown by an upward expansion of the band (*Figure 3*). Because preadipocytes express high endogenous levels of pref-1 mRNA, the constitutively expressed form increases total pref-1 RNA by at most 50%. Nonetheless, it is not the absolute level of pref-1, but rather the inability to down-regulate its expression that are addressed in this experiment. Lipid staining and levels of two RNA markers of adipocyte differentiation, stearoyl CoA desaturase and adipocyte fatty acid binding protein, demonstrate that constitutively expressed pref-1 severely inhibits adipocyte differentiation. Microscopic examination of the cultures shows a decrease in the total number of cells that differentiate into adipocytes, rather than a decrease in the amount of lipid per cell.

While the regulation of many mRNAs whose levels increase during adipocyte differentiation have been characterized in detail, very few mRNAs that are down-regulated have been identified. The sole example is the 50% decrease in the synthesis of the cytoskeletal components actin and tubulin that occurs during adipose conversion.[26] The down-regulation of pref-1 during adipose conversion may reflect the fact that

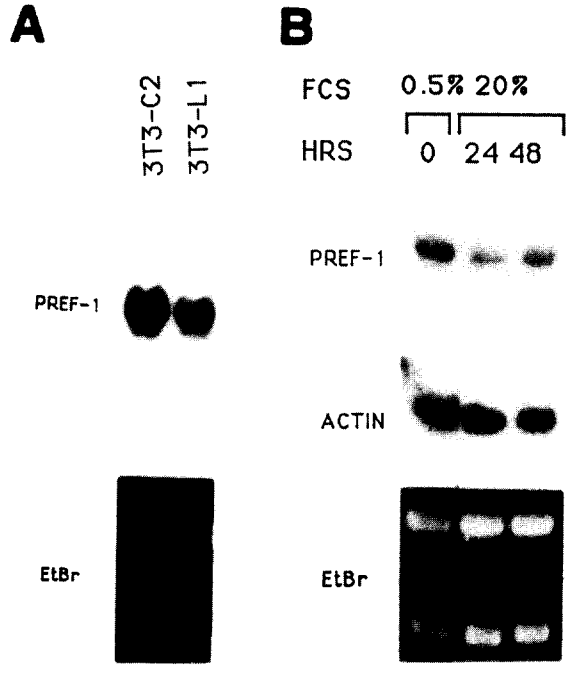

Figure 2 Differential expression of pref-1 mRNA in cell lines and by serum. A. Northern analysis of pref-1 mRNA in 3T3-C2 and 3T3-L1 cells. Ten μgs of total RNA were subjected to Northern blot and probed with ^{32}P-labeled pref-1 sequence. B. Effect of fetal calf serum on pref-1 mRNA level. Northern blot of 10 μg of RNA from 3T3-L1 preadipocytes obtained either after growth for 2 days in 0.5% FCS, at which time (0 hr) media were changed to 20% FCS and cells harvested at 24 and 48 hrs. The resultant Northern blot was sequentially hybridized to ^{32}P-labeled pref-1 and actin sequences.

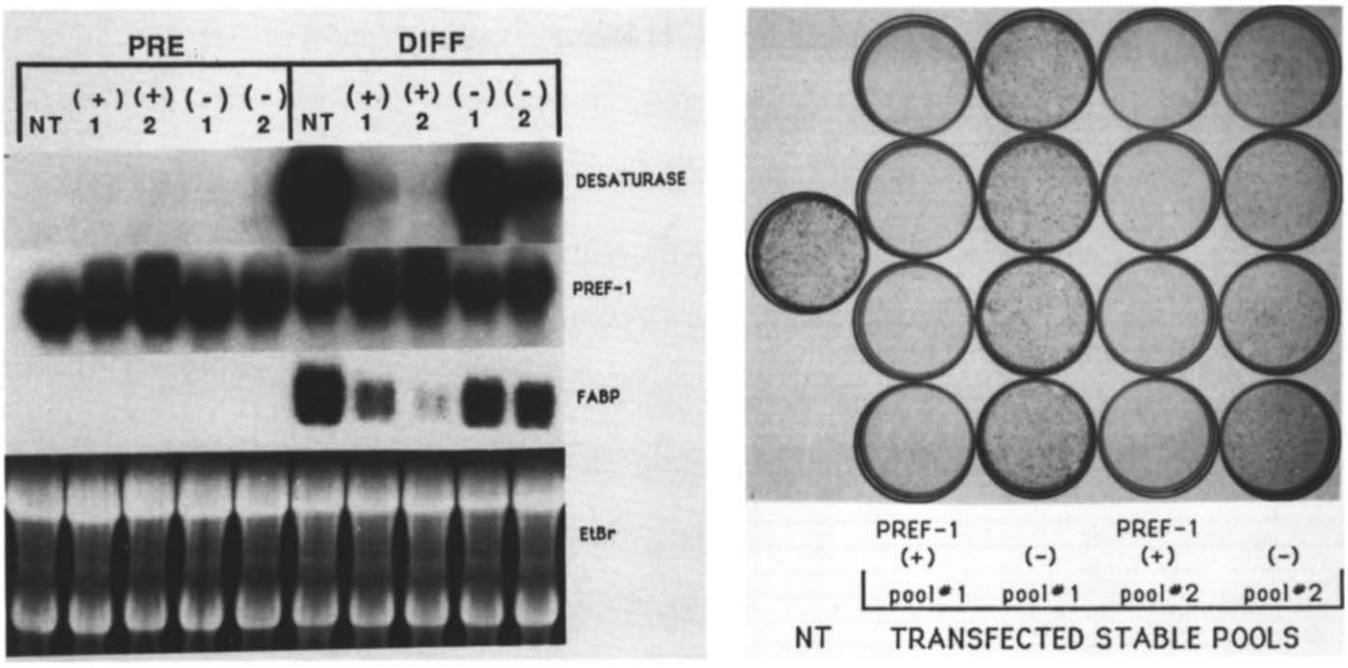

Figure 3 Constitutive pref-1 expression inhibits preadipocyte differentiation. Left panel shows Northern blots of RNA prepared from four independent stable pools of 3T3-L1 preadipocytes transfected with either the correct; (+)1, (+)2, or the reverse; (−)1, (−)2 orientation of the pref-1 ORF either at confluence (PRE) or 7 days following onset of the differentiation (DIFF). RNA was prepared from nontransfected 3T3-L1 cells after differentiation (NT). RNA was subjected to Northern blot and hybridized with ^{32}P-labeled stearoyl CoA desaturase, pref-1, and adipocyte fatty acid binding protein (aP2) sequences. Right panel shows Oil Red O Lipid staining of those four stable pools of 3T3-L1 cells transfected with the correct (+) or the reverse orientation (−) of the pref-1 ORF. Cells underwent the differentiation protocol, and 7 days post-confluence cells were fixed with 10% paraformaldehyde and stained for lipid content by Oil Red O. Quadruplicate dishes of the four independent stable pools are shown here.

is a protein that is simply no longer required in the mature adipocyte. Conversely, as indicated by the above-stated study, down-regulation of pref-1 may be required for the conversion process, either in a permissive or instructive manner. The blockage of adipocyte differentiation in cells unable to down-regulate constitutively expressed pref-1 argues for the latter hypothesis. Nevertheless, the biochemical function of pref-1 in adipocyte differentiation remains unknown. However, a number of hypotheses regarding pref-1 function in preadipocytes is suggested by the demonstrated role of EGF repeats in other molecules. If pref-1 acts as a growth factor, then its down-regulation may be involved in the cessation of cell growth, a requirement for differentiation. The fact that foci of adipocytes differentiate while surrounded by residual preadipocytes that continue to synthesize pref-1 suggests that the action of pref-1 is cell-autonomous, or at most affects only neighboring cells. This argues against a diffusible form of pref-1 and to date the only detected form is the transmembrane molecule. Pref-1 may also mediate cell-cell or cell-extracellular matrix interactions. Cell membrane components sense the microenvironment by cell-cell contact. These signals are passed to the nucleus in response to the extracellular environmental changes and can modify or control the differentiation process. Several ECM proteins contain EGF-like repeats and may even sequester soluble EGF-like proteins. Pref-1 may maintain the preadipocyte phenotype via the interaction of its EGF-like repeats with the EGF-like repeats of proteins on adjacent preadipocytes or ECM components. The determination of the molecular basis of pref-1 function will lead to a better understanding of adipose tissue development, a process for which critical regulatory genes have yet to be identified and characterized.

Conclusions

Adipose cell differentiation is probably the result of a cascade of intracellular events, possibly involving a hierarchy of regulatory genes that ultimately determine the expression pattern of genes characteristic of adipogenesis. The major step in understanding adipose tissue development involves the identification of regulatory genes for the adipose cell lineages. Unique adipocyte regulatory genes may function along with more general regulatory gene(s), such as C/EBP, in the expression of complex patterns of adipocyte-specific genes. These molecules may be regulated in concentration or by post-translational modification. Multiple positive and negative factors, which communicate information from the extracellular environment to nucleus, may be involved. Adipose tissue development will be fully understood when the integration of these multiple signals are elucidated at the molecular level.

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