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Expression and Regulation of pOb24 and Lipoprotein Lipase Genes During Adipose Conversion

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Lipoprotein lipase (LPL) and pOb24 mRNAs are known to be early markers of adipose cell differentiation. Comparative studies of the expression of pOb24 and LPL genes during adipose conversion of Ob1771 preadipocyte cells and in mouse adipose tissue have shown the following: 1) the expression of both genes takes place at confluence; this event can also be triggered by growth arrest of exponentially growing cells at the G_1/S stage of the cell cycle; 2) In contrast to glycerol-3-phosphate dehydrogenase mRNA, the emergence of pOb24 and lipoprotein lipase mRNAs requires neither growth hormone or tri-iodothyronine as obligatory hormones nor insulin as a modulating hormone; 3) in mouse adipose tissue, pOb24 mRNA is present at a high level in stromal-vascular cells and at a low level in mature adipocytes, and in contrast LPL mRNAs are preferentially expressed in mature adipocytes. Thus, these two genes do not appear to be regulated in a similar manner, as also shown by the differential inhibition of their expression by tumor necrosis factor (TNF) and transforming growth factor-β (TGF-β).

Key words: gene expression, differentiation, adipose cell

The occurrence of time-dependent events in the adipose conversion process is supported by various lines of evidence. Until recently, the early stage of adipose conversion, at the molecular level, was characterized only by the emergence of LPL activity, whereas the late and very late stages of adipose conversion were characterized by the emergence of several mRNAs and the corresponding proteins like glycerol-3-phosphate dehydrogenase (GPDH), aP2, adipsin, and phosphoenolpyruvate carboxykinase (PEPCK) [1–4].

Abbreviations used: DME, Dulbecco's modified Eagle's; GH, growth hormone; GPDH, glycerol-3-phosphate dehydrogenase; LPL, lipoprotein lipase; PEPCK, phosphoenolpyruvate carboxykinase; SVF, stromal-vascular fraction; TGF-β, transforming growth factor-β; TNF, tumor necrosis factor; T_3, tri-iodothyronine.

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Recently, we have obtained, by differential screening, an mRNA that is unique to adipose tissue in adult mice and can be considered an early marker of adipose conversion. This mRNA, namely pOb24, appears rapidly during adipocyte differentiation and is specifically expressed during the preadipose state [5]. In this paper, we report a comparative study on pOb24 and LPL gene expression during adipose conversion of Ob1771 preadipocyte cells as well as in adipose precursor cells and adipocytes from mouse adipose tissue.

MATERIALS AND METHODS

Cell Culture

Characterization of the Ob1771 clonal line has been previously described [6], and cell culture conditions were as have been described [5].

Northern-Blot Analysis

Poly(A)$^+$ RNAs from Ob1771 cells were prepared as previously described [4], and poly(A)$^+$ RNAs were prepared from adipose tissue also as described previously [7]. Poly(A)$^+$ RNAs are electrophoresed through a formaldehyde/agarose gel, transferred onto an Amersham nylon membrane filter, and hybridized with pOb24 and LPL nick-translated probes. pOb24 cDNA probe (1.2 kb) has been obtained by differential screening between exponentially growing and early confluent Ob17 cells [5]. LPL cDNA probe (2.2 kb) has been isolated from a λgt11 cDNA library derived from poly(A)$^+$ RNAs of guinea pig adipocytes [8].

Materials

Porcine TGF-β (batch 6F1471) was a product of R & D Systems, Inc. (Minneapolis, MN, USA). Recombinant mouse TNF (4×10^8 units/mg; purity > 99%) was a kind gift of Dr. Jan Tavernier (Biogent, Gent, Belgium).

RESULTS AND DISCUSSION

Expression of pOb24 and LPL mRNAs in Growth-Arrested Cells

The necessity of a growth arrest for adipose cell differentiation was clearly illustrated when Ob17 cells were transformed by the middle-T-only gene of polyoma virus. Among the different clones obtained, there was an inverse relationship in culture between their potentiality to overproliferate at low serum and their frequency to convert into adipose cells [9]. It is now well established that growth arrest during the G_1 phase of the cell cycle is required for the commitment into a given cell type [10]. As shown in Figure 1, both pOb24 and LPL mRNAs were not detectable in exponentially growing cells (lane a) and were expressed in 4-day postconfluent cells (lane b) as a 6 kb band for pOb24 mRNA and two bands of 3.3 and 3.7 kb for LPL mRNAs. Growth arrest of actively growing cells by exposure to 5 mM thymidine led within 24 h to induction of both pOb24 and LPL mRNAs (lane c). Under this condition, cells are blocked at the G_1/S stage of the cell cycle [11] and expressed early differentiation markers only as GPDH mRNA remained undetectable. When cells were allowed to resume growth by thymidine removal, pOb24 and LPL activity disappeared within 24 h [11]; similar results were obtained for LPL mRNAs (not shown). The disappearance of pOb24

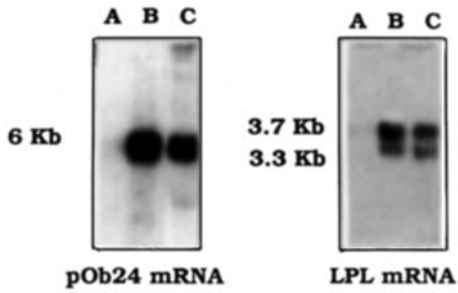

Fig. 1. Emergence in growth-arrested Ob1771 cells of pOb24 and LPL mRNAs. Growth arrest was initiated by the addition of 5 mM thymidine to Ob1771 cells actively growing in DME medium supplemented with 10% fetal bovine serum. Twenty-four hours after thymidine addition, 5 μg of poly(A)$^+$ RNAs were prepared and analyzed for the presence of pOb24 and LPL mRNAs. **A:** Exponentially growing cells; **B:** 4-day post-confluent cells; **C:** Dispersed cells growth arrested by thymidine block. The results are representative of three independent experiments performed on different series of cells.

mRNA seemed to be rapid and to occur as soon as the cells entered the S-phase and synthesized DNA [5].

These results indicate that the parallel induction of pOb24 and LPL mRNAs can occur at growth arrest at the G_1/S stage of the cell cycle, but this induction appears to be a reversible process if the cells were allowed to resume a continuous proliferation, as previously described [5,11].

Expression of pOb24 and LPL mRNAs and Hormonal Requirements

The previous experiments were carried out with cells maintained in medium supplemented with 10% fetal bovine serum, which contains a relatively high amount of growth hormone (GH) and tri-iodothyronine (T_3). In order to determine the effect of these hormones on the induction of LPL mRNAs *during differentiation,* Ob1771 cells were cultured in a Dulbecco's modified Eagle's (DME) medium supplemented with 1) 8% bovine serum containing minute concentrations of GH (<0.02 nM) [12] and supplemented with 2 nM T_3 and 17 nM insulin; 2) 10% fetal bovine serum containing GH (0.2 nM, ref. 13) and 17 nM insulin but depleted of thyroid hormones by ion exchange chromatography [14]; and 3) 10% fetal bovine serum supplemented with 2 nM T_3 but containing minute concentrations of insulin (<4 pM) [15].

As shown in Figure 2, the induction of LPL mRNAs in Ob1771 confluent cells takes place in a medium not supplemented with GH, T_3, or insulin. In a way similar to that of LPL mRNAs, and in agreement with our previous data, the induction of pOb24 mRNA was also independent of the presence of these hormones, whereas expression of GPDH mRNA required exposure of the cells to GH and T_3 and was strongly increased by insulin [5,16,17]. The results of Figure 2 indicate also that the expression of pOb24 and LPL mRNAs during differentiation was enhanced by GH, T_3, or insulin. This effect was rather weak but reproducible, in agreement with previous data showing a full expression of LPL activity in the presence of these various hormones [6,18].

Expression of pOb24 and LPL mRNAs During Adipose Cell Differentiation and in White Adipose Tissue

The emergence of pOb24 and LPL mRNAs was examined during adipose conversion of Ob1771 cells and compared with that of mRNA encoding for adipsin. As

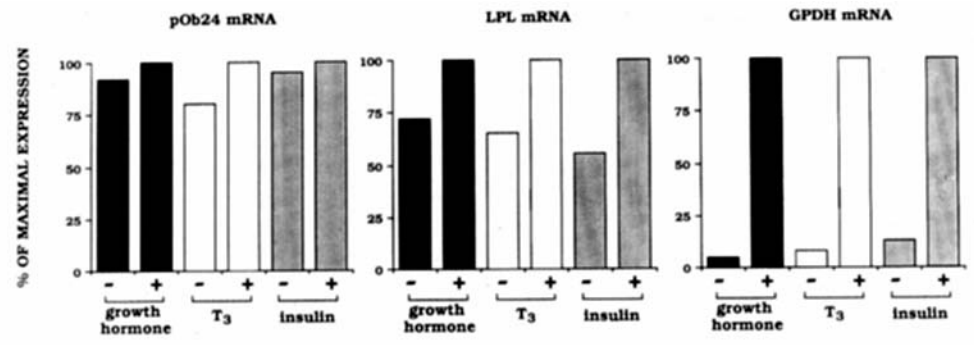

Fig. 2. Differential hormonal requirement for the emergence of pOb24, LPL, and GPDH mRNAs. Ob1771 cells were grown in the presence of 10% (T_3 depleted) fetal bovine serum or 8% (GH depleted) bovine serum. At confluence, the culture media were supplemented (+) or not (−) with 1.2 nM GH, 2 nM T_3, or 17 nM insulin. Eleven days after confluence, 5 μg of poly(A)$^+$ RNAs were prepared and analyzed with respect to pOb24, LPL, and GPDH mRNAs. Results are expressed by taking as 100% the maximal signal obtained for each probe. It is important to stress that a high and similar number of fat cell clusters was observed in the absence or presence of insulin [15], whereas only a few clusters were present when in GH- or T_3-depleted medium. The results are representative of six (for GH and insulin) and two (for T_3) independent experiments performed on different series of cells.

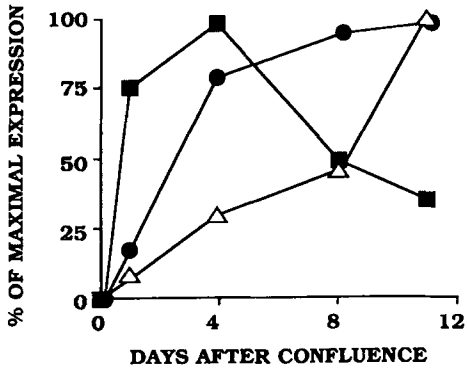

Fig. 3. Expression of pOb24, LPL and adipsin mRNAs during differentiation of Ob1771 cells. Cells were maintained since confluence in 10% fetal bovine serum supplemented with 2 nM T_3 and 17 nM insulin; 5 μg of poly(A)$^+$ RNAs were prepared at the indicated days and analyzed as described previously [5]. Values were normalized to β-actin signals and expressed by taking as 100% the maximal signal obtained for each probe. (■), pOb24 mRNA; (●), LPL mRNAs; (△), adipsin mRNA. The results are representative of three independent experiments performed on different series of cells.

shown in Figure 3, the pOb24 mRNA content attained a maximal level and then decreased rapidly at a time where the content of LPL and adipsin mRNAs was still increasing. This result suggested that pOb24 mRNA behaved as a marker of adipose cell differentiation preferentially expressed in the preadipose state; in contrast, adipsin mRNA was preferentially expressed in the adipose state. The status of LPL gene is intermediary between that of pOb24 and adipsin genes: it was expressed early but increased significantly during differentiation. This conclusion was supported by determining the relative proportion of pOb24 and LPL mRNAs in epididymal and inguinal fat tissues of OF1 mice. Stromal-vascular cells and adipocytes were isolated from adipose

tissue after collagenase digestion and centrifugation; poly(A)$^+$ RNAs from each fraction were then prepared. The Northern-blot presented in Figure 4 indicates that pOb24 mRNA was present at a high level in cells of the stromal-vascular fraction, containing adipose precursor cells [19], and at a low level in the adipocyte fraction. In contrast to pOb24 mRNA, LPL mRNAs were preferentially expressed in the fraction containing mature adipocytes. Therefore, both in vitro (Fig. 3) and in vivo (Fig. 4), these two genes appear to be differentially regulated as a function of differentiation. This assumption was supported by the inhibitory effect of TNF and TGF-β on the accumulation of pOb24 and LPL mRNAs in Ob1771 cells.

Effects of TNF and TGF-β on the Accumulation of pOb24 and LPL mRNAs in Ob1771 Cells

Because TNF and TGF-β were reported to modulate the expression of several differentiation-specific genes whose expression is characteristic of adipocytes [20], the effect of both agents on pOb24 and LPL mRNAs levels was examined.

To study the effects of TNF and TGF-β on the expression of pOb24 and LPL mRNAs, early and late postconfluent cells, i.e., 3-day and 6-day postconfluent cells, respectively, were exposed for 24 h to TNF at a concentration able to cause in 3T3-L1 cells a maximal inhibitory effect on the expression of LPL mRNAs [21] and to TGF-β at

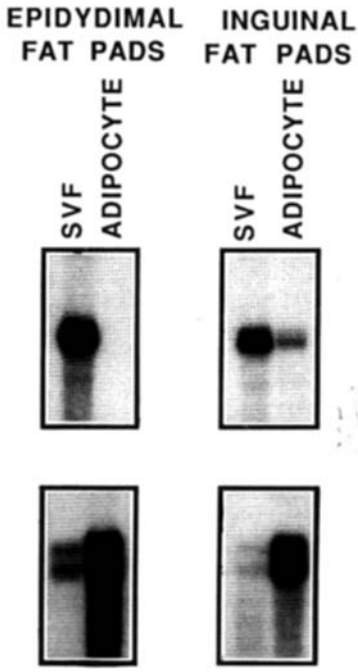

Fig. 4. Expression of pOb24 and LPL mRNAs in white adipose tissue of OF1 mice. Stromal-vascular cells (SVF) and adipocytes from epididymal and inguinal fat tissues of 3-month-old OF1 mice were separated, and poly(A)$^+$ RNAs were prepared as described [7]; 5 μg of poly(A)$^+$ RNA from stromal-vascular cells and adipocytes were quantitated for pOb24 mRNA (upper panel) and LPL mRNAs (lower panel). Similar results were obtained with 4-week-old OF1 mice as well as with 2-month-old and 4-month-old C57 BL/6J mice.

a concentration able to cause a maximal inhibitory effect on the expression of GPDH activity [22]. As shown in Figure 5, pOb24 and LPL mRNAs content were decreased in the presence of TNF or TGF-β in early confluent cells containing a low level of GPDH mRNA; this inhibitory effect was not due to growth resumption as previously described [5,20,22]. In contrast, no decrease in pOb24 mRNA content was observed in late postconfluent cells where the GPDH mRNA content was increased fourfold higher and was indicative of a higher degree of cell maturation. The lack of effect of TNF and TGF-β on pOb24 mRNA content in late cells was not due to a lack of cell sensitivity, as a twofold decrease in GPDH mRNA content could be observed in cells treated for 24 h.

In contrast to pOb24 mRNA, the LPL mRNAs content decreased in TNF and TGF-β treated cells *both* in early and late differentiated Ob1771 cells. This result is at variance with that of Ignotz and Massagué showing that, in 3T3-L1 cells, fully committed adipocytes become refractory to the action of TGF-β [22]; these results, however, are in agreement with recent results published by Torti et al. [23] in indicating that TGF-β could exert, in TA1 cells, an inhibitory effect in adipose gene expression in

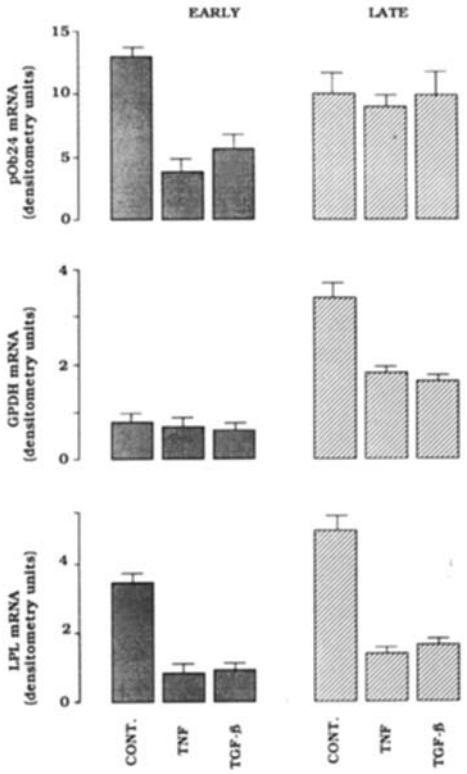

Fig. 5. Effects of TNF and TGF-β on the expression of pOb24 and LPL genes in Ob1771 cells. Early differentiating (3-day postconfluent cells; left panel) and late differentiated Ob1771 cells (6-day postconfluent cells; right panel) were treated with 1.5 nM TNF or 80 pM TGF-β or untreated (CONT.) for 24 h; 5 μg of poly(A)$^+$ RNAs were prepared under each condition, and the same blot was successively hybridized with pOb24, GPDH, and LPL cDNA probes. After autoradiography, the corresponding mRNAs were quantitated by densitometry. Each value is an average of the values from three independent experiments performed on different series of cells; the variation between different experiments is also indicated.

fully differentiated cells; As these authors have discussed, such differences may reflect differences in the cell lines or in the serum used.

The molecular actions of TNF and TGF-β on the expression of pOb24 and LPL genes are unknown, but the results indicate clearly that the two early genes are differentially regulated during adipose conversion. The expression of LPL gene is regulated by both factors in preadipose and adipose cells, whereas the expression of the pOb24 gene is refractory to the inhibitory effects of TNF and TGF-β when cells have expressed GPDH mRNA at a high level, which reflects the entry of the cell in the terminal differentiation process.

In conclusion, early events of adipose cell differentiation involve the expression of at least two sets of markers that show both similar and different pathways of regulation. At present it is premature to claim that pOb24 triggers the terminal differentiation of adipose cells. However, when one compares the kinetics of accumulation of pOb24 transcripts in differentiating adipose cells with that of myogenin, a factor regulating myogenesis, in differentiating L6 rat myoblasts [24], it is tempting to postulate that it could indeed be the case. In that respect, knowledge of the functional properties of the protein encoded by pOb24 mRNA, as a result of sequencing the full length cDNA probe, is a prerequisite now under study in our laboratory. The characterization of new cDNA probes corresponding to very early events appears necessary to determine the critical steps of the differentiation program and the hierachy of the various regulatory genes, as has been shown recently for muscle cell development.

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