Growth hormone regulation of the expression of differentiation-dependent genes in preadipocyte Ob1771 cells

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The adipose conversion of Ob1771 preadipocytes, during exposure to medium containing bovine serum and supplemented with growth hormone, is accompanied by the acquisition of phenotypic markers and the increased accumulation of specific mRNAs. The expression of lipoprotein lipase, and that of unidentified pOb24 and pGH3 mRNAs, are early events which are independent of growth hormone supplementation. By contrast, the late expression of mRNAs encoding for glycerol-3-phosphate dehydrogenase and p422 protein (a myelin-P₂ homologue) and that of glycerol-3-phosphate dehydrogenase activity require the presence of growth hormone. The abundance of β -actin mRNA does not change during differentiation. Runoff transcription by nuclei isolated from untreated or growth hormone-treated cells reveal little or no change in the rates of transcription of pOb24, pGH3 and β -actin mRNAs. By contrast, the transcription rate of the p422 gene increases markedly (> 6-fold) in nuclei of growth hormone-treated cells. However, the p422 mRNA is more abundant than would be predicted by its nuclear transcription alone, suggesting, in Ob1771 cells exposed to growth hormone, that there is a post-transcriptional level of control. These results indicate that the permissive role of growth hormone during adipose cell differentiation is related to terminal events only and that its effects can be seen both at the protein and mRNA level. These results strongly suggest that an increased rate of specific transcription is primarily responsible for the accumulation of mRNAs during exposure to growth hormone.

INTRODUCTION

The adipose conversion of Ob17 cells (Négrel *et al.*, 1978; Gaillard *et al.*, 1985; Vannier *et al.*, 1985) and other preadipocyte cell lines, i.e. mouse 3T3 and 10T1/2 cells (Green & Kehinde, 1976, 1979; Green, 1979), represents a valid model for the development of adipose tissue *in vivo*. In the process of adipose conversion, the cells develop large changes in protein composition (Sidhu, 1979). Among these profound changes are those observed in the content of lipogenic and lipolytic enzymes. These changes take place in 3T3 cells (Spiegelman & Green, 1980; Spiegelman & Farmer, 1982; Spiegelman *et al.*, 1983), in TA1 cells (Chapman *et al.*, 1984) and in Ob17 cells (Amri *et al.*, 1986; Dani *et al.*, 1986) through corresponding changes in mRNA content detected by translation and/or by hybridization using cDNA probes. Some of the mechanisms underlying the extensive differentiation-dependent changes in mRNA concentrations have been recently reported in 3T3 cells for mRNAs encoding for glycerol-3-phosphate dehydrogenase, a myelin P₂-like protein of M_r 13000 specific for adipose tissue (p422 protein), a homologue of serine proteases of M_r 28000 and unidentified proteins (Zezulak & Green, 1985; Bernlohr *et al.*, 1985a). The results obtained in 3T3 and 10T1/2 cells indicate that an increased rate of transcription of specific genes is primarily responsible for the accumulation of these mRNAs during adipose conversion (Cook *et al.*, 1985; Bernlohr *et al.*, 1985b; Chapman *et al.*, 1985; Djian *et al.*, 1985). In addition, changes at the protein and mRNA level are modulated in 3T3 cells in response to insulin and lipolytic hormones (Spiegelman & Green, 1980, 1981;

Weiss *et al.*, 1980) and in TA1 cells in response to glucocorticoids (Chapman *et al.*, 1985). From studies by different authors, it is clear that the process of adipose conversion is under multihormonal control: (i) insulin is merely active as a modulator in the expression of the differentiation programme of Ob17 cells (Djian *et al.*, 1982; Amri *et al.*, 1984) and 3T3-L1 cells (Steinberg & Brownstein, 1982), (ii) tri-iodothyronine exerts a direct and permissive role on the adipose conversion of Ob17 cells (Grimaldi *et al.*, 1982) and (iii) growth hormone exerts a direct and permissive role on the adipose conversion of 3T3-F442A cells (Morikawa *et al.*, 1982; Nixon & Green, 1984), Ob17 and Ob17MT cells (Ailhaud *et al.*, 1983; Grimaldi *et al.*, 1984), and 10T1/2 cells (Nixon & Green, 1984). The growth hormone content of foetal bovine serum has been shown on 3T3-F442A cells to account for about half of the total adipogenic activity determined by measuring the activity of glycerol-3-phosphate dehydrogenase (Nixon & Green, 1984), the remaining half being likely due to low- M_r components (Grimaldi *et al.*, 1982; Löffler *et al.*, 1983). To address the issue of the role of growth hormone in the adipose conversion process of Ob1771 cells, confluent cells have been exposed to bovine serum instead of foetal bovine serum. Under this condition a complete dependence upon exposure to growth hormone appears. In the present study, we have investigated the following points: (a) the role of growth hormone with respect to the differential expression of lipoprotein lipase, glycerol-3-phosphate dehydrogenase and acyl-CoA synthetase during adipose conversion and (b) the growth hormone-related changes in the expression of differentiation-dependent genes.

EXPERIMENTAL

Cell culture

Ob1771 cells (Amri *et al.*, 1986) were plated at 10^3 cells/cm² in 35-mm diameter dishes in the presence of Dulbecco's modified Eagle's medium supplemented with 9% (v/v) bovine serum, 200 units of penicillin/ml, 50 μ g of streptomycin/ml, 33 μ M-biotin and 17 μ M-pantothenate. This medium is termed standard medium. At confluence (day 0, approximately 5 days after seeding), cells are exposed to standard medium supplemented with 17 nM-insulin, 2 nM-tri-iodothyronine and growth hormone as indicated. In all experiments this medium, defined as differentiation medium, was supplemented only between day 0 and day 4 with 100 μ M-3-isobutyl-1-methyl-xanthine. Media were changed every 2 days. When required (see Table 1), growth hormone was removed by rinsing the cells four times at 37 °C (20 min each) with the differentiation medium not supplemented with growth hormone.

Cell-free extracts and enzyme assays

Protocols used for the preparation of cell-free extracts and for assaying lipoprotein lipase (EC 3.1.1.34) and glycerol-3-phosphate dehydrogenase (EC 1.1.1.8) have been described (Amri *et al.*, 1986). Acyl-CoA synthetase (EC 6.2.1.3) and lactate dehydrogenase (EC 1.1.1.27) activities were determined as previously described (Négre *et al.*, 1978). Enzyme activities are expressed in nmol/min per mg of protein. Inter-assay variability did not differ by more than 5%, and variability among mean values from separate dishes never exceeded 5%.

RNA isolation, quantification and analysis

Preparation and Northern-blot analysis of RNAs were performed as previously described (Amri *et al.*, 1986). Results were quantified by densitometry using a Vernon scanning densitometer. All measurements were performed within the linear response of the integrated peaks as a function of immobilized RNA.

Nuclear transcription assays

Nuclei were isolated from $(2-4) \times 10^7$ Ob1771 cells after lysis at 4 °C of the cells with 20 ml of 10 mM-Tris/HCl buffer, pH 7.5, containing 10 mM-NaCl and 3 mM-MgCl₂ (buffer A). The cells were homogenized in a tight-fitting Dounce homogenizer and Nonidet P-40 was added to a final concentration of 0.5%. After 5 min at 4 °C, nuclei were pelleted by centrifugation at 500 g for 5 min, washed once in 20 ml of buffer A containing 0.5% Nonidet P-40 and repelleted by centrifugation at 500 g for 5 min. Nuclei were resuspended in 1 ml of buffer A containing 0.5% Nonidet P-40 and pelleted at 500 g for 5 min. Nuclei were suspended in 200 μ l of 50 mM-Tris adjusted to pH 8.3 with HCl and containing 5 mM-MgCl₂, 0.1 mM-EDTA and 40% (v/v) glycerol. The transcription assay was initiated by adding to 100 μ l of nuclei suspension (approx. 10^7 nuclei) an equal volume of 10 mM-Tris adjusted to pH 8.0 with HCl and containing 5 mM-MgCl₂, 300 mM-KCl, 0.5 mM each of ATP, CTP and GTP and 200 μ Ci of [α -³²P]UTP (760 Ci/mmol) and was performed at 30 °C for 30 min with occasionally gentle shaking. Deoxyribonuclease I (Bethesda Research Laboratories) was added to a final

concentration of 60 μ g/ml. After 10 min at 30 °C, 200 μ l of a solution of 20 mM-Tris adjusted to pH 8.0 with HCl and containing 20 mM-EDTA, 1% SDS and 20 μ g of proteinase K (Sigma) was added and the reaction was continued for 10 min at 42 °C. Yeast RNA (250 μ g/ml) was added as a carrier and RNAs were extracted with 0.5 vol. of water-saturated phenol and 0.5 vol. of chloroform. The aqueous phase was adjusted to 2 M with respect to ammonium acetate and the nucleic acids were precipitated with 2 vol. of ethanol. The pellets were resuspended in 50 mM-Tris/HCl, pH 7.5, containing 10 mM-MgCl₂ and treated with deoxyribonuclease I and proteinase K as described above. RNAs were precipitated with ethanol and resuspended in water.

Nitrocellulose filters (Schleicher and Schüll) containing cDNA were prepared by denaturing 10 μ g of plasmid DNA at 100 °C in 0.1 M-NaOH for 5 min, adding 10 vol. of 1 M-ammonium acetate and spotting the mixture onto the filters (presoaked in 1 M-ammonium acetate) by using a dot-blot apparatus (Bethesda Research Laboratories). The filters were heated at 80 °C for 2 h, and then prehybridized overnight at 65 °C with 0.9 M-NaCl containing 0.09 M-sodium citrate, 50 mM-sodium phosphate, pH 6.5, 0.5% SDS, 0.1% poly(vinyl pyrrolidone), 0.1% Ficoll and 100 μ g of denatured salmon sperm DNA/ml. A sample (10^7 c.p.m.) of the ³²P-labelled reaction products was hybridized to the cDNA-containing filters in 2 ml of the prehybridization buffer at 65 °C for 40 h. After hybridization the filters were washed four times at 65 °C for 2 h with 0.3 M-NaCl containing 0.03 M-sodium citrate and 0.1% SDS, and then treated for 30 min at 37 °C with 10 μ g of pancreatic ribonuclease/ml in 0.3 M-NaCl/0.03 M-sodium citrate. In order to control the yields of hybridization, 2000 c.p.m. of ³²P-labelled single-strand cDNA (obtained by reverse transcription of rabbit globin mRNA) were added to 10^7 c.p.m. of the transcripts and hybridized in parallel with an excess of the corresponding unlabelled double-strand cDNA (prepared after insertion into the pBR322 vector and amplification in *Escherichia coli*). The filters were dried and exposed to Kodak X-AR5 film at -70 °C with an intensifying screen for 12-72 h. The results were quantified by densitometry using a Vernon scanning densitometer as described above.

Materials

[α -³²P]dCTP, tri[9,10-³H]oleoylglycerol, [1-¹⁴C]palmitic acid and nick translation kit were purchased from Amersham International (Amersham, Bucks., U.K.), and [α -³²P]UTP from New England Nuclear (Dreieich, Germany). Culture media and bovine serum were from Gibco (Cergy-Pontoise, France). Guanidinium monothiocyanate was a product of Fluka (Buchs, Switzerland). Globin mRNA was purchased from Bethesda Research Laboratories (Bethesda, MD, U.S.A.). Plasmids pA21, pC8, pAL422, pGH3 and pOb24 have been previously described (Amri *et al.*, 1986) as well as a plasmid pRGAPDH-13 containing a full-length cDNA probe of 1.3 kilobase encoding rat glyceraldehyde-3-phosphate dehydrogenase (Fort *et al.*, 1985). Bovine growth hormone was obtained through the National Hormone and Pituitary Program (NIADDK, Baltimore, MD, U.S.A.).

Fig. 1. Long-term effects in Ob1771 cells of exposure to growth hormone on the activities of enzyme markers for adipose conversion

Cells were grown in the standard medium and transferred at confluence in the differentiation medium in the presence or absence of 1.2 nM-growth hormone as described in the Experimental section. Left: the enzyme activities were determined on 2-day pre-confluent cells (Exp., open bars) and on 12-day post-confluent untreated cells (—, hatched bars) or treated cells (+ solid bars). The 100% values correspond to the activities obtained with growth hormone-supplemented cells: they are expressed in munits/mg and they were equal to 12.3, 8, 233 and 2300 for lipoprotein lipase (A), acyl-CoA synthetase (B), glycerol-3-phosphate dehydrogenase (C) and lactate dehydrogenase (D), respectively. The experiments were performed with two independent series of cells; error bars are $\pm$ changes. Right: micrographs of Ob1771 cells before confluence (a) and after confluence when maintained in the absence (b) or the presence (c) of 1.2 nM-growth hormone. The bar is equivalent to 25 μ m.

RESULTS AND DISCUSSION

Differential effects of growth hormone treatment on the activities of enzyme markers of adipose conversion

Lipoprotein lipase and acyl-CoA synthetase were determined by using a sensitive radioactivity assay. As shown in Fig. 1, acyl-CoA synthetase, in contrast to lipoprotein lipase, was present at detectable levels in exponentially-growing cells. The spectrophotometric assay used for glycerol-3-phosphate dehydrogenase did not allow us to detect the low activity levels present in growing cells. In order to observe a growth hormone dependence, if any, bovine serum was used instead of foetal bovine serum, which contains high concentrations of growth hormone (Nixon & Green, 1983). The histograms in Fig. 1 indicate that the activities of lipoprotein lipase were increased only 2-fold when confluent cells were chronically exposed to a physiological concentration of growth hormone. By contrast, the activities of acyl-CoA synthetase and glycerol-3-phosphate dehydrogenase are increased under the same conditions by 15-fold and 33-fold, respectively. Lactate dehydrogenase, which is not directly related to lipogenesis and which can be used as an internal control, had a similar activity in exponentially-growing cells and in cells treated in the presence or absence of growth hormone. The lack of dependence of growth hormone with respect to the expression of lipoprotein lipase is also illustrated in Fig. 2, which shows that its emergence takes place unimpaired in the absence of growth hormone addition. At later times, the steady-state activities of lipoprotein

lipase were slightly increased in growth hormone-supplemented cells, in agreement with the results of Fig. 1. Thus, a transient effect of growth hormone on the activities of lipoprotein lipase, which could be limited to a period of time during which the enzyme is actually expressed, can be excluded. The late expression of glycerol-3-phosphate dehydrogenase, on the other hand, is clearly dependent upon chronic exposure of the cells to growth hormone. Not surprisingly, lactate dehydrogenase activities remain fairly constant with time in culture whether or not the cells are exposed to growth hormone. No significant change in cell number could be observed if cells were chronically exposed to growth hormone. This is not surprising since, although not shown in this paper, post-confluent mitoses do occur identically whether or not Ob1771 cells are exposed to growth hormone. If we assume that similar events take place with time in Ob1771 and Ob1754 cells (Amri *et al.*, 1986), this lack of growth hormone effect in post-confluent mitoses should indicate that this hormone plays a permissive role in the expression of glycerol-3-phosphate dehydrogenase *per se* but not on the limited proliferation which precedes the enzyme expression.

The dose-response relationships of added growth hormone to the activities of lipoprotein lipase, glycerol-3-phosphate dehydrogenase and lactate dehydrogenase are presented in Fig. 3. Once again the curves show the differential effect of growth hormone on the expression of these enzymes. Lipoprotein lipase is expressed in the absence of growth hormone addition and the 2-fold increase is maximum at 1.2 nM-growth hormone. The

Fig. 2. Development of enzyme markers of adipose conversion in Ob1771 cells exposed or not to growth hormone

Cells were exposed since confluence (day 0) to the differentiation medium supplemented (◆, ●, ■, ▲) or not (◇, ○, □, △) with 1.2 nM-growth hormone. The specific activities of lipoprotein lipase (◆, ◇) and glycerol-3-phosphate dehydrogenase (●, ○) in (a) and the specific activity of lactate dehydrogenase (■, □) and the cell number (▲, △) in (b) were determined at the times indicated as described in the Experimental section. Similar results were obtained in a further independent experiment.

Fig. 3. Dose-response relationship of growth hormone to the activities of enzyme markers of adipose conversion

After growth in the standard medium, confluent cells were maintained in the differentiation medium in the presence or absence of growth hormone as indicated. Lipoprotein lipase (◆), glycerol-3-phosphate dehydrogenase (●) and lactate dehydrogenase (■) activities were determined at day 8 post-confluence. The curves are representative of experiments performed on three independent series of cells.

expression of glycerol-3-phosphate dehydrogenase is also near maximum at 1.2 nM and the half-maximally effective concentration is close to 0.8 nM; the latter concentration is in the range of the growth hormone concentration determined in fetal bovine serum (Gluckman *et al.*, 1981). No significant effect of growth hormone addition was observed on the activities of lactate dehydrogenase.

Taken together, the results of Figs. 1–3 show that the presence of growth hormone is not required for the early expression of lipoprotein lipase but it is required for the late expression of glycerol-3-phosphate dehydrogenase. The low activities of glycerol-3-phosphate dehydrogenase observed in the absence of growth hormone addition were most likely caused by a small percentage of cells that are able to express this enzyme at high activity and to differentiate under this stringent condition (Fig. 1, micrograph *b* compared with micrograph *c*) rather than a very low rate of expression of this enzyme in the whole cell population.

Table 1 describes a more detailed study of the importance of the exposure time to growth hormone for the expression of glycerol-3-phosphate dehydrogenase activity. The data indicate that exposure of confluent cells between day 0 and day 6, when lipoprotein lipase is almost fully expressed whereas glycerol-3-phosphate dehydrogenase is expressed only at a low level (Fig. 2), is sufficient to induce the late expression of glycerol-3-phosphate dehydrogenase activity on day 11 at a level similar to that of cells continuously exposed to growth hormone for 11 days.

Table 1. Effects of exposure time to growth hormone on the activities of enzyme markers of Ob1771 cells

Ob1771 cells were maintained after confluence (day 0) in the differentiation medium alone (day 0, no addition) or supplemented with 1.2 nM-growth hormone for the time indicated. The enzyme activities, expressed in munits/mg of protein, were determined 11 days after confluence; they are quoted as means $\pm$ ranges for two independent experiments.

Exposure time (days) . . .	Activity (munits/mg)					
	0	0-2	0-4	0-6	0-8	0-11
Lipoprotein lipase	7 $\pm$ 1	7.2 $\pm$ 1	8 $\pm$ 1	7.7 $\pm$ 1	9.2 $\pm$ 1.5	12 $\pm$ 1.5
Glycerol-3-phosphate dehydrogenase	10 $\pm$ 5	62 $\pm$ 10	115 $\pm$ 15	265 $\pm$ 20	325 $\pm$ 35	350 $\pm$ 35
Lactate dehydrogenase	2220 $\pm$ 120	2210 $\pm$ 145	2300 $\pm$ 150	2350 $\pm$ 150	2275 $\pm$ 130	2500 $\pm$ 170

Accumulation of specific mRNAs by exposure to growth hormone

Different cloned probes have been used to examine, by Northern blotting, the changes in the content of specific mRNAs which are related to growth hormone exposure.

This examination included two mRNAs which appeared both early and simultaneously with lipoprotein lipase at early times during adipose conversion of Ob1771 cells; these two mRNAs of 6 and 5 kilobases were detected with pOb24 and pGH3 cDNA probes, respectively (our unpublished work). This examination also included other mRNAs which were expressed later in a co-ordinate manner during adipose conversion of 3T3 cells (Spiegelman *et al.*, 1983). These mRNAs encode for glycerol-3-phosphate dehydrogenase (detected with pC8 cDNA probe; Kozak & Birkenmeier, 1983) and for an homologue of the myelin-P₂ protein of *M.* 13000 (detected with pAL422 cDNA probe; Bernlohr *et al.*, 1984). It can be seen in Fig. 4 that the pOb24 and pGH3 mRNAs were detected in early confluent cells, in the presence or absence of growth hormone. These two mRNAs, which encode for unknown proteins, were not detected or detected in very small amounts in exponentially-growing cells. The emergence of pOb24 and pGH3 mRNAs paralleled that observed at the protein level for lipoprotein lipase (Figs. 1-3). The decrease in pOb24 mRNA content is also parallel with that observed at the protein level for this enzyme (Fig. 2). As shown in Fig. 4 and as previously described (Dani *et al.*, 1986), there is a single mRNA species encoding for glycerol-3-phosphate dehydrogenase with a size of 3.5 kilobases. This mRNA species was absent from growing and confluent untreated cells at day 4 and day 10. A similar situation applies for the mRNA of 0.6 kilobase encoding for the myelin P₂-like protein. Clearly, at day 4 post-confluence and in contrast with untreated cells, growth hormone-treated cells did contain low but detectable concentrations of these two mRNAs. At day 10 or at later days (results not shown), the mRNAs encoding for these two proteins were much increased compared with control cells. Similar concentrations of the mRNAs encoding for glycerol-3-phosphate dehydrogenase and myelin P₂-like protein were never reached in control and growth hormone-treated cells, excluding a mere acceleration of adipose conversion by hormone exposure, in agreement with the low and high activities of glycerol-3-phosphate dehydrogenase in late control and growth hormone-treated cells, respectively (Fig. 2). Under all conditions actin mRNA, of 2.2 kilobases in length (detected with a cDNA probe

encoding for mouse β -actin), did not decrease in content during growth and differentiation. This observation is in agreement with that made for actin mRNA during adipose conversion of TA1 cells (Chapman *et al.*, 1985), but it differs from that made with 3T3-F442A cells in which a 2-3-fold decrease has been reported (Spiegelman *et al.*, 1983). The absence of a significant change in the actin mRNA is similar to the situation observed for lactate dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase mRNAs (Dani *et al.*, 1986). The results of Fig. 4 have been analysed by densitometry and quantified after normalization to signals generated by probing with the cDNA probe for mouse β -actin, in order to correct for possible differences in the amounts of poly(A)-containing mRNAs loaded and in transfer efficiency. The contents of pOb24 and pGH3 mRNAs at day 10 were only increased 1.3-fold and 1.4-fold, respectively, when confluent cells are chronically exposed to growth hormone. By contrast, the contents of mRNAs encoding for glycerol-3-phosphate dehydrogenase and myelin P₂-like protein were increased in these cells 50-fold and 33-fold, respectively.

Transcription of isolated nuclei of confluent cells exposed to growth hormone

The relative transcription rates of different genes were measured by using a nuclear transcription assay. Nuclei were isolated from untreated cells at day 18 after confluence or from those treated chronically with 1.2 nM-growth hormone and then incubated with [α -³²P]UTP at 30 °C to allow extension and labelling of nascent RNAs. Under these conditions, transcriptional initiation does not occur (Cox, 1973; Wu & Zubay, 1974); therefore incorporation of UTP into newly synthesized RNA reflects the number of RNA polymerase molecules transcribing the gene of interest at the time of nuclei isolation (McKnight & Palmiter, 1979).

To assay the transcription of specific genes, control experiments of transcription were performed on exponentially-growing cells and on 11-day post-confluent cells maintained in the presence or absence of growth hormone. The results show: (i) an identical time course of UMP incorporation into transcripts synthesized by nuclear RNA polymerases (the incorporation reaches a plateau at 30 min under each condition); this time course is also identical in nuclei used immediately or after storage in liquid N₂ for at least 2 months; (ii) the ³²P-labelled transcripts from nuclei of both exponentially-growing, undifferentiated cells and of differentiated cells

Fig. 4. Long-term effects of exposure to growth hormone on the accumulation of pOb24, pGH3, glycerol-3-phosphate dehydrogenase, pAL422 and actin mRNAs in Ob1771 cells

Ob1771 cells were grown in the standard medium and transferred at confluence to the differentiation medium in the presence or absence of 1.2 nM-growth hormone. At the time indicated, poly(A)-containing RNAs were isolated, electrophoresed, transferred to nitrocellulose and hybridized to the different cDNA probes (see the Experimental section for details). The symbols are: Exp. exponentially-growing cells; -, untreated cells; +, cells treated with growth hormone. The different cDNA probes used (left) and the length of the corresponding mRNAs in kilobases (kb) (right) are indicated. The size of the mRNA bands were determined from standards of ribosomal RNAs and *Hinf*I fragments of digested pBr322 DNA. Similar results were obtained in three further experiments.

treated with growth hormone range in length from 50 to 1000 bases; (iii) nuclei preincubated with α -amanitin at 2 μ g/ml for 10 min before addition of labelled UTP and ribonucleoside triphosphates do not transcribe the pGH3 mRNA and the mRNAs encoding for glycerol-3-phosphate dehydrogenase, actin and glyceraldehyde-3-phosphate dehydrogenase through the action of RNA polymerase II, whereas they transcribe normally ribo-

Fig. 5. Transcriptional induction of specific genes in isolated nuclei of cells exposed to growth hormone

Confluent Ob1771 cells were exposed for 18 days to differentiation medium in the absence (-) or in the presence (+) of 1.2 nM-growth hormone. Nuclei were isolated and [α - 32 P]UTP was incorporated into nascent RNA chains as described in the Experimental section. Labelled RNA (10^7 c.p.m.) was then hybridized for 40 h to 10 μ g of each plasmid DNA immobilized on nitrocellulose filters. After washing and treatment with pancreatic ribonuclease, filters were exposed to X-ray film for 3 days for the radioactive transcripts and only for 12 h for the radioactive single strand control for the hybridization yields (see the Experimental section). The results were normalized to globin signals.

somal 18 S and 28 S RNA through the action of RNA polymerase I; (iv) after completion of the hybridization experiment, no further hybridization of RNA transcripts to a second set of identical filters can be detected. Dot-blot analysis shows no difference for the transcription of the actin gene in nuclei of control and growth hormone-treated cells (Fig. 5). This result is not unexpected, owing to the lack of effect of growth hormone exposure on the actin mRNA content (Fig. 4). The results obtained with the pBr322 DNA plasmid

exclude any significant unspecific hybridization. Slight differences were observed in the transcription activity for the genes corresponding to pOb24, pGH3 and pRGAPDH-13 mRNAs in control and growth hormone-stimulated cells. This contrasts to the large difference (> 6-fold) for the transcription from the pAL422 gene. The actual enhancement is likely to be higher since, in order to detect a pAL422 signal in nuclei of control cells, the 3-day exposure leads to an underestimation of the same signal in nuclei of growth hormone-treated cells. Although cells at different ages in culture have been used for transcription studies and measurement of mRNA concentrations, the results indicate that a good correlation is observed between transcription signals and poly(A)-containing RNA concentrations for the pA21, pGH3 and pAL422 genes. When confluent cells were treated with growth hormone, a lower transcription signal of the pOb24 gene was observed in nuclei from cells at 18 days post-confluence. On the other hand, the pOb24 mRNA content was slightly increased in the same cells but 8 days earlier. These observations could suggest either that the transcription rate decreases in growth hormone-treated cells aging in culture compared with untreated cells or that the stability of the pOb24 mRNA might increase in growth hormone-treated cells. Nevertheless, it is evident that the expression of pOb24 mRNA in Ob1771 cells is not dramatically affected by exposure to growth hormone. In conclusion, the transcription of genes expressed early during adipose conversion, i.e. the pOb24 and pGH3 genes, does not require exposure of Ob1771 cells to growth hormone and it is affected only slightly by chronic treatment with physiological concentrations of this hormone. By contrast, the transcription of a gene expressed late during adipose conversion, i.e. the pAL422 gene, strictly requires exposure of the cells to growth hormone. It seems that an increased transcription of the pAL422 gene is primarily, if not exclusively, responsible for the observed increase in steady-state mRNA level. However changes in mRNA stability cannot be excluded in growth hormone-treated cells since it should be remembered that the regulation by insulin of the expression of the genes encoding for glycerol-3-phosphate dehydrogenase and myelin P₂-like protein clearly involves a stabilization of the corresponding mRNAs in Ob1771 cells (Dani *et al.*, 1986; our unpublished work). In conclusion, this is the first demonstration that terminal adipose differentiation, which is under the direct control of growth hormone, involves enhanced transcription of a specific differentiation-dependent gene.

We thank Drs. P. Fort (Montpellier), A. Kahn (Paris), L. Kozak (Bar Harbor) and D. Lane (Baltimore) for the kind gift of pRGAPDH-13, pGH3, pC8 and pAL422 probes, respectively. The authors are grateful to B. Barhanin for expert technical help and G. Oillaux for efficient secretarial assistance. This work was supported by the Centre National de la Recherche Scientifique (LP 7300).

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