Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 5440–5444, September 1984
Cell Biology

ob17 cells transformed by the middle-T-only gene of polyoma virus differentiate *in vitro* and *in vivo* into adipose cells

(oncogenes/growth requirements/adipogenic factors)

PAUL GRIMALDI*, DOROTA CZERUCKA*, MINOO RASSOULZADEGAN†, FRANÇOIS CUZIN†, AND GÉRARD AILHAUD*

*Laboratory of Biology of Adipose Tissue Development, Centre National de la Recherche Scientifique and †Laboratory of Molecular Genetics of Papovaviruses, Institut National de la Santé et de la Recherche Médicale, Centre de Biochimie, Parc Valrose, Université de Nice, 06034 Nice Cédex, France

Communicated by Howard Green, April 23, 1984

ABSTRACT **Cell lines were derived from ob17 preadipocyte cells by focus formation after transfer of the complete early region of polyoma virus (ob17PY) or of a modified genome encoding only the middle T protein (ob17MT). Both ob17PY and ob17MT cell lines exhibited a high cloning efficiency in agarose medium containing 10% fetal bovine serum. Fully transformed ob17PY cells grew to high saturation densities and did not differentiate *in vitro* and *in vivo*. ob17MT cells and derived subclones did not grow in the absence of serum and were able to differentiate *in vitro* and to give rise *in vivo* to adipose tumors. Among these different clones an inverse relationship was observed in culture between their potentiality to overproliferate at low serum and their potentiality to convert into adipose cells. The expression of enzyme markers of adipose conversion was strictly dependent upon the presence of growth hormone. In addition, the hormonal requirements for differentiation were simpler than those of the original ob17 cells and the adipose conversion could take place in serum-free hormone-supplemented medium.**

The mechanisms controlling cellular growth and differentiation are intimately related. This tight relationship is well illustrated by the behavior in culture of preadipocyte cell lines. Adipose conversion of ob17 (1) and of other preadipocyte cell lines from different origins (2–6) proceeds after confluence with the formation of fat cell clusters. These clusters arise from a limited number of post-confluent mitoses of committed cells (7, 8). However, no differentiation occurs when ob17 cells are forced to cycle in the presence of mitogens such as prostaglandin $F_{2\alpha}$ (9) or fibroblast growth factor (10). Genetically defined alterations of the growth control mechanisms can be induced by transferring cloned oncogenes into cells in culture. In several instances, this was shown to interfere with the adipose cell differentiation: murine sarcoma viruses block adipocyte differentiation of bone marrow preadipocytes and of 3T3-L1 cells (11). A similar block was observed in 3T3-L1 cells transformed by simian virus 40 (G. Serrero-Davé, personal communication). Recently two main groups of oncogenic determinants with different effects on cell growth regulation have been recognized (12, 13, 29, 30). A first group includes the gene for the large T protein of polyoma virus, the Ela proteins of adenoviruses, and the *myc* oncogenes. All of these genes confer on cells in culture the ability to grow in the presence of low concentrations of serum and, in the case of rodent primary embryonic cells, an unlimited potential for growth in culture (14, 15). A second group includes the genes encoding the polyoma virus middle T (*pmt*), the adenovirus Elb proteins, and the *ras* cellular oncogenes. They confer on already immortalized cells the terminal transformation properties and tumorigenicity.

Experimental systems provided by cells expressing only one type of oncogene have the additional interest for the study of differentiation that only part of the transformed phenotype is expressed or, in several instances, the transformed phenotype is expressed in a conditional manner. For instance, rat fibroblast cells transformed with the *pmt* gene (MTT lines) exhibit fully transformed properties at high serum concentration but revert to a normal growth control and cell morphology in low serum medium (12). As complete transformation usually interferes with cell differentiation (16), such partially, or conditionally, transformed cells may still maintain the expression of differentiated properties.

In this study, we have examined the properties of cell lines derived from the preadipocyte ob17 line after transfer of the *pmt* gene of polyoma virus. By contrast with cells transformed with the wild-type virus, these cells could still undergo adipose conversion under the conditions in which a partially normal growth control was maintained in culture (low serum medium) but interestingly, differentiation did not exhibit in this case the serum requirements previously observed for the original cell line (10).

MATERIALS AND METHODS

Cell Culture. Cultures were carried out, as described (1), with media supplemented with 10% (ob17 cells) or 3% fetal bovine serum (transformed ob17 cells) (Seromed, Munich, F.R.G.). The medium was changed every other day and the cells were subcultured every 4–6 days. Cell enumeration was performed with a Coulter Counter.

Cell Transformation. The ob17 cell line was seeded at a density of 2×10^5 cells per 35-mm dish in Dulbecco's modified Eagle's medium (DME medium) supplemented with 10% serum. After 24 hr, the cells were fused as described (17) with bacterial protoplasts of *Escherichia coli* bacteria carrying plasmid pPY1 encoding a complete wild-type viral genome (17) or pPyMT1 carrying only the gene encoding middle T (*pmt*) (18). Three days later, cells were trypsinized and plated at a density of 10^5 cells per 60-mm dish in DME medium containing 10% serum. Foci of transformed cells were observed after 3 wk of culture. The efficiency of transformation was in the range of 10^{-4} foci per cell with both plasmids. In each case, cells from one focus of cells were selected and designated ob17PY1 and ob17MT1, respectively. Nine subclones were derived from isolated colonies of ob17MT1 cells and designated ob17MT11 to ob17MT19.

Growth Experiments. Exponentially growing cells were harvested after tryptic dissociation [0.25% trypsin/0.03% EDTA in Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline at pH 7.4 (P_i/NaCl)], suspended in DME medium containing 10% serum, and then plated at a density of 8×10^3 cells per 35-mm dish. Fifteen hours later, cells were washed three

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GPDH, glycerol-3-phosphate dehydrogenase; T3, triiodothyronine.

times for 30 min at 37°C with DME medium and then incubated in DME medium containing different concentrations of serum. Colony formation in suspension was carried out at a density of 250–1000 cells per ml of DME medium as described (1). Plates were incubated for 2 wk without further feeding, and colonies were scored under a microscope.

Adipose Conversion. Cells were inoculated in multi-well plates (Linbro) at a density of 10^4 cells per 25-mm diameter well and grown in DME medium containing 10% (ob17 cells) or 3% serum (transformed ob17 cells). One day before confluence, cells were washed three times as described above and shifted to DME medium/F12 medium, 1:1 (vol/vol), supplemented with 0.85 μM insulin/50 nM transferrin/0.15 nM triiodothyronine (T3)/0–12 nM growth hormone (defined as serum-free medium)/0–10% serum. This medium was changed every other day. Enzyme activities were determined at least in duplicate as described (1). Protein was estimated by the method of Lowry *et al.* (19).

RESULTS

Growth Behavior. Exponential ob17MT1 cells grown in the presence of 3% serum exhibited a fibroblastic appearance identical to that of ob17 cells (see Fig. 3*E*). ob17 cells and ob17MT1 cells did not grow in the absence of serum (Fig. 1 *A* and *B*). Growth of ob17 cells only became significant above 1% serum with a doubling time of 28 hr. As expected, the growth rate of ob17MT1 cells was higher at any serum concentration than that of original cells. In contrast to ob17MT1 cells, ob17PY1 cells (Fig. 1*C*) did show a significant growth in the absence of serum. At 0.1% serum, the doubling time decreased to 20 hr and did not change much by increasing the serum concentration up to 3%.

ob17MT1 cells and the subclones ob17MT11 to ob17MT19 were tested for their ability to divide in the absence of anchorage: in each case, the colony-forming ability in agarose medium at 10% serum was 100% and the resulting colonies were >500 cells per colony, whereas the original ob17 cells had a limited ability to divide (minute colonies of 2–8 cells). Immunoprecipitation experiments (Fig. 2) indicated the presence of the expected T-antigen species in ob17MT13 cells—namely, the M_r 56,000 middle T protein. As expected

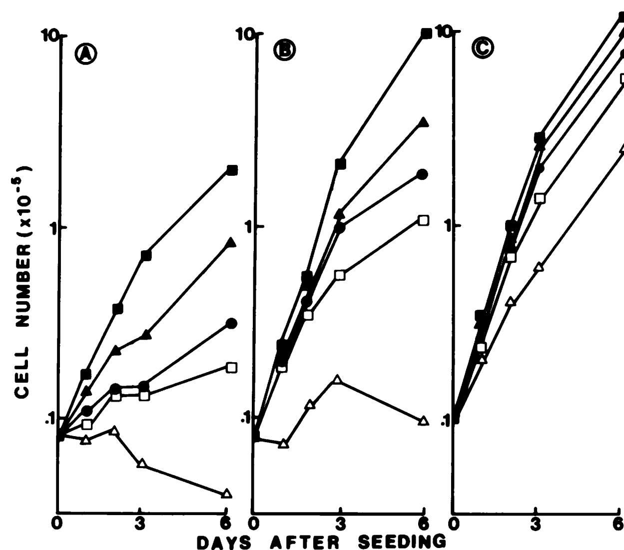

FIG. 1. Growth curves of original and transformed ob17 cells. Cells were plated in 10% serum and shifted (day 0) in media at different serum concentrations. △, 0%; □, 0.1%; ●, 0.3%; ▲, 1%; ■, 3%. Each point represents the average of three separate determinations. Media were changed at days 2 and 4. (*A*) ob17; (*B*) ob17MT1; (*C*) ob17PY1. No significant cell death or detachment was observed in ob17MT1 and ob17PY1 cultures at any serum concentration.

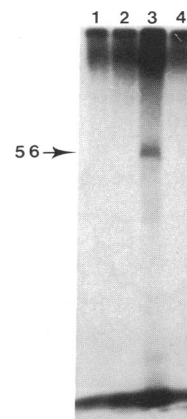

FIG. 2. Immunoprecipitation analysis of the polyoma middle T protein synthesized in ob17MT13 cells. Before labeling, the cells were seeded at a density of 10^5 cells per 10-cm dish and grown for 2 days in the presence of 3% serum. Labeling was for 2 hr in the presence of 250 μCi (1 Ci = 37 GBq) of [^{32}P]orthophosphate in 1 ml of Tris-buffered isotonic saline solution. Extracts were prepared and analyzed after immunoprecipitation with anti-polyoma T-antigen antibodies from rat ascites fluid (20) as described in ref. 17. The indicated electrophoretic mobility corresponding to an apparent M_r of 56,000 (shown as $M_r \times 10^{-3}$) was deduced from the position of protein markers on the same gel. Lane 1, ob17, immune serum; lane 2, ob17 cells, control serum; lane 3, ob17MT13 cells, immune serum; lane 4, ob17MT13 cells, control serum.

for polyoma virus-transformed mouse cells (see ref. 17 for review), immunoprecipitation after ^{32}P labeling *in vivo* evidenced the presence of obMPY1 cells of both the middle T and the large T proteins and also of a truncated form (M_r 65,000) of the large T protein (data not shown).

The tumorigenicity of original ob17 cells and transformed cells (ob17MT1, ob17MT12, ob17MT13, ob17MT15, ob17-MT18, and ob17PY1) was examined by injecting in each case a minimum of five 6-week-old male nude mice with 10^5 to 2×10^5 cells. Under these conditions, ob17 cells did not give rise to tumors after 4 months. In contrast, as illustrated in Fig. 3 *A* and *B*, 2×10^5 injected ob17MT1 cells gave rise within 3 wk to fibrosarcomas in which mature adipocytes were clearly recognized. ob17PY1 cells also gave rise to tumors, but these remained undifferentiated fibrosarcomas.

Adipose Conversion. Original ob17 cells are known to convert at high serum concentrations into adipose cells with a high frequency (1). The adipose conversion was important at 10% serum but not apparent on a morphological basis below 1% serum (Fig. 3 *C* and *D*), whereas ob17 cells lost viability below 0.3% serum. In contrast, ob17MT1 cells, which were able to proliferate at low serum concentrations (Fig. 1*B*), had also the ability to convert significantly into adipose cells in serum-free medium after growth in the presence of 3% serum. The adipose conversion of ob17 cells has been shown to require the presence of T3 (21) and also, as originally shown for 3T3-F442A preadipocyte cells (22), the presence of growth hormone. The adipogenic effect of growth hormone on ob17 cells was observed in bovine plasma- or in bovine serum-supplemented medium (23) but, in addition to this hormone, other factors are required for differentiation (10, 24). It is of interest to see that ob17MT1 cells, when compared to ob17 cells, remained fully viable in serum-free medium (Fig. 3*F*) and could convert into adipose cells provided there is an addition of physiological concentrations of growth hormone (Fig. 3*G*). However, increasing the serum concentration to 10% abolished adipose conversion (Fig. 3*H*). Cell overgrowth with the appearance of foci occurred under this condition. These observations were assessed on a quantitative basis by using glycerol-3-phosphate dehydrogenase (GPDH) as a late differentiation marker (25). As

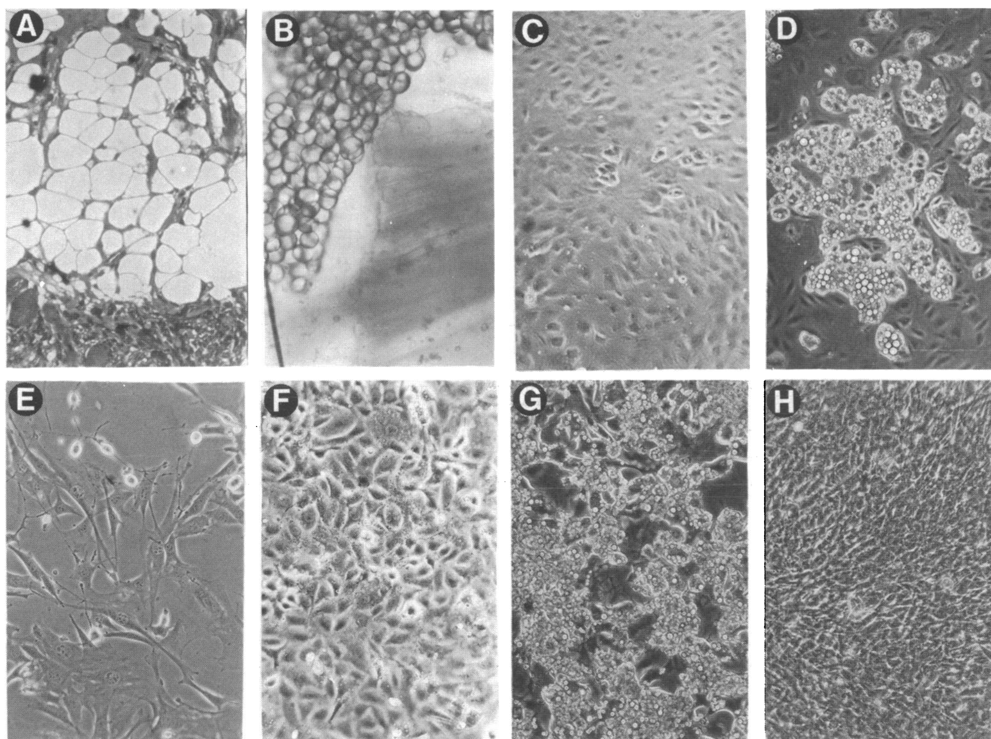

FIG. 3. Adipose conversion of ob17MT1 cells *in vivo* and *in vitro*. (*A*) Semi-thin section of the tumor after treatment with Boin's fixative; (*B*) explant of adipose tumor from ob17MT1 cells (3 wk after injection); (*C* and *D*) 14-day post-confluent ob17 cells maintained in serum-free medium supplemented with 0.3% serum (*C*) or with 10% serum (*D*); (*E*) exponentially growing ob17MT1 cells; (*F*) 10-day post-confluent ob17MT1 cells maintained in serum-free medium deprived of growth hormone; (*G*) the same cells as in *F* but in the presence of 1.2 nM growth hormone; (*H*) the same cells as in *G* supplemented with 10% serum. (*A* and *C–G*, ×240; *B*, ×120.)

shown in Fig. 4*A*, significant levels of GPDH activity were attained for ob17 cells at and above 1% serum. Since fetal bovine serum contains significant amounts of growth hormone, the adipogenic effect of the latter was no longer visible above 3% serum. On the other hand, ob17MT1 cells showed high GPDH activity levels when maintained in serum-free medium. The expression of GPDH was strictly dependent upon the presence of growth hormone. The activity levels began to decrease above 0.1% serum and became negligible at 3% serum. This result was in agreement with the important overgrowth of ob17MT1 cells observed at this serum concentration (Fig. 1*B*).

The expression of GPDH, its dependency upon the addition of growth hormone, and its disappearance at high serum

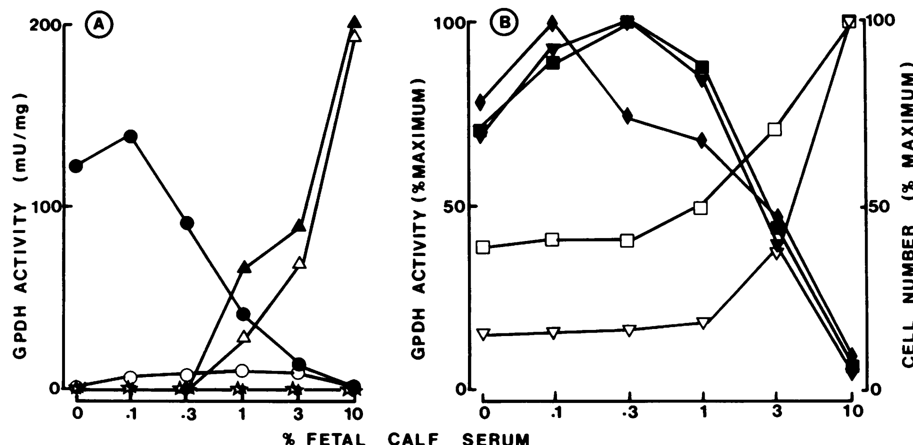

FIG. 4. GPDH activity levels of ob17, ob17PY1, and ob17MT1 cells and related subclones as a function of serum concentration in the culture medium. (*A*) ob17 cells (▲, △), ob17MT1 cells (●, ○) and ob17PY1 cells (★, ☆) were seeded and cultured; at confluence, cells were shifted in serum-free medium containing (▲, ●, ★) or not containing (△, ○, ☆) 12 nM growth hormone and increasing concentrations of fetal bovine serum, as indicated. GPDH activities were determined 10 days later. (*B*) ob17MT12 cells (◆), ob17MT13 cells (▼), and ob17MT18 cells (■) were cultured as in *A* and shifted at confluence in serum-free medium containing 12 nM growth hormone and increasing concentrations of fetal bovine serum, as indicated. GPDH activities were determined at day 8 (ob17MT12 and ob17MT13 cells) and at day 12 (ob17MT18 cells). One-hundred percent corresponds in milliunits/mg to 452 for ob17MT12 cells, 667 for ob17MT13 cells, and 1841 for ob17MT18 cells. Growth hormone showed no mitogenic effect on ob17 and ob17MT1 cells grown in serum-free hormone-supplemented medium. The values reported are the mean of duplicate determinations from two separate dishes. Each curve is representative of at least three separate experiments. The number of ob17MT13 cells (▽) and of ob17MT18 cells (□) was determined at day 9. One-hundred percent corresponds to 3.45×10^5 cells per cm^2. Each point is the mean of triplicate dishes from a single experiment.

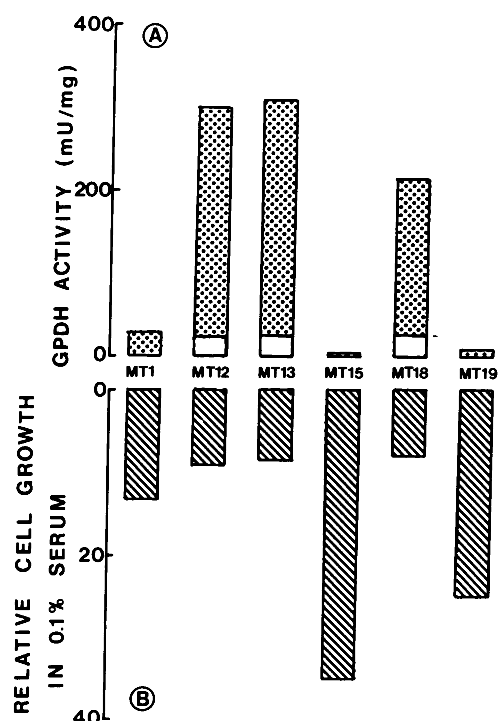

FIG. 5. Inverse relationship of ob17MT1 and derived subclones between their ability to differentiate and their ability to grow at a low serum concentration. (*A*) Cells were cultured and shifted at confluence in serum-free medium in the absence (white bars) or in the presence (dotted bars) of 1.2 nM growth hormone. GPDH activities (milliunits/mg) were determined 9 days later. (*B*) Cells were seeded at day 0 at a density of 8×10^3 cells per 35-mm dish and treated as described in the legend to Fig. 1. Values determined in triplicate are ratios of cell number at day 6 to that at day 0.

concentrations were extended to clones ob17MT12, ob17-MT13, and ob17MT18. The decrease in the specific activities of GPDH began to take place when the increase in cell overgrowth just appeared, and the expression of GPDH was almost abolished at 10% serum (Fig. 4*B*). Among subclones of ob17MT1 cells exhibiting different abilities to proliferate in low serum medium, those growing at the fastest rates were the least susceptible to undergo adipose conversion, as shown by the expression of GPDH (Fig. 5). This inverse relationship between the growth and the differentiation potentials is also well illustrated in Fig. 4*B* for clones ob17MT13 and ob17MT18. The lack of expression of GPDH when cell overgrowth occurred was extended to other enzyme markers of adipose conversion such as lipoprotein lipase and acid: CoA ligase (acyl-CoA synthetase) (Table 1), whereas lactate dehydrogenase, not directly related to lipogenesis, remained unchanged. Thus, it is not surprising that ob17PY1 cells, able to grow significantly in the absence of serum (Fig. 1*C*), could not convert into adipose cells *in vitro* when maintained in serum-supplemented or in serum-free medium, in the absence or in the presence of growth hormone (Fig. 4*A*).

DISCUSSION

Cell lines were derived from the mouse preadipocyte line ob17 by focus selection after transfer of plasmids that contained either of the wild-type early region polyoma virus genome (ob17PY1) or a modified genome encoding only the middle T protein (18) (ob17MT1). Growth properties of ob17MT1 cells were in general agreement with previous observations made on rat fibroblast lines (12). This similarity suggests that these properties are typical of cells expressing only middle T. Both in the rat and the mouse cells, the presence of the middle T protein resulted in the conditional expression of the transformed phenotype, dependent upon the serum concentration of the culture medium. The *wt*-transformed and *pmt*-transformed lines exhibited significant and interesting differences in their differentiation potential *in vitro* and in the type of tumors induced *in vivo*.

Both ob17PY1 and ob17MT1 cells could grow up to high cell densities in medium supplemented with 10% serum. By contrast, in medium containing either no serum or 0.1% serum, only ob17PY1 cells could still grow past confluence, as expected from the known serum independence of polyoma transformants (12, 26). Under these conditions, ob17MT1 cells could grow (unlike the original ob17 cells), but only up to the monolayer stage. Growth rates measured at low serum medium were somewhat different among subclones (Fig. 5*B*). It would be of interest to check whether these differences correlate with the level of expression of the middle T protein. However, quantitative estimates of the latter are difficult to obtain, in view of the low level of expression characteristic of all polyoma virus-transformed lines (17). The partially transformed state of ob17MT1 cells in the presence of low concentrations of serum did not prevent their differentiation into mature adipocytes. As expected from their highly transformed phenotype, neither ob17MT1 cells in high serum medium nor ob17PY1 cells at any serum concentration could differentiate in culture.

As described previously for rat cell lines transformed by *pmt* (18), ob17MT1 cells produced tumors in nude mice as efficiently as ob17PY1 cells, whereas the original ob17 cells gave rise only to fat pads that did not develop into tumors (27). A striking difference was revealed, however, by the examination of the tumors: ob17PY1 cells evolved into undifferentiated fibrosarcomas but ob17MT1 cells and subclones produced tumors containing fully mature adipose cells.

It is of interest to notice that the expression of the *pmt* gene led to the disappearance of serum requirement for adipose conversion. It must be recalled that growth of ob17 cells can be supported by a medium supplemented with a combination of four factors: insulin, transferrin, fibroblast growth factor, and a fraction of apparent M_r 24,000–28,000

Table 1. Activity levels of enzyme markers in proliferative or nonproliferative ob17MT1 cells

Days relative to confluence	Serum added after confluence	Growth hormone	GPDH	Acid:CoA ligase	LPL	LDH
−2		−	0	0.7	0	4800
+12	−	−	0	1.05	0	5500
+12	−	+	120 (3)	14 (0.5)	6.3	5830
+12	+	−	0	0.64	0	6150
+12	+	+	<0.5	0.8	0	6200

Activity levels of enzyme markers in proliferative or nonproliferative ob17MT1 cells. Cells were grown in the presence of 3% serum and, where indicated, maintained after confluence in the absence or in the presence of 3% serum. Growth hormone (12 nM) was added or not added, as indicated. Values obtained for cells treated with bromodeoxyuridine during the exponential phase of growth (1) are given in parentheses. Enzyme activities were determined at the indicated days and are expressed in milliunits/mg. LPL, lipoprotein lipase; LDH, lactate dehydrogenase.

partially purified from rat submaxillary glands. Addition of serum is required for differentiation (10). The serum-free medium able to support the growth of ob17MT1 cells and related clones is qualitatively but not quantitatively similar to that of ob17 cells. In contrast to the latter cells, the mere addition of T3 and growth hormone to this medium is sufficient for their adipose conversion. Thus, these cell lines, as already observed for the 1246 preadipocyte cell line (28), could undergo adipose differentiation in serum-free medium. These reduced requirements for growth and differentiation might well be related, at least in part, with the previous observation by Kaplan and Ozanne (26) that rat MTT transformants secrete growth factors of the transforming growth factor type. In that respect, one cannot exclude that factors active on growth might be the same as those active on differentiation. These cell lines should allow a more precise determination of the changes in growth factor requirements induced by a variety of oncogenes such as the *plt* gene of polyoma virus (large T protein) or the cellular *myc* and *ras* oncogenes. These cell lines offer also, by slight changes in the mitogenic potency of the medium, the opportunity to study the fine balance between the progression in the cell cycle and the susceptibility of precursor cells to convert into adipose cells.

Thanks are due to Dr. G. Serrero-Davé for a kind gift of bovine growth hormone and to Dr. L. Austin for careful reading of the manuscript. The skillful assistance of Mrs. B. Barhanin and G. Oillaux is gratefully acknowledged. This work has been supported by grants from the Ministère de l'Industrie et de la Recherche (Grants 82-V-0010, 82-V-1372, and 83-V-0093), from the Institut National de la Santé et de la Recherche Médicale (CRE 831006), from the Association pour le Développement des Recherches sur le Cancer, and from the Fédération Nationale des Centres de Lutte contre le Cancer.

1. Négrel, R., Grimaldi, P. & Ailhaud, G. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6054–6058.
2. Green, H. & Kehinde, O. (1975) *Cell* **5**, 19–27.
3. Green, H. & Kehinde, O. (1976) *Cell* **7**, 105–113.
4. Darmon, M., Serrero, G., Rizzino, A. & Sato, G. (1981) *Exp. Cell Res.* **132**, 313–327.
5. Forest, C., Grimaldi, P., Czerucka, D., Négrel, R. & Ailhaud, G. (1983) *In Vitro* **4**, 344–354.
6. Hiragun, A., Sato, M. & Mitsui, H. (1980) *In Vitro* **15**, 685–693.
7. Pairault, J. & Green, H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 5138–5142.
8. Djian, P., Grimaldi, P., Négrel, R. & Ailhaud, G. (1982) *Exp. Cell Res.* **142**, 273–281.
9. Négrel, R., Grimaldi, P. & Ailhaud, G. (1981) *Biochim. Biophys. Acta* **666**, 15–24.
10. Gaillard, D., Négrel, R., Serrero-Davé, G., Cermolacce, C. & Ailhaud, G. (1984) *In Vitro* **20**, 79–88.
11. Greenberger, J. S., Davisson, P. B. & Gans, P. J. (1979) *Virology* **95**, 317–333.
12. Rassoulzadegan, M., Cowie, A., Carr, A., Glaichenhaus, N., Kamen, R. & Cuzin, F. (1982) *Nature (London)* **300**, 713–718.
13. Ruley, H. A. (1983) *Nature (London)* **304**, 602–606.
14. Rassoulzadegan, M., Naghashfar, Z., Cowie, A., Carr, A., Grisoni, M., Kamen, R. & Cuzin, F. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 4354–4358.
15. Mougneau, E., Lemieux, L., Rassoulzadegan, M. & Cuzin, F. (1984) *Proc. Natl. Acad. Sci. USA*, in press.
16. Holtzer, H., Bichl, J., Yeoh, G., Meganathan, R. & Kaji, A. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 4051–4055.
17. Rassoulzadegan, M., Gaudray, P., Canning, M., Trejo-Avila, L. & Cuzin, F. (1981) *Virology* **114**, 489–500.
18. Treisman, R., Novak, U., Favalaro, J. & Kamen, R. (1981) *Nature (London)* **292**, 595–600.
19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 173–181.
20. Goldman, E. & Benjamin, T. L. (1975) *Virology* **66**, 372–384.
21. Grimaldi, P., Djian, P., Négrel, R. & Ailhaud, G. (1982) *EMBO J.* **6**, 687–692.
22. Morikawa, M., Nixon, T. & Green, H. (1982) *Cell* **29**, 783–789.
23. Ailhaud, G., Amri, E., Cermolacce, C., Djian, P., Forest, C., Gaillard, D., Grimaldi, P., Khoo, J., Négrel, R., Serrero-Davé, G. & Vannier, C. (1983) *Diabete Métab.* **9**, 125–133.
24. Nixon, T. & Green, H. (1984) *Endocrinology*, in press.
25. Forest, C., Czerucka, D., Grimaldi, P., Vannier, C., Negrel, R. & Ailhaud, G. (1983) in *The Adipocyte and Obesity: Cellular and Molecular Mechanisms*, eds. Angel, A., Hollenberg, C. H. & Roncari, D. A. K. (Raven, New York), pp. 53–64.
26. Kaplan, P. L. & Ozanne, B. (1982) *Virology* **123**, 372–380.
27. Vannier, C., Gaillard, D., Grimaldi, P., Amri, E., Djian, P., Cermolacce, C., Forest, C., Etienne, J., Négrel, R. & Ailhaud, G. (1984) in *Recent Advances in Obesity Research IV*, eds. Hirsch, J. & Van Italie, T. (Libbey, London), in press.
28. Serrero, G. & Khoo, J. C. (1982) *Anal. Biochem.* **120**, 351–359.
29. Houweling, A., Van den Elsen, P. J. & Van der Eb, A. J. (1980) *Virology* **105**, 537–550.
30. Land, H., Parada, L. F. & Weinberg, R. A. (1983) *Nature (London)* **304**, 596–602.