A preadipose 3T3 cell variant highly sensitive to adipogenic factors and to human growth hormone

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SUMMARY

We describe a new Swiss 3T3 preadipose clone, 3T3-F442A/C4, which shows higher sensitivity to serum adipogenic factors and to human growth hormone as compared to other 3T3 preadipose clones. The 3T3-F442A/C4 clone exhibited several characteristics different from the parental 3T3-F442A cells, mainly a high extent of adipose conversion under culture conditions that are non-adipogenic for the parental cells. The 3T3-F442A/C4 cells are not committed to undergo adipose differentiation, since they do not differentiate into adipocytes under serum-free or low-serum culture conditions, unless adipogenic factors or growth hormone are added into the culture medium. The 3T3-F442A/C4 cells showed 1.5- to 3.6-fold higher sensitivity to serum adipogenic factors and 5- to 6-fold higher sensitivity to human growth hormone as compared to the 3T3-F442A cells. The 3T3-F442A/C4 variant clone also differed from the parental clone by having a shorter population doubling time, an increased saturation density, and lower activity levels in some biochemical markers of adipose differentiation. On the other hand, the new variant clone has a similar proportion of cells susceptible to become adipocytes, and a similar response to insulin as compared to the parental cells. Our results show that the 3T3-F442A/C4 cells represent a new 3T3 preadipose clone that could be useful as a bioassay to evaluate growth hormone activity, as well as to purify and characterize hormones, adipogenic factors, and those compounds that affect mammalian adipogenesis.

Key words: 3T3 adipocyte, adipogenic factor, human growth hormone

INTRODUCTION

The 3T3 preadipocytes are a useful model to study adipose cell differentiation and its regulation by hormones and growth factors (Watt, 1991). The 3T3-F442A cells exhibit high ability to differentiate into adipocytes when they become quiescent under appropriate culture conditions (reviewed by Ailhaud, 1990; Kuri-Harcuch and Castro-Muñozledo, 1984). The adipose differentiation of 3T3-F442A cells is strictly dependent upon adipogenic factors found in the serum added to the culture medium (Kuri-Harcuch and Green, 1978). Sera from different animal species vary in their adipogenic activities: fetal bovine serum (FBS) shows the highest adipogenic activity, whereas serum from adult domestic cat does not support adipose differentiation in these cells due to its lack or low concentrations of adipogenic factors (Kuri-Harcuch and Green, 1978). Growth hormone (GH) promotes 3T3-F442A adipogenesis and is responsible for one third to one half of the adipogenic activity in FBS (Nixon and Green, 1984). Other adipogenic factors found in the serum are still poorly known, although a great effort has been devoted in our laboratory (Kuri-Harcuch et al., 1985; Ramírez-Zacarias et al., unpublished), and in others (Grimaldi et al., 1982; Hauner and Löffler, 1986; Zaitsu and Serrero, 1991), to purifying these molecules and to characterizing their role on mammalian adipogenesis.

We have isolated a group of 3T3-F442A variant clones by mutagenesis with nitrosoguanidine and/or selection with high retinoic acid concentration (Salazar-Olivo et al., 1994). The clone 3T3-F442A/C4 is a spontaneous variant obtained directly by selection of 3T3-F442A cells with high retinoic acid concentration. This variant clone showed significant differences with respect to the parental cell line: high resistance to retinoic acid cytotoxicity, higher colony forming efficiency (Salazar-Olivo et al., 1994), and a high capacity to undergo adipose conversion under culture conditions that are non-adipogenic for the parental cells. In this report, we studied the response of these cells to serum adipogenic factors and to other agents that modulate adipose phenotype expression; we also determined some of the kinetic and biochemical characteristics of this new 3T3 clone. Our results show that the variant clone 3T3-F442A/C4 exhibited differences in metabolism and cell growth as compared to the parental 3T3-F442A cells, remarkably a higher sensitivity to adipogenic factors from FBS and to human GH. Therefore, the new 3T3-F442A/C4 preadipose clone could be useful as a bioassay with high sensitivity to evaluate the activity of GH as well as for the purification and characterization of other serum adipogenic activities.
MATERIALS AND METHODS

Materials
We obtained fetal bovine serum (FBS) from Microbiological Associates (Bethesda, MD), calf serum from HyClone Labs Inc. (Logan, UT), and cat serum from Colorado Serum Co. (Denver, CO). Human growth hormone (hGH) was obtained from the National Hormone and Pituitary Program (Baltimore, MD). Epidermal growth factor (EGF) was from IMCERA Bioproducts Inc. (Terre Haute, IN). Insulin, d-biotin, bovine serum albumin (BSA), all-trans-retinoic acid, and Oil Red O were from the Sigma Chemical Co. (St Louis, MO). All other reagents were analytical grade.

Obtaining of 3T3-F442A/C4 variant
The 3T3-F442A/C4 variant clone was isolated as a spontaneous retinoic acid-resistant 3T3-F442A subpopulation as previously described (Salazar-Olivo et al., 1994). In brief, 3T3-F442A preadipocytes were inoculated at 100 cells per 60 mm dish in medium supplemented with 10% calf serum. After three days, cultures were refed with medium containing 5% calf serum and 0.1 mM retinoic acid. Individual colonies were isolated ten days after and tested for their resistance to retinoic acid cytotoxicity and for their ability to undergo adipose differentiation (Salazar-Olivo et al., 1994).

Cell culture conditions
Preadipocytes were inoculated in Corning or Linbro tissue culture dishes at 7×10^3 cells/cm^2 in Eagle’s medium modified by Dulbecco and Vogt (DMEM) supplemented with 5% (v/v) calf serum (adipogenic medium) or with 4% (v/v) cat serum (non-adipogenic medium), both containing 5 µg/ml insulin and 1 µM d-biotin (Kuri-Harcuch et al., 1978). Medium was changed every other day. Four serum adipogenic activities were obtained by ion exchange chromatography of FBS: fraction containing bovine growth hormone (FGH), adipogenic fraction 1 (AF1), adipogenic fraction 2 (AF2), and adipogenic fraction 3 (AF3) (Ramírez-Zacarías et al., unpublished) and they were tested in preconfluent cultures refed with DMEM containing 2% (v/v) cat serum, 0.2% calf serum, 5 µg/ml insulin, 1 µM d-biotin, 5 µg/ml transferrin, 2 nM triiodothyronine, 40 µM 2-mercaptoethanol, and 0.01 ng/ml EGF (definitive medium) (Nixon and Green, 1984). Insulin concentration was assayed in the same medium containing 5% FBS. For experiments in serum-free medium, preconfluent cultures were washed three times with serum-free DMEM, refed with DMEM-F12 (1:2, v/v) containing 1 mg/ml BSA, 10 µg/ml transferrin, 0.15 nM triiodothyronine, 50 ng/ml EGF, 5 µg/ml insulin and 1 µM d-biotin, and maintained during 7-10 days without further changes of medium. All cultures were incubated in a humidified 90% air, 10% CO2 atmosphere at 37°C.

Enzyme assays and quantitation of adipose differentiation
Cell extracts were prepared as previously reported (Kuri-Harcuch and Green, 1977). Glycerophosphate dehydrogenase (EC 1.1.1.8) and malic enzyme (L-malate; NADP oxireductase (decarboxilating), EC 1.1.1.40) were assayed as described (Castro-Muñozledo et al., 1987). Glycerophosphate acyltransferase (acyl-CoA:L-glycerol-3-phosphate O acyltransferase, EC 2.3.1.15) activity was determined as described previously (Kuri-Harcuch and Marsch-Moreno, 1983). The extent of adipose differentiation was quantitated by staining intracellular lipids with Oil Red O (Ramírez-Zacarías et al., 1992). We estimated the activity of adipogenic factors by defining one unit of adipogenic activity as the amount of protein needed to produce 0.1 of absorbance at 510 nm in isopropanol extracts of Oil Red O-stained cultures.

RESULTS
In experiments to analyze the mechanism by which retinoic acid inhibits 3T3 adipogenesis, we isolated some 3T3-F442A clones resistant to retinoic acid cytotoxicity (Salazar-Olivo et al., 1994). The 3T3-F442A/C4 cells are one of these cloned subpopulations that, in addition to their previously reported high resistance to retinoic acid cytotoxicity and high colony forming efficiency (Salazar-Olivo et al., 1994), showed some other distinctive characteristics: mainly a high capacity to undergo adipose conversion under culture conditions that are non-adipogenic for the parental 3T3-F442A cells (see below). Therefore, we analyzed some of their kinetic and metabolic parameters to obtain a more detailed characterization of this new clone.

The 3T3-F442A/C4 clone exhibited faster proliferative rate and higher cell saturation density than parental cells: the 3T3-F442A/C4 clone had a population doubling time of 13.8 hours and a saturation density of 1.17×10^5 cells/cm^2 as compared with 3T3-F442A cells that showed a population doubling time of 18.8 hours and a saturation density of 0.8×10^5 cells/cm^2.

Nearly 50% of 3T3-F442A cells are susceptible to becoming adipose in surface cultures (Kuri-Harcuch and Marsch-Moreno, 1983). Under the same culture conditions, fully differentiated 3T3-F442A/C4 cultures showed a more intense staining with Oil Red O and an apparent greater proportion of adipocytes than parental cells. To determine whether these observations indicated a higher number of cells susceptible to undergoing adipose differentiation in the 3T3-F442A/C4 clone, we stimulated quiescent cultures of both clones to differentiate and then we estimated the percentage of susceptible cells as previously described for 3T3-F442A cultures (Kuri-Harcuch and Marsch-Moreno, 1983). The results showed that the proportion of cells susceptible to undergoing adipose differentiation was about 51% for 3T3-F442A and 42% for 3T3-F442A/C4 cells. After adipose conversion the increase in total cells was the same (2.9-fold) for both clones, suggesting a similar susceptibility to differentiation for both cell types. Therefore, the more intense Oil Red O staining in 3T3-F442A/C4 cultures was not due to a higher proportion of cells susceptible to becoming adipose in this clone, but to a higher number of adipose cells per dish (13.7×10^3) as compared to the parental cell line (8.5×10^3). These results are in agreement with the higher saturation density exhibited by the variant clone.

<table>
<thead>
<tr>
<th>Precursor</th>
<th>Lipid fraction</th>
<th>3T3-F442A</th>
<th>3T3-F442A/C4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic acid</td>
<td>Triglyceride</td>
<td>30.4</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>9.8</td>
<td>8.7</td>
</tr>
<tr>
<td>Sodium acetate</td>
<td>Triglyceride</td>
<td>48.7</td>
<td>20.2</td>
</tr>
<tr>
<td></td>
<td>Cholesterol</td>
<td>1.9</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>Phospholipid</td>
<td>8.6</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Cultures of adipocytes of both clones were incubated with [14C]oleic acid or [14C]sodium acetate during three hours in serum-free DMEM. Then, cell monolayers were washed three times with cold PBS, cells were harvested with a rubber spatula and the radioactivity incorporated into lipid fractions analyzed by thin layer chromatography. Results from one typical experiment are shown. Data were reproduced in three different experiments.
Under phase contrast microscopy, 3T3-F442A/C4 cells displayed lower accumulation of intracytoplasmic lipids. To determine the capacity of the variant cells for lipid accumulation, we analyzed the incorporation of radioactive precursors into lipid fractions in both cell types. The results showed about 2.4-fold lower incorporation into triglycerides of [14C]oleic acid and of [14C]sodium acetate in 3T3-F442A/C4 than in 3T3-F442A adipocytes (Table 1). This lower incorporation of lipid precursors into triglycerides could be explained by lower lipogenic enzyme activities. We analyzed the levels of glycerophosphate dehydrogenase (GPDH), malic enzyme, and glycerophosphate acyl transferase (GAT) activities in both clones cultured under adipogenic and non-adipogenic conditions. The GPDH and malic enzyme activities were lower in the variant clone when both clones were maintained in adipogenic culture conditions (Fig. 1A-C). Also, the rate of increase for these enzyme activities was slower in the variant clone (Fig. 1A-C). A third enzyme, GAT, reached similar activity levels and expression pattern in both clones under adipogenic and non-adipogenic conditions. The high extent of adipose differentiation exhibited by the 3T3-F442A/C4 cells under non-adipogenic conditions could be only the result of a higher clonal amplification of committed cells.

We have previously showed that the variant clones are not committed to adipose differentiation since they are unable to reverse retinoic acid-inhibition when cultured with non-adipogenic medium (Salazar-Olivo et al., 1994). To further study the dependence of 3T3-F442A/C4 differentiation upon adipogenic factors, we tested their ability to become adipocytes when cultured in a serum-free medium or in a low-serum, hormone-supplemented medium (definitive medium) that does not support 3T3 adipogenesis while allowing the survival of confluent cultures for several weeks without further medium changes (Nixon and Green, 1984). Under both culture conditions, the 3T3-F442A/C4 preadipocytes as well as the

The 3T3-F442A/C4 preadipocytes exhibit high responsiveness to serum adipogenic factors

The 3T3-F442A/C4 preadipocytes cultured under non-adipogenic conditions (DMEM containing 4% cat serum, insulin and biotin, changed every other day) underwent adipose conversion and were able to express biochemical markers of differentiation, unlike the parental 3T3-F442A preadipocytes that did not differentiate under such conditions (Fig. 1). Therefore, the variant cells could be either already committed to adipose differentiation and thus independent from serum adipogenic factors to undergo terminal differentiation, or they could be more sensitive to these factors and therefore stimulated by the very low concentrations that might be found in the cat serum. Alternatively, the high extent of adipose differentiation exhibited by the 3T3-F442A/C4 cells under non-adipogenic conditions could be only the result of a higher clonal amplification of committed cells.

We have previously showed that the variant clones are not committed to adipose differentiation since they are unable to reverse retinoic acid-inhibition when cultured with non-adipogenic medium (Salazar-Olivo et al., 1994). To further study the dependence of 3T3-F442A/C4 differentiation upon adipogenic factors, we tested their ability to become adipocytes when cultured in a serum-free medium or in a low-serum, hormone-supplemented medium (definitive medium) that does not support 3T3 adipogenesis while allowing the survival of confluent cultures for several weeks without further medium changes (Nixon and Green, 1984). Under both culture conditions, the 3T3-F442A/C4 preadipocytes as well as the
parental 3T3-F442A cells were unable to differentiate into adipocytes, unless calf serum or hGH was present (Table 2). This result indicated that the 3T3-F442A/C4 cells, like the parental 3T3-F442A cells, are still dependent on serum adipogenic activities to undergo adipose conversion.

We then compared the response of 3T3-F442A and 3T3-F442A/C4 preadipocytes to serum adipogenic activities by stimulating their differentiation with various concentrations of FBS or partially purified serum adipogenic factors (Ramírez-Zacarías et al., unpublished); we calculated the sensitivity of each clone to the different factors as described in Materials and Methods. As Fig. 2 shows, 3T3-F442A/C4 preadipocytes exhibited higher responsiveness to all adipogenic fractions, as well as to FBS, than the parental 3T3-F442A cells. The sensitivity of the variant cells to these adipogenic activities was 1.5- to 3.6-fold higher (Table 3). Moreover, to further compare the sensitivity of both clones to adipogenic activities, we evaluated their response to GH. Growth hormone is the only serum adipogenic factor that induced differentiation of both clones.

Table 2. Adipose differentiation of 3T3 preadipocytes in low serum or serum-free medium

<table>
<thead>
<tr>
<th>Culture conditions</th>
<th>3T3-F442A (A510/culture)</th>
<th>3T3-F442A/C4 (A510/culture)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Definitive medium*</td>
<td>0.04±0.01</td>
<td>0.04±0.02</td>
</tr>
<tr>
<td>+ 5% calf serum*</td>
<td>0.06±0.01</td>
<td>0.10±0.03</td>
</tr>
<tr>
<td>Serum-free medium†</td>
<td>0.02±0.002</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>+ 25 ng/ml hGH‡</td>
<td>0.07±0.01</td>
<td>0.39±0.04</td>
</tr>
</tbody>
</table>

Table 3. Sensitivity of 3T3-F442A/C4 cells to serum adipogenic factors

<table>
<thead>
<tr>
<th>Adipogenic fraction</th>
<th>Adipose activity*</th>
<th>Sensitivity index†</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBS</td>
<td>230</td>
<td>150</td>
</tr>
<tr>
<td>AF1‡</td>
<td>2200</td>
<td>1200</td>
</tr>
<tr>
<td>AF2‡</td>
<td>155</td>
<td>100</td>
</tr>
<tr>
<td>AF3‡</td>
<td>12.2</td>
<td>8.2</td>
</tr>
<tr>
<td>FGH‡</td>
<td>130</td>
<td>36</td>
</tr>
<tr>
<td>hGH</td>
<td>0.012</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Fig. 2. Effect of serum adipogenic fractions on adipose differentiation of 3T3 preadipocytes. Confluent 3T3-F442A (○) or 3T3-F442A/C4 (●) preadipocytes were stimulated to differentiation with definitive medium containing four adipogenic fractions obtained by ion exchange chromatography of FBS: fraction containing bovine growth hormone (FGH), adipogenic fraction 1 (AF1), adipogenic fraction 2 (AF2), and adipogenic fraction 3 (AF3) (Ramírez-Zacarías et al., unpublished). Control cultures received definitive medium alone or supplemented with FBS. Seven days after, the cultures were fixed and stained with Oil Red O and adipose conversion was quantitated by intracytoplasmic lipid accumulation (see Materials and Methods).

Preconfluent cultures of 3T3-F442A or 3T3-F442A/C4 preadipocytes were induced to differentiation and processed as described in Fig. 2.

*One unit of adipogenic activity was defined as the amount of protein needed to elicit 0.1 units of absorbance to 510 nm in isopropanol extracts of Oil Red O-stained cultures or, in the case of hGH, the amount of protein needed to produce 60 adipose cell clusters per dish.
†Expressed as the quotient of the concentration of protein needed to elicit one unit of adipogenic activity on 3T3-F442A cells by the concentration of protein needed to elicit the same extent of adipogenic activity on 3T3-F442A/C4 cells for the same adipogenic fraction.
‡Adipogenic fractions obtained by chromatographic fractionation of FBS on a Carboxymethyl-Sephadex C50 column.
Similar to the previous results, the new clone 3T3-F442A/C4 was 6-fold more sensitive to hGH adipogenic activity (Table 3), as evaluated by the activity of GPDH enzyme (Fig. 3A) and by the number of adipose cell clusters per dish (Fig. 3B). It is known that each adipose cell cluster arises from a committed preadipose cell by several rounds of clonal multiplication (Pairault and Green, 1979; Kuri-Harcuch and Marsh-Moreno, 1983).

The similar increases in total cells exhibited by both clones after differentiation (see above), and the higher number of adipose cell clusters showed by the 3T3-F442A/C4 cells with increasing hGH concentrations, strongly argue against the possibility that the higher levels of differentiation observed in the 3T3-F442A/C4 cells were the result of a higher clonal amplification of committed cells. To further rule out this possibility we also analyzed the effect of insulin, a hormone that modulates the transient amplification of immature 3T3 adipocytes (Green and Kehinde, 1975; Steinberg and Browstein, 1982). Preconfluent cultures of 3T3-F442A or 3T3-F442A/C4 preadipocytes were stimulated to differentiate with definitive medium supplemented with 5% FBS and the indicated insulin concentrations. An insulin concentration of 0.25 µg/ml elicited the highest differentiation in both clones, as determined by intracytoplasmic lipid staining (Fig. 4A), GPDH activity (Fig. 4B), and the number of adipocytes per dish (Fig. 4C). Higher concentrations of insulin did not further increase any of these parameters either in the parental or in the variant clone (Fig. 4), clearly showing that both clones have a similar response to insulin concentration. These results suggest that both clones have similar patterns of clonal amplification in response to hormones that modulate the selective and transient multiplication of committed cells.

**DISCUSSION**

The Swiss mouse embryo derived 3T3 preadipocytes are a useful model for in vitro studies of adipose tissue development and its regulation by hormones and growth factors (Watt, 1991). Several 3T3 clones described have diverse ability to undergo adipose differentiation in the presence of serum adipogenic factors or growth hormone. Until now, the 3T3-F442A
clone showed the highest frequency of adipose differentiation among the 3T3 clones (Green and Kehinde, 1976). We previously obtained a set of 3T3-F442A cell variant clones which should be useful in the study of several aspects of adipose cell physiology and differentiation (Salazar-Olivo et al., 1994). One of these variants, the clone 3T3-F442A/C4, has several characteristics that distinguish it from the parental 3T3-F442A cell line, mainly the ability to differentiate into adipocytes under culture conditions that are non-adipogenic for the parental cells.

We found that the 3T3-F442A/C4 cells exhibited a lower population doubling time, an increased saturation density, but the same proportion of cells susceptible to undergo adipose differentiation compared with the parental cell line. The variant cells also expressed biochemical markers of adipose differentiation, although two of them (GPDH and malic enzyme) reached lower activity levels in the variant cells as compared to the parental clone. The lower activity levels of these key lipogenic enzymes could explain the lower de novo synthesis of fatty acids also shown by the variant clone. A third marker (GAT) reached similar activity levels in both clones. On the other hand, unlike the parental cells, the variant 3T3-F442A/C4 clone exhibited high activity levels for the three enzymes under non-adipogenic culture conditions.

The 3T3-F442A/C4 variant clone showed an unusually high extent of adipose differentiation and expressed differentiation markers when cultured with repeated changes of non-adipogenic medium. This result suggested that the variant cells were either already committed to differentiation and therefore independent from adipogenic factors to undergo terminal differentiation, or they were more sensitive to such factors, or to other hormones that modulate phenotype expression. The possibility that 3T3-F442A/C4 cells were already committed to differentiation was ruled out by our results showing that they were unable to differentiate into adipocytes when cultured under serum-free medium, or under limited serum supplement (definitive medium), unless calf serum, or hGH, were present. This conclusion agrees with our previous studies showing that retinoic acid inhibits both the commitment and the expression of differentiation of the 3T3-F442A/C4 clone, in a similar manner to parental cells (Salazar-Olivo et al., 1994).

An alternative explanation, the higher clonal amplification of 3T3-F442A/C4 cells after commitment to differentiation was also ruled out, since both clones exhibited similar responses to increasing insulin concentrations, as evaluated by intracytoplasmic lipid accumulation, GPDH activity, or proportion of adipocytes per culture. These similar responses to insulin showed that the high level of differentiation exhibited by 3T3-F442A/C4 cells under non-adipogenic conditions was not due to an augmented clonal amplification, and further supported the view that the variant cells mainly have higher sensitivity to serum adipogenic activities.

We confirmed such higher sensitivity of 3T3-F442A/C4 cells to the adipogenic factors when we compared the cellular response of both clones to several serum adipogenic activities obtained after fractionation of FBS (Ramírez-Zacarías et al., unpublished), as well as to hGH and FBS. In all cases, the 3T3-F442A/C4 variant cells showed a higher sensitivity to adipogenic activities than parental 3T3-F442A cells, reaching values as high as 6-fold for hGH. The results of Fig. 3B showing that hGH increased the number of adipose cell clusters also strongly support the conclusion that the increased sensitivity to hGH or the other adipogenic factors is due to the formation of newly committed cells to differentiate.

The pathway by which GH affects adipose tissue development is still largely unclear, and the precise identities of the various adipogenic factors present in the serum remain to be known. Until now, we cannot establish the mechanisms that mediate the higher sensitivity of 3T3-F442A/C4 cells to hGH and the other serum adipogenic factors. We can hypothesize, however, a common pathway for signal transduction for GH and the other adipogenic factors, since the variant cells showed a general augmented sensitivity to each one of them. In such a case, an augmented affinity for a common receptor for GH and the other adipogenic factors, or a more efficient activation of another common intermediary molecule capable of interacting with different receptors of the same family, such as the tyrosine kinases of the Janus family (Argentsinger et al., 1993; Ihle, 1994), could explain the general increase in the sensitivity to adipogenic factors.

The higher sensitivity of the 3T3-F442A/C4 preadipocytes to serum adipogenic activities seems to be an exclusive characteristic of this new variant clone. Another extensively used 3T3 preadipose clone, 3T3-L1 (Green and Kehinde, 1974), exhibited a similar responsiveness and sensitivity to FBS, hGH and the AF2 adipogenic fraction as 3T3-F442A cells (our unpublished results). Other clones with altered responses to hormones that modulate the adipose differentiation were also recently derived from 3T3-F442A preadipocytes. Guller et al. (1989) obtained a 3T3-F442A variant clone that also shows high sensitivity to adipogenic capacity of calf serum. However, these cells are independent from GH to undergo adipose conversion, which suggest that they are already committed to differentiation, in contrast to the 3T3-F442A/C4 cells.

Several bioassays for GH have been described, some of which are technically arduous and relatively insensitive, requiring microgram quantities of partially purified GH and long time for testing (Greenspan et al., 1949; Ellis et al., 1978). Other bioassays, like the Nb-2 lymphocyte proliferation assay, are not specific for GH but also respond to other lactogenic hormones such as prolactin (Tanaka et al., 1980). Recently, Foster et al. (1993) have proposed a new GH bioassay that takes advantage of the GH responsiveness of 3T3-F442A cells. The proposed bioassay is based on the suppression of lipid accumulation in terminally differentiated 3T3-F442A adipocytes (Foster et al., 1993) and has some disadvantages, mainly the use of radioactive precursors and the prolonged time of cultivation to obtain terminally differentiated mature adipocytes (usually two weeks). The 3T3-F442A/C4 cells with a higher sensitivity to GH and a faster growth ability, offer the opportunity to develop a more sensitive, faster and simpler assay to evaluate the biological activities of GH, as well as the differences in biological activities among GH isoforms to which 3T3-F442A cells are also less sensitive than 3T3-F442A/C4 cells (Castro-Muñozledo et al., unpublished). On the other hand, the ability of 3T3-F442A/C4 cells to respond to very low concentrations of adipogenic activities makes this clone an optimal cell system to characterize these activities, their mechanism of action, and their role on normal and pathological adipose development.

In conclusion, we described here a new 3T3 preadipose clone, named 3T3-F442A/C4, which differs from the parental
cells in a number of kinetic and biochemical traits, such as a higher sensitivity to adipogenic factors and to hGH. Consequently, current work in our laboratory shows that this clone could serve as an excellent bioassay for in vitro studies directed towards purifying these factors and to characterize their role on adipose tissue development. Also, this new clone could be used as a sensitive and rapid assay to determine the biological activity of different GH samples.

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