

Control by Fibroblast Growth Factor of Differentiation in the BC₃H1 Muscle Cell Line

BRIAN LATHROP, ERIC OLSON, and LUIS GLASER

*Department of Biological Chemistry, Division of Biology and Biomedical Sciences, Washington University
School of Medicine, St. Louis, Missouri 63110*

ABSTRACT The regulation of creatine phosphokinase (CPK) expression by polypeptide growth factors has been examined in the clonal mouse muscle BC₃H1 cell line. After arrest of cell growth by exposure to low concentrations of serum, BC₃H1 cells accumulate high levels of muscle-specific proteins including CPK. The induction of this enzyme is reversible in the presence of high concentrations of fetal calf serum, which cause quiescent, differentiated cells to reenter the cell cycle. Under these conditions, the rate of CPK synthesis is drastically reduced. We show in the present communication that either pituitary-derived fibroblast growth factor (FGF) or brain-derived FGF are as effective as serum in repressing the synthesis of CPK when added to quiescent, differentiated cells. The decrease in the rate of synthesis of CPK occurs within 22 h after the addition of pituitary FGF to the cells. Pituitary FGF had very little effect, if any, on the rate CPK degradation. The overall rate of protein synthesis and the pattern of synthesis of the major polypeptides made by these cells was not altered by the addition of FGF. Although pituitary FGF was mitogenic for BC₃H1 cells, the rate of cell growth was not absolutely correlated with the extent of repression of CPK. Brain-derived FGF fully repressed CPK induction under conditions where it showed no significant mitogenic activity. These results show that the expression of a muscle-specific protein, CPK, can be controlled by a single defined polypeptide growth factor in fully differentiated cultures, and that initiation of cell division is not required for their regulation to take place.

The relationship between cell growth and differentiation has been a subject of extensive investigation in a number of systems. Muscle cells in culture have been a preferred system for the investigation of this relationship (3, 11, 19, 26, 28). After depletion of mitogens from the culture medium (16), muscle cells cease exponential growth and undergo differentiation (13, 21). Morphological differentiation is usually accompanied by fusion of mononucleated myoblasts into multinucleated myotubes and by the expression of a number of muscle-specific proteins including myosin and other components of the contractile apparatus as well as creatine phosphokinase (CPK)¹ and the acetylcholine receptor (3, 18, 19, 28).

In examining the relationship between growth and induction, a number of investigations have demonstrated the usefulness of causing quiescent, differentiated myoblasts under

nonfusing conditions to reenter the cell cycle in response to a mitogenic stimulation (3–5, 22–25). A cell line particularly suitable for these studies is the muscle-like cell line, BC₃H1. After growth is arrested at moderate cell densities by exposure to low levels of fetal calf serum (FCS) the cells differentiate as defined by the expression of high concentrations of CPK as well as other muscle-specific proteins including the acetylcholine receptor; however, unlike myoblasts, these cells do not fuse (20, 30). Differentiation in this system has been shown to be reversible. That is, nongrowing cells that are induced to synthesize muscle-specific proteins will reinitiate cell growth in response to high levels of serum with a concomitant loss of the differentiated phenotype (23–25). This repression² appears to involve both transcriptional (23, 24) and posttrans-

¹ *Abbreviations used in this paper:* B-CPK, brain isozyme of CPK; CPK, creatine phosphokinase; DME, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; FGF, fibroblast growth factor; LSM, low-serum medium; M-CPK, muscle isozyme of CPK.

² Induction and repression are used to indicate situations in which the cells synthesize or fail to synthesize muscle-specific proteins. No particular mechanism of regulation is implied by these terms. Furthermore, repression in this context should not be confused with the use of the term to represent a temporal delay of differentiation.

lational regulation, particularly at the level of subunit assembly of the acetylcholine receptor (25).

The use of serum as a mitogenic stimulus has significant disadvantages in the study of the molecular events associated with the loss of the differentiated phenotype. Serum is a complex mixture of molecules, and a biological effect brought about by the addition of serum to cells cannot be assigned unequivocally to a particular serum component. We therefore have developed a medium for BC₃H1 cells which supports cell viability and differentiation in the presence of trace concentrations of serum. We present evidence in this paper that this medium supplemented with pituitary fibroblast growth factor (FGF) specifically induces cell growth in quiescent BC₃H1 cells, and that the synthesis of a muscle-specific protein, CPK, is rapidly repressed. Repression is also observed after addition of another polypeptide growth factor, the acidic form of brain FGF,³ but without significant stimulation of cell growth.

MATERIALS AND METHODS

Materials: Trypsin (Type III), bovine insulin, human transferrin, fatty acid-free bovine serum albumin (BSA) (No. A-7511), and collagen (Type III) were from Sigma Chemical Co. (St. Louis, MO). FGF was from Collaborative Research, Inc. (Waltham, MA). FCS and antibiotics were from Gibco Laboratories (Grand Island, NY). High glucose/high bicarbonate Dulbecco's modified Eagle's medium (DME) was from the Washington University Basic Cancer Research Center (St. Louis, MO). Culture dishes and flasks were from Falcon Labware (Oxnard, CA). Epidermal growth factor was prepared by the method of Savage and Cohen (29). Highly purified platelet-derived growth factor (PDGF) was the generous gift of Dr. Thomas F. Deuel (Washington University). Acidic brain FGF (31) purified by high-performance liquid chromatography was the kind gift of Dr. Kenneth A. Thomas (Merck Sharp & Dohme Div., Rahway, NJ).

Insulin was dissolved in 12 mM HCl to a concentration of 1 mg/ml and was stored at -70°C. Transferrin was dissolved in phosphate-buffered saline (PBS) to 10 mg/ml and was stored at -20°C. FGF was prepared and stored according to manufacturer's instructions. Fatty acid-free BSA was dissolved in PBS at 25 mg/ml and was stored at 4°C. All solutions were stored in polypropylene containers and were filter sterilized when appropriate. Repeated freeze-thawing was avoided.

Cell Culture: The BC₃H1 muscle-like cell line (30) was grown in DME supplemented with 20% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin in a humidified atmosphere of 10% CO₂ and 90% air at 37°C. Cultures were re-fed every 2 d. Subconfluent stocks were subcultured every 4-5 d at a split ratio of ~1:20. To subculture, we first washed cultures with calcium- and magnesium-free solution at 37°C. 2 ml of 0.05% trypsin, 0.02% disodium ethylenediamine tetraacetic acid in calcium- and magnesium-free solution at 37°C was spread evenly over the cells and was immediately aspirated. After 90 s, cells were resuspended by gentle agitation in warm 20% FCS. Cells were either pelleted at 600 rpm for 3 min and resuspended, or plated directly in 20% FCS with no apparent difference in growth or morphology. 35-mm culture dishes were used and were collagen coated as described previously (20). Cell numbers were determined with a Coulter counter (Coulter Electronics, Inc., Hialeah, FL).

Biochemical Procedures: CPK was assayed spectrophotometrically as described previously (26). Cell homogenates were prepared in 1 ml of 50 mM glycylglycine, pH 6.75, 1% Nonidet P40 and were assayed under conditions of activity that were linear with respect to time and protein concentration.

To determine the rate of CPK synthesis, we labeled cells for 3 or 4 h with L-[³⁵S]methionine (New England Nuclear, Boston, MA) as described (23). At the end of the labeling period, cells were harvested and extracted with Triton X-100 into a final volume of 400 µl, and CPK was immunoprecipitated as described previously (23). The commercial antiserum used for the precipitation of CPK precipitates both the brain isozyme (B-CPK) and the muscle isozyme (M-CPK). The isozymes were easily separated by SDS polyacrylamide gel electrophoresis (14) on the basis of their relatively large difference in molecular weights (26). The gels were subjected to fluorography, and the relative rate of synthesis was determined by densitometry of the M- and B-CPK bands as

described (23). Individual cultures were used for each immunoprecipitation, and equal volumes of immunoprecipitated material were used for electrophoresis. Rates of total protein synthesis were determined by precipitation of labeled extracts with trichloroacetic acid (23).

RESULTS

To study the role of purified growth factors in the regulation of growth and differentiation in BC₃H1 cells, we developed a simplified medium that would allow the cells to retain viability and normal rates of CPK synthesis in the absence of mitogens, but upon supplementation with mitogens, would allow the cells to grow at levels comparable to that seen with DME that contained 20% FCS. The medium that was developed contains a trace quantity of FCS, 0.25% without which the viability of the cells could not be maintained (data not shown). This medium, designated as LSM (low-serum medium), contains DME and F12 in a ratio of 3:1, 50 µg/ml transferrin, 250 µg/ml BSA, and antibiotics. As shown in Fig. 1A, the morphology of quiescent cells in this medium resembled that of differentiated cells in DME that contained 1% FCS (see reference 30). As will be shown below, induction of CPK and overall protein synthesis continued at a rate comparable to that in DME that contained 1% FCS. Collagen coating allowed excellent adhesion of cells to the substratum but had no effect on the rate of growth or induction in this system.

A variety of growth factors that have been shown to be mitogenic for muscle cells (6, 9, 10, 12, 33) or other cell types were tested for their ability to reinitiate growth of quiescent cultures of BC₃H1 cells in LSM. Significant cell growth, comparable to that observed upon the addition of 20% FCS, was observed only when a combination of FGF and insulin was added to the cells (Fig. 2A). The substances tested that had no mitogenic activity included PDGF, epidermal growth factor, dexamethasone, and linoleic acid bound to fatty acid-free BSA. These compounds added to the medium either alone or in various combinations had no effect on CPK activity. For example, 48 h after addition of pituitary FGF to LSM that contained insulin, there was a 60% increase in cell number; addition of 50 ng/ml PDGF to LSM + insulin (1 µg/ml) resulted in no increase in cell number relative to LSM that contained insulin. In the same experiment, addition of 20% FCS resulted in a doubling of cell number in 48 h. PDGF also had no effect on the rate of synthesis of M-CPK, measured as described in Materials and Methods, 20 h after addition of PDGF.

FGF dramatically decreased the rate of accumulation of CPK when added to differentiated cells in LSM (Fig. 2B). As in the case of 20% FCS, CPK activity in the culture continued to increase for ~1 d after addition of FGF and then remained constant. We have previously demonstrated that all cells in differentiated cultures are synthesizing CPK (23), thus repression of CPK activity must represent an effect on cells that have already entered the differentiation process rather than an inhibition of entry of additional cells into that process.

The effects of different combinations of insulin and FGF on cell growth and CPK activity are summarized in Table I. FGF repressed CPK activity if added alone, when the mitogenic effect was weak, or with insulin, when the rate of growth was comparable to that observed in 20% FCS. In contrast, insulin had a small but significant mitogenic effect comparable to that observed upon addition of FGF alone, but it did not inhibit the accumulation of CPK. Rather, it showed a small increase in the level of CPK. These observations indicate

³ Unless otherwise specified, all references to FGF refer to the pituitary form.

FIGURE 1 Morphological changes in response to hormonal supplements. Cell growth was arrested and cultures were allowed to differentiate as described in Materials and Methods. Cultures were then re-fed with the experimental media, and fresh medium was added at 20 h. Photographs show culture morphology after 40 h in the medium indicated. (A) Cultures in LSM; (B) cultures in LSM supplemented with 200 ng/ml FGF; (C) LSM with FGF and 1 μ g/ml insulin; (D) LSM with acidic brain FGF at 10 ng/ml and 1 μ g/ml insulin; (E) 20% serum. Phase-contrast pictures, $\times 27$.

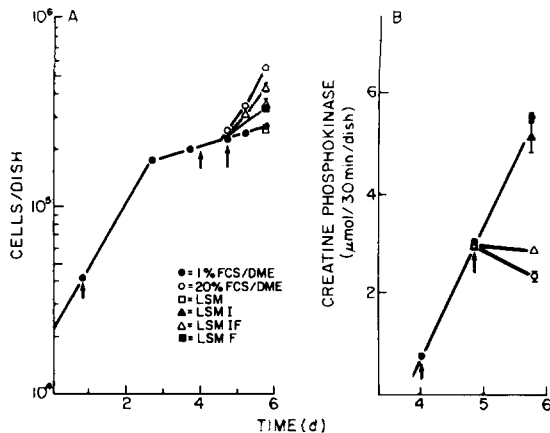
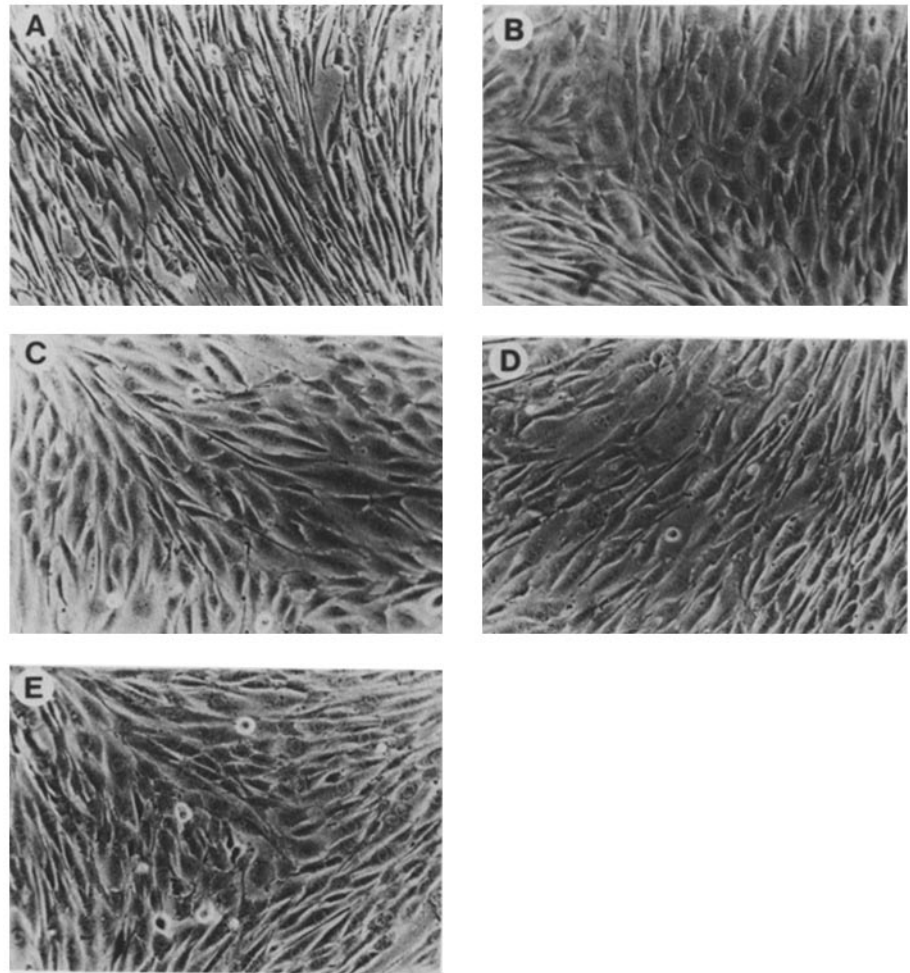


FIGURE 2 Effects of hormones on cell growth and CPK activity accumulation. Cultures were plated in 20% serum as described. On day 1, cultures were re-fed with medium that contained 1% serum (arrow). Cell number is shown in A and CPK activity in B. The culture medium was then replaced at the times indicated by the arrows with the various experimental media. Cell numbers were determined in duplicate with a Coulter counter. CPK was determined as described in Materials and Methods from duplicate extracts. Unless otherwise indicated, error bars were smaller than symbols. Insulin was present at 1 μ g/ml and FGF at 200 ng/ml.

that the degree of repression of CPK activity is not absolutely correlated with the rate of cell growth. The concentration dependence of FGF for the repression of CPK and for cell

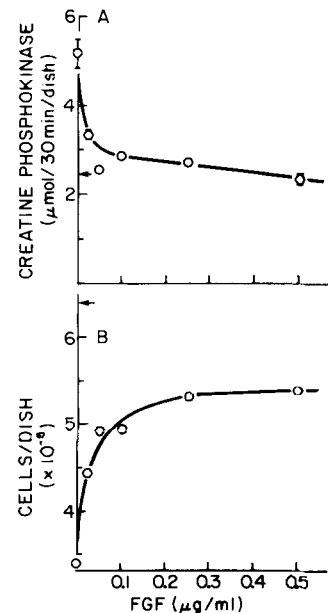


FIGURE 3 Dependence of cell growth and CPK activity on concentration of FGF. Induced cultures were treated with LSM supplemented with 1 μ g/ml insulin and FGF at concentrations indicated. Fresh medium was added 20 h later. Cell numbers were determined 45 h after re-feeding. CPK activity was determined from duplicate extracts 40 h after re-feeding. Unless otherwise indicated, error bars are smaller than symbols. CPK activity and cell number in 20% FCS controls are indicated by arrows.

TABLE I
Effect of Fibroblast Growth Factor on CPK Activity and Cell Growth

Medium	CPK (% maximal activity)	Percent increase in cell number
LSM	75 ± 3 (n = 6)	—
LSM + insulin	100 ± 4 (n = 8)	29 ± 6 (n = 9)
LSM + insulin + FGF	55 ± 5 (n = 7)	75 ± 8 (n = 7)
LSM + FGF	49 ± 1 (n = 3)	28 ± 5 (n = 3)
1% FCS/DME	96 ± 15 (n = 6)	—
20% FCS/DME	41 ± 4 (n = 7)	128 ± 18 (n = 8)

Quiescent, differentiated cultures were re-fed as in Fig. 2 with the experimental medium indicated. CPK activity was determined at 40 h after the medium change. Results are expressed as relative units normalized to the activity in LSM + insulin (*n* represents the number of separate experiments). The error between determinations from duplicate extracts was <10% and usually ~4%. Cell number is expressed as the percent increase over control levels by 48 h. In the case of LSM + insulin and LSM + insulin + FGF, the reference value is the culture condition in LSM. In the case of 20% serum, the reference value is the culture in 1% FCS. Cell number typically showed no increase after 48 h in either LSM or 1% FCS; however, in some experiments cell number increased by as much as 20% in these media. Insulin was present at 1 µg/ml and FGF at 0.2 µg/ml.

growth in shown in Fig. 3. The concentration dependence for both effects is similar; a half-maximal response is observed at ~20 ng/ml.

FGF affected the morphology of the cells, as shown by comparison of *A* and *B* of Fig. 1. Cultures treated with FGF developed a flattened, fibroblastic morphology characteristic of exponentially growing cells (30). A similar change in morphology was seen when 20% FCS was added to the cells, as shown in *E*. Since FGF produced this effect in the presence or absence of insulin, a strong mitogenic effect does not seem to be required for this change to occur. Inspection of Fig. 1 *B* shows that most cells in the culture assumed a fibroblastic morphology in response to FGF.

FGF could affect the rate of accumulation of CPK by affecting either the rate of synthesis or the rate of degradation. Fig. 4 shows the effects of 20% FCS and LSM supplemented with insulin and FGF on the rate of CPK synthesis at various times after their addition to induced cultures. 5 h after the addition of either 20% FCS or FGF, the rate of M-CPK synthesis was equal to or higher than that for controls. By 22

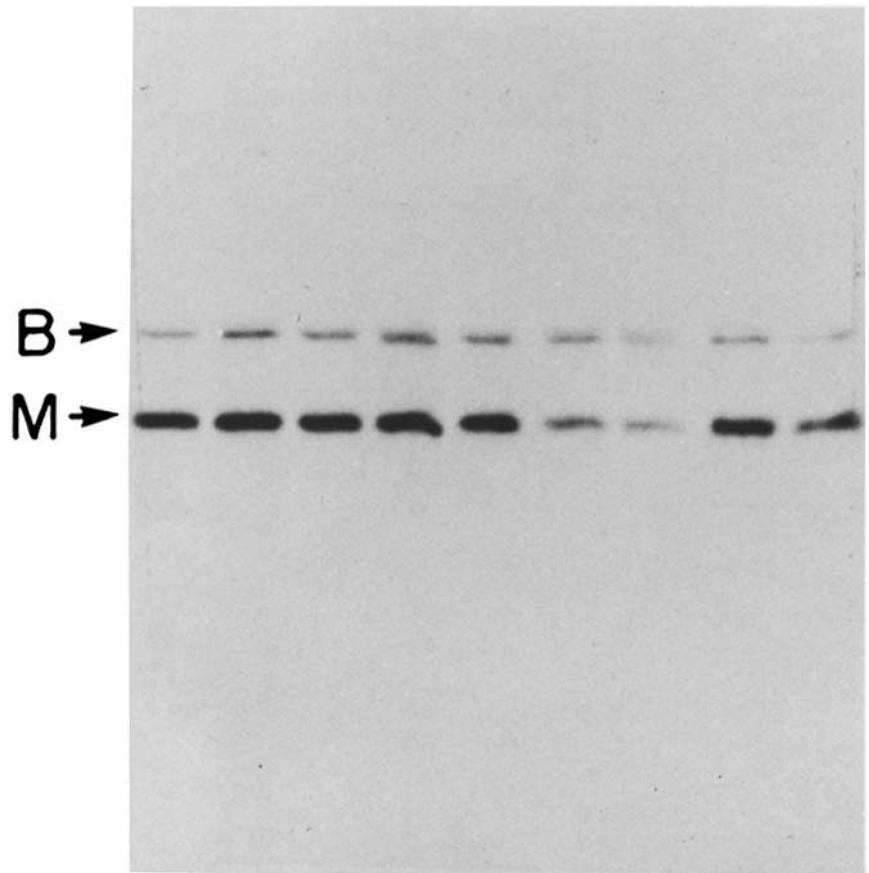
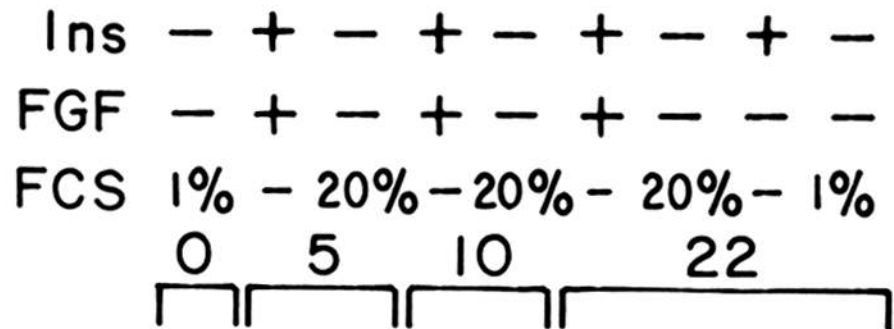


FIGURE 4 Time dependence of the effects of FGF and FCS on the rate of M-CPK synthesis. Cultures were re-fed with DME supplemented with FCS at the concentrations indicated or with LSM supplemented with 1 µg/ml insulin or 200 ng/ml FGF as indicated. Duplicate cultures were pulse-labeled for 3 h with [³⁵S]methionine, CPK was immunoprecipitated, and the isozymes were resolved by gel electrophoresis. The numbers above the lanes indicate the time (in hours) at the midpoint of each pulse period. (*M*, muscle isozyme of CPK; *B*, brain isozyme of CPK.)

h, however, the rate of synthesis in both cases was much lower than in control cultures. Table II summarizes several experiments in which the rate of CPK synthesis was measured 22 h

TABLE II
Effect of Growth Factors and FCS on the Rates of CPK Synthesis and Protein Synthesis

Medium	Relative rate of M-CPK synthesis	Relative rate of overall protein synthesis
LSM	80 ± 6 (n = 4)	76 ± 3
LSM + insulin	100 ± 9 (n = 5)	100 ± 6
LSM + insulin + FGF	35 ± 6 (n = 5)	102 ± 2
1% FCS/DME	78 ± 15 (n = 5)	59 ± 2
20% FCS/DME	20 ± 6 (n = 5)	122 ± 7

The rate of M-CPK synthesis was determined by densitometry of fluorographs prepared as described in Fig. 2. All values have been normalized to LSM + insulin. Abbreviation used are the same as those in Fig. 2. *n* represents the number of experiments performed; duplicate extracts were made in each experiment. The rate of overall protein synthesis was determined by trichloroacetic acid precipitation of 7.5 μl from cell extracts and are duplicate determinations from a representative experiment. Counts per minute have been normalized to LSM + insulin (100% = 4 × 10⁶ cpm of [³⁵S]methionine/dish).

after the addition of various mitogens or hormones to induced BC₃H1 cells. FGF, either alone or in the presence of insulin, repressed the rate of M-CPK synthesis to levels comparable to that observed in the presence of 20% FCS. FGF did not significantly affect the rate of synthesis of the major polypeptides in BC₃H1 cells, as shown by one-dimensional gel electrophoresis (Fig. 5), which indicates that repression of M-CPK synthesis does not reflect a general decrease or alteration in protein synthesis. The relative increase in total protein synthesis due to addition of insulin or 20% FCS varied in different experiments, but in no case did addition of FGF cause a significant change in the overall rate of protein synthesis. In most experiments, insulin stimulated the rate of overall protein synthesis by 20–30%. The lower levels of B-CPK and the occasional presence of co-migrating trace contaminants precluded precise quantitation of this isozyme, but its level was less sensitive to addition of FGF than that of M-CPK (see, for example, Fig. 4). These results show that the rate of M-CPK synthesis in the presence of FGF is reduced to a level comparable to that observed after the addition of 20% FCS.

To determine whether the rate of degradation of M-CPK was also affected by the addition of mitogens, we pulse labeled

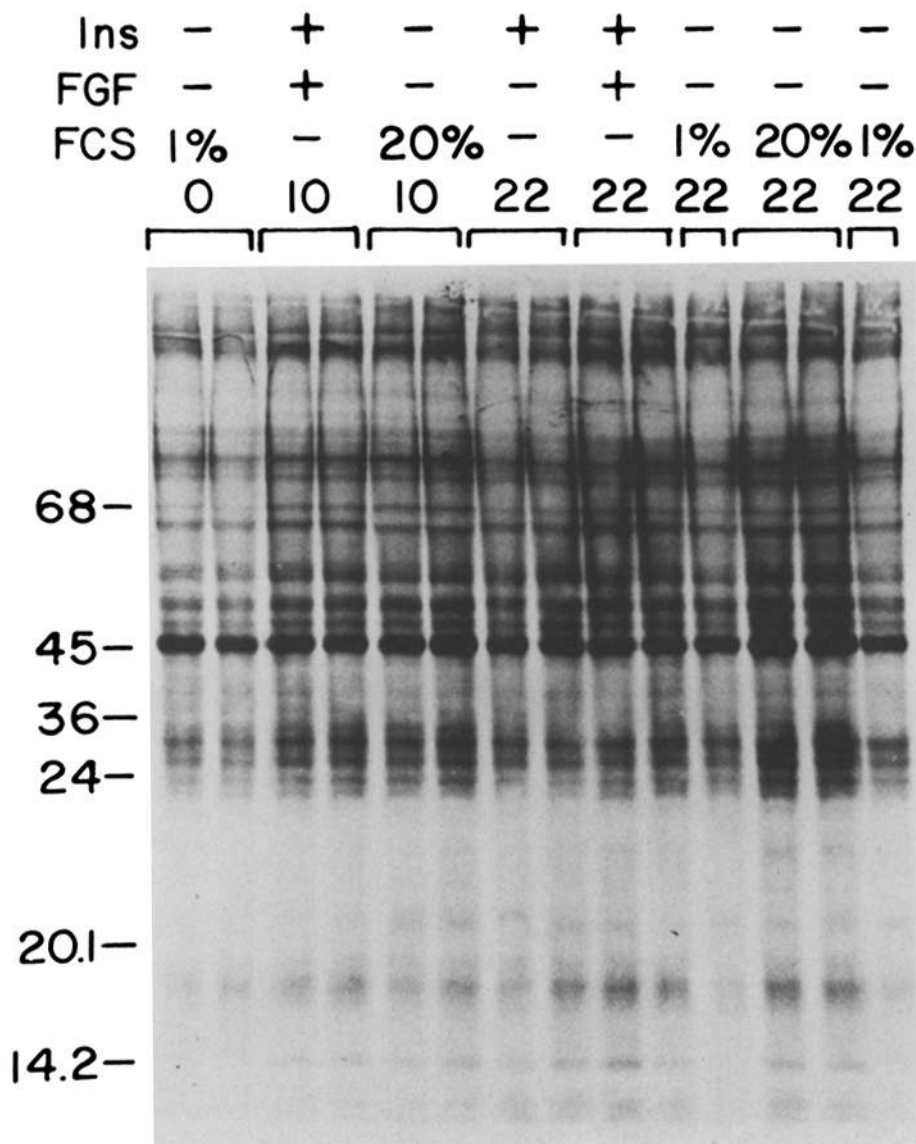


FIGURE 5 Effect of growth factors on the overall pattern of protein synthesis. Quiescent, differentiated cultures were re-fed with the medium indicated. At the times indicated (in hours), cultures were labeled with [³⁵S]methionine for 4 h and extracted. A 10-μl aliquot from an extract of 400 μl volume was subjected to electrophoresis.

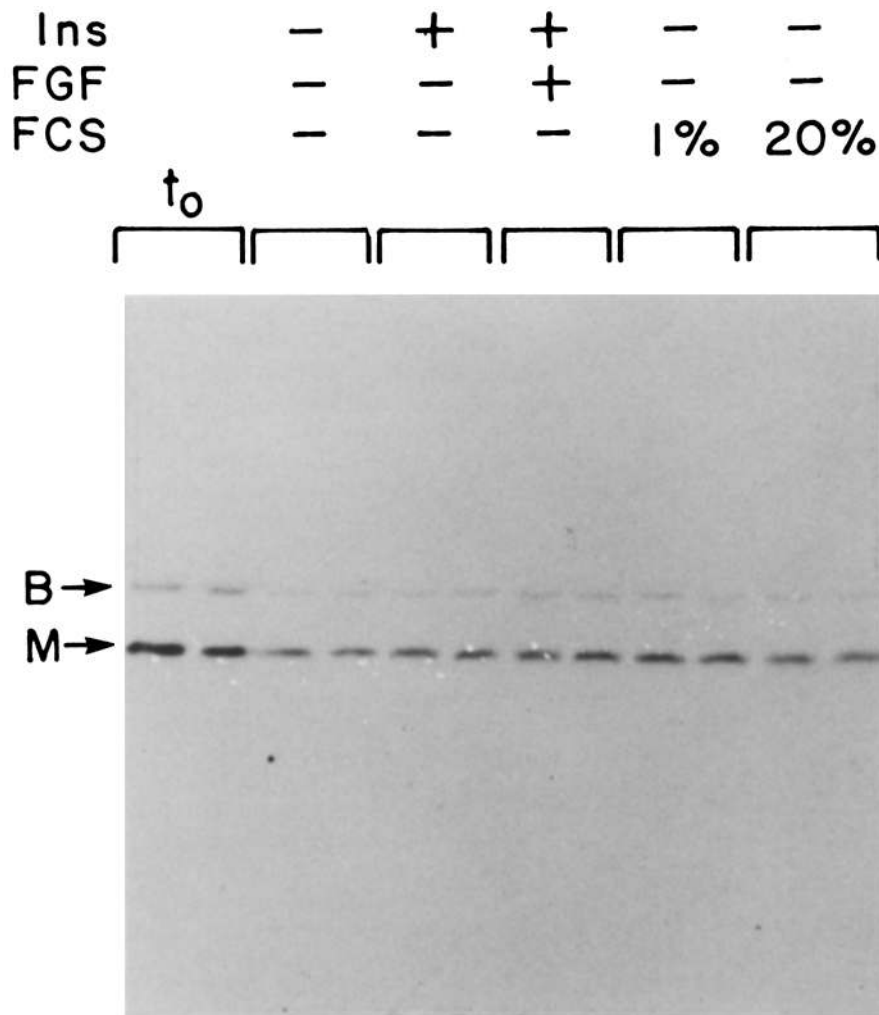


FIGURE 6 Effect of growth factor on CPK turnover. Quiescent, differentiated cultures were washed with methionine-free DME and were pulse-labeled for 4 h with 100 $\mu\text{Ci/ml}$ L-[^{35}S]methionine in 0.5 ml methionine-free DME containing 1% dialyzed fetal calf serum and 20 μM unlabeled L-methionine. The pulse medium was removed, the cultures were washed with DME, and the various experimental media were added. Cells were harvested and extracted 20 h later. CPK was immunoprecipitated and equal volumes from each extract were electrophoresed. t_0 represents cultures that were harvested and immunoprecipitated immediately at the end of the pulse period.

BC₃H1 cells with [^{35}S]methionine for 4 h. At the end of the labeling period, the pulse medium was removed, and cultures were re-fed with media that contained FCS or with LSM that contained various hormonal supplements. After 20 h, extracts were prepared and CPK was immunoprecipitated. As shown in Fig. 6, the rate of turnover, as determined by the amount of undegraded radiolabeled CPK, was approximately the same in all conditions. Approximately 50% of the CPK radioactivity was lost after 20 h. Turnover was slightly enhanced in 20% FCS, as has been seen previously in the laboratory (Caldwell, K., unpublished observations).

Pituitary FGF and brain FGF are distinct molecules (1, 21) and, although both are mitogens for fibroblasts (8, 31), the exact biological relationship between them is as yet unclear. The possibility that brain FGF is a minor contaminant of the pituitary FGF preparation cannot be excluded. We have tested brain FGF to determine whether it could also repress the synthesis of CPK in BC₃H1 cells, and, as shown in Fig. 7, the acidic form of brain FGF at a concentration of 10 ng/ml was at least as effective as 20% FCS in repressing CPK synthesis. Densitometry of the fluorograph shown in Fig. 7 indicated that the relative rate of M-CPK synthesis in LSM that contained insulin and brain FGF was 12% the rate observed in cells that contained LSM plus insulin (Table II). Brain FGF had no effect on the rate of overall protein synthesis (not shown), and it promoted the same change in morphology as that observed with pituitary FGF and FCS (Fig. 1C). Fig. 8

illustrates the dependence of the rate of M-CPK synthesis on the concentration of brain FGF added 20 h previously. Under these conditions, the addition of 5 ng/ml of brain FGF to LSM or LSM supplemented with 1 $\mu\text{g/ml}$ insulin did not cause any detectable increase in cell number.

DISCUSSION

A variety of environmental factors modulate cell differentiation. Cells interact with the substratum, with other cells, and with a variety of media components (hormones and mitogens), all of which influence the developmental process. To investigate these effects, it is important to examine the effects of single defined environmental components on cell differentiation and, if possible, to quantitate the effect by measurements of the quantity or activity of well-characterized molecules associated with the differentiated phenotype. The observations presented in this paper describe a unique system for such investigations. Two defined mitogens (one highly purified, brain FGF) influence differentiation in BC₃H1 cells, defined by the synthesis of a muscle-specific protein, the M isozyme of CPK. These mitogens drastically decrease the rate of synthesis of M-CPK to levels comparable to that observed upon addition of 20% FCS to the same cells. The rate of degradation of M-CPK and the rate of overall protein synthesis in these cells remain relatively unaffected by these mitogens. The decreased rate of M-CPK synthesis after addition

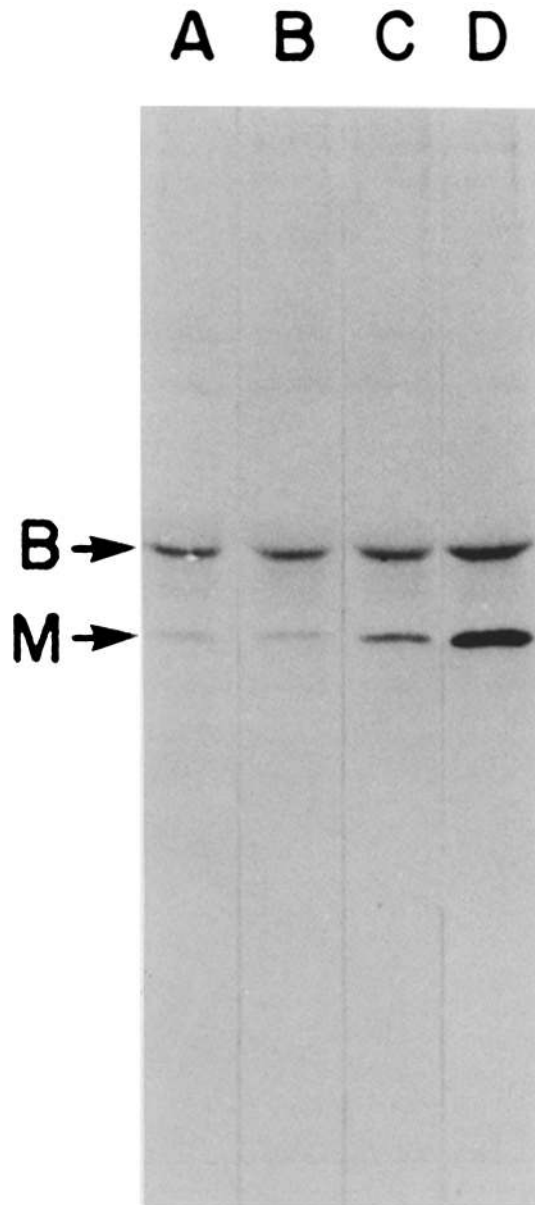


FIGURE 7 Effect of brain FGF on the rate of CPK synthesis. Cultures and extracts were treated exactly as described in Fig. 4. Cultures were pulse-labeled 22 h after the addition of the experimental media. A, 20% serum; B, LSM + 1 $\mu\text{g}/\text{ml}$ insulin and supplemented with 10 ng/ml acidic brain FGF; C, LSM + 1 $\mu\text{g}/\text{ml}$ insulin + 200 ng/ml pituitary FGF; and D, LSM + 1 $\mu\text{g}/\text{ml}$ insulin.

of growth factor observed in these studies, coupled with the turnover of M-CPK, adequately accounts for the cessation of CPK accumulation after addition of mitogen.

In previous experiments from this laboratory, we examined the rate of M-CPK synthesis 48 h after addition of 20% FCS to differentiated cultures (23) and observed an increased rate of CPK synthesis even though the level of CPK in these cultures was reduced. It appears that the increased level of CPK synthesis represented the early stages of reinduction of these cells as they reached high cell densities in 20% FCS (20), which had not yet led to the accumulation of additional CPK in the cells. All measurements of the rate of M-CPK synthesis in this paper were confined to early times (≤ 20 h) after addition of mitogens.

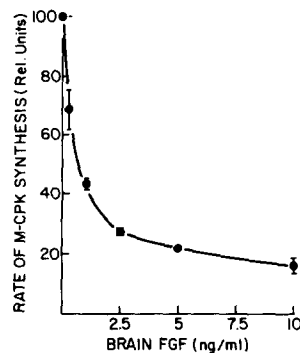


FIGURE 8 Dependence of the rate of M-CPK synthesis on the concentration of brain FGF. Induced cultures in 1% serum were re-fed with LSM supplemented with 1 $\mu\text{g}/\text{ml}$ insulin and brain FGF at the concentrations indicated. The rate of M-CPK synthesis was determined from duplicate extracts 20 h after re-feeding, as described in Materials and Methods. The solvent added with 10 ng/ml brain FGF (2 μM trifluoroacetic acid, 0.01% acetonitrile) had no effect on the rate of M-CPK synthesis.

Investigations in a number of laboratories have examined the relationship between cell growth and differentiation in muscle cells. Nguyen et al. (22) have isolated a temperature-sensitive muscle cell line which, at the nonpermissive temperature, synthesizes muscle-specific proteins but does not irreversibly commit to differentiation. Similar to what is observed with BC₃H1 cells, the addition of serum reinitiates growth in these cells and represses muscle-specific gene expression. The factors in serum that were responsible for repression were not identified in these experiments; however, the addition of pituitary FGF has been shown to be sufficient to delay the onset of myogenic induction by prolonging exponential growth (9, 15, 16, 32), although high rates of cell proliferation were shown to be unnecessary for FGF to block the onset of differentiation (17).

An inhibitor of muscle differentiation that is not mitogenic has been described by Evinger-Hodges et al. (7), but its mechanism of action is not known. Similarly, previous results from this laboratory have described a crude serum fraction which is not mitogenic for cells but blocks differentiation in BC₃H1 cells (20).

Because of the apparent inverse correlation between cell growth and cell differentiation, we examined a variety of known mitogenic molecules to ascertain whether they could replace serum as a factor which causes repression of CPK synthesis, and found both the pituitary FGF and the acidic form of brain FGF to be effective in this regard, even though they are distinct molecules with rather different properties.

Even though these molecules were selected for study because of their potential mitogenic activity, the correlation between mitogen activity and repression of CPK synthesis is poor, and brain FGF appears not to be mitogenic for BC₃H1 cells under our assay conditions. Thus, while the subcellular signals generated by the binding of these mitogens to a receptor may be the same for cell growth and for repression of cell differentiation, cell growth per se appears not to be required for the control of the synthesis of M-CPK and, by inference, for the control of the repression of other gene products characteristic of differentiated BC₃H1 cells.

Mitogenic polypeptides, when added to quiescent cells such as fibroblasts in the G₀ phase of the cell cycle, induce a series of sequential events which, 8–12 h later, result in the initiation of DNA synthesis and ultimately cell division (27). Differentiating BC₃H1 cells are arrested early in the G₁ phase of the cell cycle (Lathrop, B., unpublished observation). We may therefore speculate that synthesis of differentiated proteins such as M-CPK is only possible in the metabolic environment of cells in G₀. Addition of mitogenic polypeptides alters this

environment so that synthesis of M-CPK is no longer possible, presumably because mRNA synthesis for this molecule ceases, and existing mRNA is degraded. We have shown previously that addition of 20% FCS to differentiating BC₃H1 cells decreases the level of translatable M-CPK messenger extractable from these cells, and the decrease in the rate of M-CPK synthesis observed in the present experiments presumably is a measure of M-CPK message in these cells. In this simple model, transit through the cell cycle is not required for FGF to exert its effect, and simply changing the cellular composition (proteins, ions, pH) from that in cells at G₀ will have the effect of preventing further synthesis of M-CPK. In other words, partial transit through G₁ will block differentiation. Experiments are in progress to critically test this model. As an example, there have been recent observations that adenovirus 2E1A protein, a c-myc-like protein, can enhance as well as block transcription of different genes (2). The increase in the concentration of such proteins brought about as a result of the addition of growth factor to cells might serve both to initiate transit through the cell cycle while simultaneously blocking transcription of genes associated with cell differentiation.

Although the receptor or receptors for FGF in these and other cells remain to be characterized, it seems likely that they will share characteristics in common with better characterized mitogen receptors, which are tyrosine-specific protein kinases and rapidly stimulate a number of metabolic events in cells, including activation of ion fluxes, etc. It would be of considerable interest, in light of the specific effects of FGF on the differentiation of BC₃H1 cells, to better characterize the interaction of FGF with these cells, including characterization of the receptor and of the early events that follow the interaction of FGF with its receptor(s).

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