Differential Phenotypic Expression Induced in Cultured Rat Astroblasts by Acidic Fibroblast Growth Factor, Epidermal Growth Factor, and Thrombin*

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We compared the effects of three growth factors, acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), and thrombin, on rat astroblast proliferation, morphology, glutamine synthetase-specific activity, and phenotypic expression of proteins. In vitro experiments were made on 20-day-old primary cultures. Astroblast proliferation was stimulated transiently (after 48 h treatment) by the three growth factors, while the cell glutamine synthetase activity began to increase significantly only after 3 days of treatment. Acidic FGF and EGF, but not thrombin, modified the cell morphology. The effects on phenotypic expression were first determined after 5 days of treatment to minimize the mitogenic effect of the factors. Proteins synthesized during the last 18 h of the treatments were separated by two-dimensional polyacrylamide gel electrophoresis. About 600 spots were compared, 54 were modulated by the various treatments, 13 were altered similarly by all three factors, 28 by aFGF and EGF, 7 by only aFGF, 3 by only EGF, and 3 by only thrombin.

These results indicate a large similarity of effects between aFGF and EGF (41 proteins) and show that these factors elicit a more extended modulation of the phenotypic expression than thrombin (13 proteins). Each of the three factors has a few specific effects, which suggests that even for aFGF and EGF, which are supposed to elicit their effects through membrane receptor-associated tyrosine kinase activity, some specificity appears in their mechanism of action.

A model is proposed to suggest that cell maturation is characterized by the modulation of the synthesis of many proteins which can be grouped into classes. Each class appears to be under the control of one regulatory element. The specificity of the effect of a growth factor should result from the activation of a specific combination of such regulatory elements.

Analysis of the proteins after only 18 h of treatment, when neither proliferation nor maturation were significantly affected, showed that 11 proteins were regulated only at that time. These proteins could be related to intermediate steps of the growth factor signal transduction.

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Rat astroblasts in primary culture provide a good model for such investigations because they are responsive to numerous growth factors, particularly aFGF, EGF, and thrombin (6-8). It was reported that in other cell types aFGF and EGF receptors are tyrosine kinases whereas thrombin receptor is not (3, 9-11). In the present work we compared the effects of these three growth factors on cell proliferation, cell morphology, glutamine synthetase activity, an enzyme specific for astrocytes in the central nervous system (12), and individual protein synthesis analyzed by two-dimensional polyacrylamide gel electrophoresis.

This study was designed to obtain new information about the effects of growth factors at the cellular level and about the differences between effects elicited by different factors. The aim, in terms of mechanisms, was to obtain indirect evidence of some specificity in the pathways of the growth factor action and to test the hypothesis that maturation proteins, regulated by the growth factors, can be grouped into a few classes or sets, each of which should be under the control of one regulatory element (putative regulatory transcription factor).

1 The abbreviations used are: EGF, epidermal growth factor; PDGF, platelet-derived growth factor; aFGF, acidic fibroblast growth factor; LDS, lithium dodecyl sulfate; CHAPS, 3-(3-cholamidopropyl)dimethylammonio)-1-propanesulfonic acid; IdUrd, iododeoxyuridine.

8319
EXPERIMENTAL PROCEDURES

Materials and Reagents—Falcon tissue culture Petri dishes were purchased from Becton and Dickinson; Waymouth's MD 760/1 medium and fetal calf serum were from Gibco. Bovine insulin (1-5600), fatty acid-free bovine serum albumin (A-6003), Nonidet P-40 (N-4500), and EGF (E-1257) were from Sigma. Highly purified human thrombin, 3600 units (NIH)/mg, 1 mg/ml in 0.75 M NaCl, was prepared at the Centre National de Transfusion Sanguine de Strasbourg, France, by Dr. J. M. Freyssinet (INSERM U311). Acidic fibroblast growth factor (aFGF) was purified from bovine brain (6). [35S]Methionine was supplied by CEA, France, and [3H]thymidine was from New England Nuclear, Great Britain. Lithium dodecyl sulfate (LDS) and CHAPS were from Serva.

Preparation of Adult Rat Brain Neurons—Rat brains, and [125I]iododeoxyuridine was from New England Nuclear, Great Britain. In some experiments, rat brains were dissociated by passage through a 2-mm diameter needle and used for electrophoretic experiments.

Astroblast Cultures—Astroblasts were prepared as described by Garrels (18) with some modifications (19). The cells were dissociated by passage through a 2-mm diameter needle and cultured in a medium consisting of Waymouth's medium supplemented with 5 mg/ml insulin and 0.5 mM EDTA, antibiotics, and sodium pyruvate (14). Acidic FGF (4 ng/ml), EGF (10 ng/ml), or thrombin (0.25 units/ml) were added to the cultures just after the nutrient medium change. Eighteen hours after harvesting, the medium was changed again to a low methionine-containing medium (1 mg/ml instead of 50 mg/liter). Only 1 ml of nutrient medium was added. At the same time growth factors were added and eventually the radioactive precursors [35S]methionine or [3H]IdUrd. For cells treated for 5 days a further medium change and treatment was done at day 23, days after the beginning of the treatment.

Glutamine Synthetase Assay and Protein Determination—Cells were rinsed three times with 0.9% NaCl, sedimented, and frozen at −20 °C. A small volume of 10 mM imidazole, 0.5 mM EDTA, pH 7, was added to the cell pellets and vortexed. Cell suspensions were then sonicated three times for 10 s with a MSE Ultrasonic disintegrator Mk2. The glutamine synthetase assay was performed as described by Miller et al. (15) with slight modifications. Final concentrations in the reaction mixture were 40 mM imidazole HCl, pH 7, 30 mM glutamine, 0.4 mM sodium ADP, 0.5 mM MnCl2, 20 mM sodium arsenate, and 65 mM hydroxylamine HCl. Between 50 and 250 μl of the sonicated sample were added in a total volume of 0.4 ml and incubated at 37 °C for 20 min. The reaction was stopped by addition of 1 ml of 220 mM Fe(NO3)3 in 2.5% trichloroacetic acid (16). The reaction mixture was centrifuged at 4000 × g for 5 min and degassed. The γ-glutamyl hydroxamate produced was determined from the optical density at 500 nm. Proteins were measured by the method of Lowry et al. (17) using bovine serum albumin as standard.

First Dimension: the acrylamide isoelectric focusing gel consisted of 9.2% urea, 3.78% (w/v) acrylamide, 0.22% (w/v) bisacrylamide, 2% (w/v) Nonidet P-40, 2% (w/v) amyllopectin (same mixture as for the lysis buffer). Gels were prerun as described by Garrels (18): initial voltage was 300 V until current stabilization and 1000 V thereafter. Isoelectric focusing was performed at 1000 V for 1 h, and then the gels were placed at once in LDS sample buffer containing 10% (w/v) glycerol, 2.3% (w/v) LDS, and 0.0625 M Tris-HCl, pH 6.8, prior to freezing at −80 °C.

Second dimension: LDS-polyacrylamide gel electrophoresis was performed as described by Lasenmull (21). Gels (200 × 200 × 1 mm) consisted only of a separation gel containing 15% (w/v) acrylamide (bisacrylamide/acrylamide = 0.006) and were run at 20 mA/gel constant current overnight until the dye front reached the bottom. The gels were then soaked in 50% methanol overnight, dried, and exposed to Kodak diagnostic film X-Omat AR at 4 °C for 24 h, 4, or 15 days.

RESULTS AND DISCUSSION

Stimulation of Astroblast Proliferation and Maturation by Growth Factors—Quiescent astroblasts (at 20 days in vitro) were treated with aFGF, EGF, and thrombin for 5 days, and incorporation of [3H]IdUrd was determined every day after 18 h of labeling. Fig. 1A shows that the three growth factors produced a sharp transient stimulation of astroblast proliferation. Acidic FGF had a much stronger mitogenic activity than EGF and thrombin although the three factors were used at their optimum doses.

Glutamine synthetase activity, which is a biochemical indicator of astroblast maturation, was determined at the same time intervals as used for the proliferation experiments. Fig.

![Fig. 1. Time course of the effects of aFGF, EGF, and thrombin on the incorporation of [3H]IdUrd (A) and on glutamine synthetase (GS)-specific activity (B) in rat astroblasts in culture. Astroblasts were treated with the growth factors from day 20 in vitro as described under "Experimental Procedures." [3H]IdUrd (0.5 μCi/ml) was added 18 h before harvesting. Glutamine synthetase activity was determined on parallel cultures. A representative experiment is shown. Cells were treated with aFGF (A), EGF (B), and thrombin (C). Error on triplicate dishes was less than 10% for incorporation and less than 15% for glutamine synthetase activity.](image-url)
1B shows that the three factors elicited a progressive increase in glutamine synthetase activity. The effect of EGF was more drastic than that of aFGF, whereas the effect of thrombin was very weak. Two remarks can be advanced concerning the comparison of the effects of the growth factors on proliferation and glutamine synthetase activity of astroblasts. First, the patterns are different: mitogenic effect is expressed only transiently between 24 and 48 h of treatment while the biochemical effect increases with time after 48 h of treatment. Second, the efficiencies of the growth factors are not parallel on the two phenomena: decreasing order is aFGF, EGF, and thrombin for proliferation and EGF, aFGF, and thrombin for the glutamine synthetase activity.

These results show that the three growth factors stimulate first proliferation and then maturation. To study the effects of growth factors on maturation, with minor interference of the effects on cell proliferation, a treatment of 5 days is the most effective under our experimental conditions.

Only aFGF and EGF induced a morphological change (Fig. 2). This change was hardly visible at 48 h but it increased with time. It was not possible to keep, more than 5 days, cultures treated with aFGF and to a lesser extent with EGF because these cells become loosely attached to the substratum at that time and begin to come off. The decreased cell adhesion could be related to the modification of the cell morphology.

Regulation of the Protein Synthesis by the Growth Factors—Using the same conditions as in the previous experiments, but after 5 days of treatment, synthesis of individual proteins was analyzed by two-dimensional polyacrylamide gel electrophoresis after incorporation of [35S]methionine for 18 h and exposure to x-ray film. Fig. 3 shows the protein pattern on an autoradiogram exposed for 15 days. About 600 spots are intense enough to be compared visually on different films.

With the various treatments the synthesis of 54 proteins was reproducibly modulated. These proteins are circled and numbered on Fig. 3. Outlined areas have been enlarged to show in more detail the variations of the synthesis of proteins after the treatments. In Fig. 4, protein 19 is not visible in control cells, but is induced by aFGF and EGF. Proteins 5 and 6 are increased by the three treatments, whereas protein 11 is decreased. In Fig. 5, proteins 32, 33, and 34 are stimulated by aFGF and EGF; however, protein 41 almost disappears and protein 13 is repressed by the three growth factors. Fig. 6 shows some specific regulations: protein 51 is inhibited only by EGF, 43 is induced only by aFGF, and proteins 52 and 53 are stimulated only by thrombin.

Classification of the Regulated Proteins—Proteins that had their synthesis modified were classified according to their modulation by the growth factors (Table I). Some proteins were regulated by the three factors (13 proteins), some by aFGF and EGF (28 proteins), and others by only one treatment (13 proteins). The most prominent feature of these results is the great homology between the effects of aFGF and EGF and their difference with thrombin. The majority of the proteins regulated by thrombin are also regulated by the other two factors, and none are modulated by thrombin and only one other factor.

Other differences between thrombin and the two other factors are known and possibly related to the observations made. Acidic FGF and EGF are thought to bind membrane receptors with tyrosine kinase activity, although this property has not been established for astroblasts, while the thrombin receptor has no such activity and consequently is thought to act through a different pathway. Another difference is that aFGF and EGF are known to be endogenous in brain and supposedly play a role in brain development. However, thrombin is not endogenous and probably plays a role in reactive gliosis because it is released after injury during the process of blood clotting.

However, even if the two types of factors do not act through the same pathway and under the same physiological circumstances, it can be seen that most proteins modulated by thrombin are also modulated by aFGF and EGF. The two growth factor types should share some common mechanism of action to induce similar effects. The common effect involves 13 proteins, and the effect common only to aFGF and EGF involves 28 proteins. The difference between aFGF and EGF is given by 10 proteins out of 51 modulated by the two factors. The ratio is small, but these modulated proteins, specifically regulated by either aFGF or EGF, suggest that the mechanism of action of these two factors is not identical. Thus, the effects observed are not the result of only a tyrosine kinase activation. Some specificity must arise from other properties of the membrane receptor or from the existence of other (different) membrane receptors.

From the results of Table I, a scheme can be constructed showing five distinct classes of proteins (Fig. 7). If each class of proteins has its expression modulated by one regulatory element, then the action of the growth factors results in the activation of a small number of such regulatory elements and thus the specificity of each growth factor effect arises from the activation of different combinations of these elements.

Early and Late Protein Modulation by Growth Factors—After cells were treated with the growth factors and [35S]methionine for 18 h, the proteins were analyzed and compared with those of control cells (Table II). Only 16 proteins were found to have their rate of synthesis regulated during the first
FIG. 3. Autoradiogram after electrophoresis on two-dimensional polyacrylamide gel of [35S]methionine-labeled proteins from rat astroblasts treated with aFGF for 5 days. Proteins that varied after 5 days of treatment with the growth factors are numbered as indicated in Table I. Outlined areas are shown enlarged in other figures.

18 h of treatment. Comparison of these proteins with those modified after 5 days of treatment showed that of the 16 proteins concerned, 11 were regulated only within 18 h. Of these 11 proteins, 4 are specific to aFGF and 7 are modulated by all three factors. This finding suggests that these 7 proteins, which are modulated transiently, are involved in an intermediate step common for the three factors, triggering proliferation or maturation. All these proteins are shown and numbered in Fig. 8. Only 5 of the 54 proteins that are regulated at day 5 have their synthesis modified at 18 h. They could be early induced maturation proteins or proteins related to other regulatory functions.

Protein Modulation by aFGF at Three Delays—A more detailed study was undertaken with aFGF. Cells were also treated for an intermediate time of 48 h. Proteins were compared with those of control cells taken at the same time and classified according to the various patterns observed (Table III). Under these conditions, a total of 83 proteins were modulated. The main feature of this experiment is the modulation of 23 new proteins only at 48 h. One (protein 51) was seen before, modulated by EGF. Since at 48 h proliferation was optimum and maturation minimum, one can assume that some of these 23 proteins are involved in the proliferation process, while others are related to intermediate steps of the maturation mechanism. All these proteins are shown and numbered in Fig. 8. Among the 43 maturation proteins not regulated at 18 h, 10 were already modified at 48 h. Among the 11 “early” proteins modulated at 18 h and not at 5 days, 6 were still modified at 48 h, the 5 that show a decrease in amount were modulated by the three factors at 18 h (data not shown). For these 5 proteins an intermediate role in the common maturation pathway can be assumed.

Effect of Serum Withdrawal on Protein Synthesis—For all the experiments described above, astroblasts were initially grown in the presence of serum for 20 days and then transferred to a serum-free defined medium before being treated with the growth factors. In the course of the study of the effects of the growth factors, it appeared that in untreated control cells, harvested at the same delays, modulation of the synthesis of some proteins was observed. This modulation was obviously due to an effect of time after serum withdrawal and also possibly to an effect of time after the renewal of the
culture medium, independently of its composition. Autoradiograms obtained 18 h, 48 h, and 5 days after the culture medium change were compared. A classification was established on the basis of the variation of the spot intensities between 48 and 18 h and between day 5 and 48 h (Table IV). A total of 40 proteins had their syntheses changed, including 13 new proteins not changed in other circumstances. These 13 proteins are numbered in Fig. 8. An area of the autoradiogram is shown enlarged in Fig. 9. The most dramatic changes are proteins 64 and 96 which disappear after 48 h and 5 days, respectively, and proteins 38 and 72 which appear after 48 h without serum.

The high ratio of 13 of 40 proteins modulated specifically by serum, compared with 7 of 48 for aFGF, 3 of 44 for EGF, and 3 of 16 for thrombin, may be related to the presence in serum of a variety of growth factors, hormones, and other active molecules, each one eliciting some specific effects on the astroblasts through various mechanisms.

Concluding Remarks—In this report evidence was presented that growth factors elicit a variety of effects (pleiotropic responses) on rat astroblasts and that the mitogenic effect can be largely dissociated from the maturation effect. The first one is seen mainly at 48 h of treatment (Fig. 1) while cell morphology and glutamine synthetase were affected only after 48 h. Concerning the effect of aFGF on protein synthesis, out of 83 proteins modulated, 35 putative “proliferation” proteins were modulated at 18 or 48 h but not at day 5, whereas 36 putative “maturation” proteins were regulated only at day 5 (Table III). The 12 proteins left were regulated at day 5 and sooner.
Growth Factor-induced Phenotypic Expression

Rat astroblasts were treated in vitro from day 20 to 25 with the growth factors. [35S]Methionine was incorporated for the last 18 h of treatment. Proteins separated by two-dimensional gel electrophoresis were compared with those from untreated cells.

Table I

| Modulation of protein synthesis by growth factor | Number of proteins involved in various classes | Protein numbers
|-----------------------------------------------|----------------------------------------------|------------------|
| aFGF  | EGF  | Thrombin | Number | Subtotal | Total |  |
| +     | +    | +        | 5      | 13      | 41    | 1−8 |
| −     | −    | −        | −5     | 9−13    |       |     |
| +     | −    | +        | 21     | 28      | 14–34 |     |
| −     | +    | −        | 7      | 33−41   |       |     |
| +     | −    | −        | 3      | 42–44   |       |     |
| −     | +    | +        | 4      | 45–48   |       |     |
| −     | −    | +        | 2      | 49–50   |       |     |
| +     | −    | −        | 3      | 51      |       |     |
| −     | +    | −        | 3      | 51–54   |       |     |

* + indicates increase and − indicates decrease.

The localization of these proteins can be seen on the autoradiogram in Fig. 3.

Fig. 7. Scheme for possible organization of the phenotypic expression. Sets or classes of proteins defined in Table I are composed of proteins similarly regulated by more than one growth factor or regulated by only one growth factor. Arrows indicate the sets of proteins activated. Each set of protein should be under the control of a regulatory element noted R1, R2, R3, R4, R5. Numbers in the circles indicate the number of proteins involved. Thr, thrombin.

All three growth factors tested modified the rate of synthesis of a high number of proteins, about 100 proteins were involved out of 600 proteins analyzed. This number of modifications is presumably underestimated because the proteins whose variation was not visually obvious were eliminated. However, since our results come from two fully independent experiments, run in duplicate, our main findings appear to be well established, namely the large similarity between the effects of aFGF and EGF, the less extensive effect of thrombin, and the observation that most proteins altered by thrombin were also modified by the two other factors.

The membrane receptors for FGF, EGF, and thrombin pertain to the two main types of growth factor membrane receptors, each one employing different transducing mechanisms. The receptors for FGF and EGF are endowed with protein tyrosine kinase which becomes activated after ligand binding (3, 9–11). This activation leads to the autophosphorylation of the receptor as well as to the phosphorylation of some substrates (for review, see Ref. 22). The receptor for thrombin does not harbor a tyrosine kinase activity, its early response to activation is hydrolysis of phosphatidylinositol bisphosphate leading to the release of diacylglycerol, which activates protein kinase C (23), and of inositol trisphosphate, which mobilizes Ca2+ from internal stores (5). How the activation of the two receptor types leads to DNA replication is still not clear. Either the two different signaling pathways can act through different ways or there may be a common step somewhere in the cascade of events triggered. For instance, elevation of intracellular calcium could be involved since it also happens after activation of tyrosine kinase-bearing receptors, through influx from external medium (24). Also tyrosine kinase activity present as soluble enzyme in the cytoplasm of various tissue (25) could be activated by thrombin, although no data sustain such possibility. However, it has been reported recently that these two intracellular events are not sufficient to trigger cell proliferation since a PDGF receptor, after deletion of a domain without known activity, is still able to enhance intracellular calcium and to stimulate its tyrosine kinase activity after PDGF binding, without effect on DNA replication (26). To add to the puzzle, the reality of distinct pathways induced by the two types of membrane receptors has been questioned. For instance, although FGF and EGF are inactive on phosphoinositide hydrolysis in some cells (9, 27, 28), EGF also activates this pathway in other cell types (24, 29, 30).

For the regulation of phenotypic expression the situation is even more confused, since some effects elicited by different growth factors are similar and others are different. At the present time, the most obvious hypothesis we can propose is that correlations between the different types of regulation of the protein synthesis (corresponding to the so-called sets), seen after action of the three growth factors studied, and some events of the poorly known intracellular pathways, are relations of cause and effect. Then the few proteins specifically regulated by thrombin could be related to the phosphoinositol

Table II

| Protein synthesis modulation, relative to control cells, after treatments for 18 h or 5 days | Number of proteins modulated as indicated by More than one treatment | Numbers of the new proteins involved |
|-----------------------------------------------------------------|--------------------------|----------------------------------|
| Only one treatment                                             | aFGF and EGF             | aFGF, EGF, and thrombin          | Total                        |
| 18 h 5 days                                                   |                          |                                 |                              |
| ± ±                                                           | ± ±                      | ± ±                             | ± ±                          |
| 0 ±                                                          | 0 ±                      | 0 ±                             | 0 ±                          |
| ± ±                                                          | ± ±                      | ± ±                             | ± ±                          |

Comparison of the modulation of the synthesis of the proteins after 18 h and after 5 days of treatments

Astroblasts were treated with the growth factors for 18 h or for 5 days. The synthesis of the proteins was compared with that of corresponding untreated cells. Three classes of proteins are revealed: those modulated in the same way at 18 h and at 5 days, those modulated only at 5 days (all these proteins have been numbered in Table I), and those modulated only at 18 h. These latter new proteins are numbered.
FIG. 8. Autoradiogram of a polyacrylamide gel after two-dimensional electrophoresis of rat astroblast proteins. Cells at 20 days in vitro were switched to serum-free medium and incubated with [35S]methionine for 18 h. Proteins numbered from 55 to 65 (Δ) were modulated by growth factors only after 18 h of treatment (see Table II). Proteins numbered from 66 to 88 (□) were regulated transiently by aFGF (see Table III). Proteins numbered from 89 to 101 (○) were modulated after serum withdrawal as a function of time, in untreated cells (see Table IV). The outlined area is shown enlarged in Fig. 9.

TABLE III
Kinetics of the modulation of the synthesis of the proteins, relative to control cells, under the effect of aFGF
Astroblasts after 20 days in culture were treated with aFGF for 18 h, 48 h, or 5 days. The synthesis of each protein, evaluated by the incorporation of [35S]methionine, was compared with that seen in untreated cells grown and harvested like the treated cells.

| Modulation of synthesis after treatment for | Number of proteins found in various patterns | Numbers of the proteins modulated |
|-------------------------------------------|---------------------------------------------|---------------------------------|
| 18 h | 48 h | 5 days | Number | Subtotal | Total |
| --- | --- | --- | --- | --- | --- |
| − | − | − | 2 | 2 | 2 | 11, 36 |
| 0 | + | + | 7 | 10 | 46 | 5, 6, 8, 20, 26, 27, 30 |
| 0 | − | − | 3 | 36 | 46 | 10, 13, 39 |
| 0 | 0 | + | 25 | 46 | 46 | 1-4, 14-19, 21-25, 28, 29, 31-34, 42-44 |
| 0 | 0 | − | 11 | 24 | 24 | 9, 12, 37, 38, 40, 41, 45-48 |
| 0 | + | 0 | 13 | 6 | 11 | 51, 66-77 |
| 0 | − | 0 | 11 | 6 | 65 | 78-88 |
| + | + | 0 | 1 | 6 | 11 | 65 |
| − | − | 0 | 5 | 6 | 60-64 |
| + | 0 | 0 | 2 | 5 | 5 | 60-64 |
| − | 0 | 0 | 3 | 5 | 5 | 60-64 |
breakdown. The specific effects elicited by FGF or EGF could originate from an action of the growth factor molecules themselves, after internalization, or from some specificity of the membrane receptors. Recent data support the possibility of unconventional transduction pathways. First, FGF and EGF molecules have been shown to reach the nucleus and to bind chromatin (31–33). They could then elicit specific effects. Second, specific receptor domains like that of the PDGF can modulate the rate of transcription. This assumption has several limitations; for instance, the present work did not investigate a regulatory role at the level of translation or of the modulation of the mRNA stability. It only took into account the long term effects of the factors but did not consider the early events that mediate the final effects in a complicated way. Comparative synthesis of the proteins was evaluated only by a “+” (increase) or “−” (decrease), fine quantitative variations were not taken into account. The classes of proteins that we proposed could actually be composed of several subclasses that should be revealed after treatment with other growth factors or hormones. The regulatory factors themselves could be composed of several proteins since there is now evidence of multiple protein-binding sites on the promoter region of genes (for review see Ref. 34), suggesting that at least some genes are under the control of several regulatory proteins that can modulate the rate of transcription.

The comparison of phenotypic expression, under the effect of various extracellular inducers, through the use of two-dimensional polyacrylamide gel electrophoresis, could provide general information on the process of cell maturation. Correlations between the activation of sets of proteins and the previous appearance or modification (phosphorylation) of nuclear proteins, after the action of various growth factors and hormones, could help us identify the putative regulatory transcription factors involved in this phenomenon.

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Growth Factor-induced Phenotypic Expression

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