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Acidic and basic fibroblast growth factors similarly regulate the rate of biosynthesis of rat astroblast proteins

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Quiescent rat astroblasts in culture have been treated for various periods of time with acidic and basic fibroblast growth factors. Both factors elicited similar effects on the cell proliferation and glutamine synthetase activity. The rate of biosynthesis of the proteins analyzed on autoradiograms of polyacrylamide gels after two-dimensional electrophoresis was also similarly modulated by the two growth factors. These results suggest that the two fibroblast growth factors act through the same membrane receptors on rat astroblasts in culture.

Fibroblast growth factor; Astroblast; Two-dimensional gel electrophoresis; Cell maturation

1. INTRODUCTION

The basic fibroblast growth factor (bFGF) is the main representative of a family of heparin-binding growth factors [1-6]. It is found in nearly all tissues and synthesized by various cell types [1-3], while the second factor of the family, the acidic fibroblast growth factor (aFGF), is found mainly in the nervous system [7]. Only recently the presence of aFGF has been detected in another tissue, the kidney, in very low amounts [8]. Up to now in most works on the nervous system or on neural cells, only one of the two FGFs was tested and most often bFGF [9-12]. On other cell types no specificity for one of these factors has yet been found [13-15]. All cells tested respond to both factors although usually a higher dose (10-100 times) of aFGF is needed to elicit the same effect as bFGF [13-15]. The high degree of amino acid sequence homology (about 53%) [16] suggests the possibility that both factors bind to the same cell membrane receptors. Indeed, it has been found that each FGF can compete with the order for binding on various cells or cell membranes [17,18]. No such studies have been made on neural cells. Since in a previous work we observed some transient differences between the morphological effects elicited by aFGF and bFGF on rat astroblasts in culture [19], we investigated the effects of the two factors in more detail. We used the technique of two-dimensional polyacrylamide gel electrophoresis to search for a possible difference between the effects of the two factors at the level of the modulation of individual protein synthesis. We did not find any significant difference.

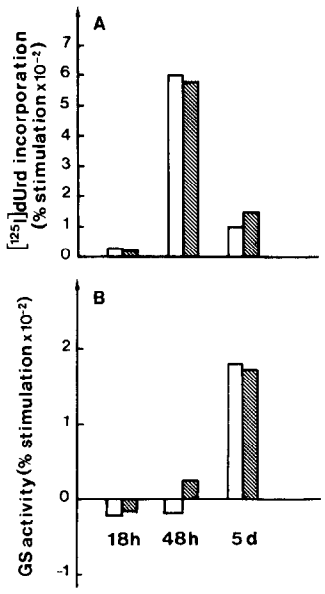

Fig.1. Effects of aFGF (open columns) and bFGF (hatched columns) on [^{125}I]dUrd incorporation (A) and glutamine synthetase activity (B) in rat astroblasts grown for 20 days in culture before treatment. Cells were treated with FGFs (5 ng/ml) for 18 h, 48 h and 5 days as described in section 2. In control cells, incorporation of [^{125}I]dUrd was 2500 cpm and glutamine synthetase activity was 62 nmol · min^{-1} · mg^{-1} protein. Results are means of triplicate dishes and of two independent experiments. Errors were less than 15%.

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Abbreviations: CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; FGF, fibroblast growth factor; GS, glutamine synthetase; [^{125}I]dUrd; [^{125}I]iododeoxyuridine; IEF, isoelectric focusing; LDS-PAGE, lithium dodecylsulfate-polyacrylamide gel electrophoresis

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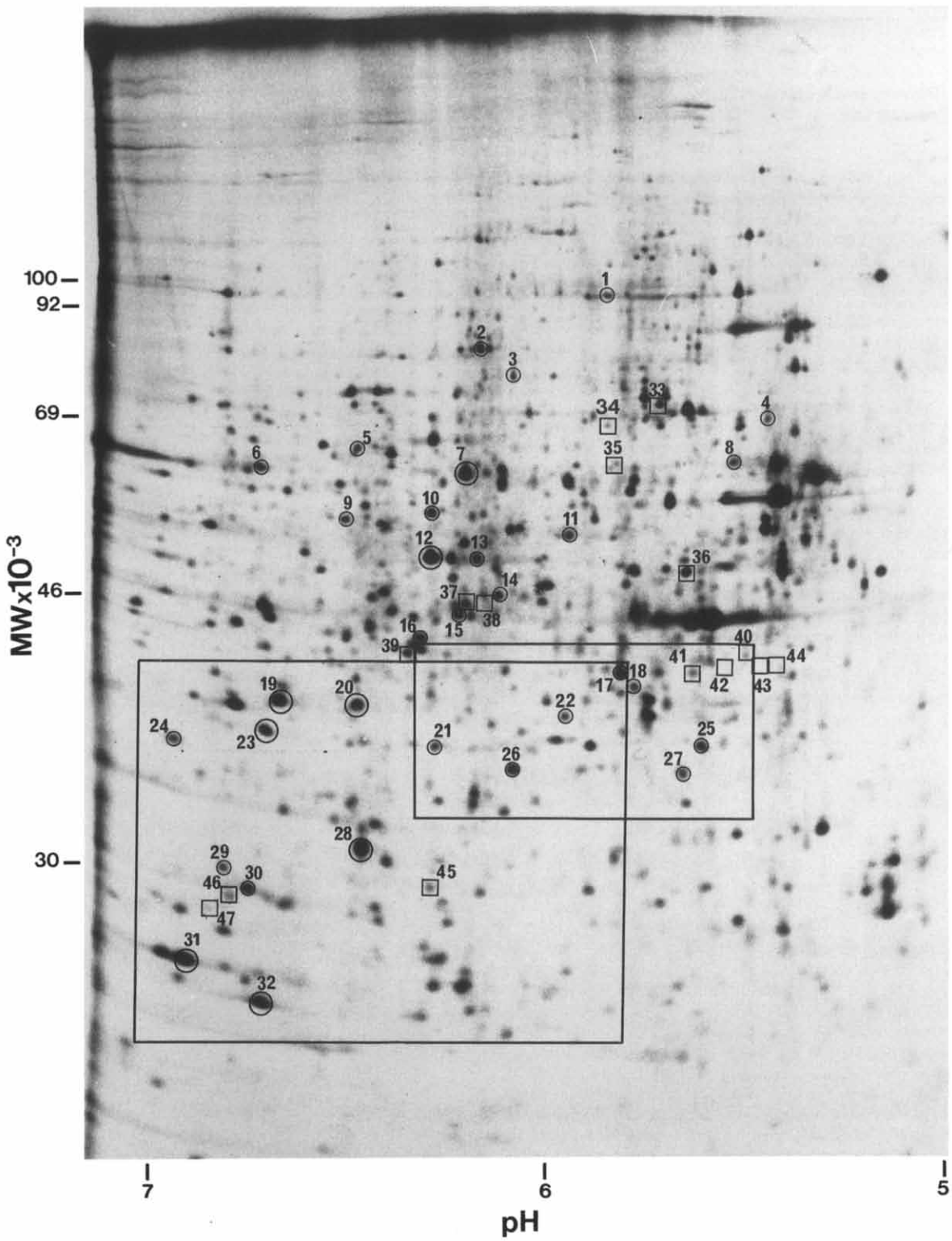

Fig.2. Autoradiogram of a polyacrylamide gel after two-dimensional electrophoresis of rat astroblast proteins. Cells were grown for 20 days in the presence of 10% fetal calf serum, and then switched to a serum-free medium. They were treated with 5 ng/ml aFGF for 5 days. [^{35}S]Methionine was added 18 h before harvesting. X-ray film was exposed for 15 days. Proteins whose rate of synthesis was increased under the effect of aFGF are in circles. Those which were repressed are shown in squares. Delimited areas, the large one and the small one, are shown in fig.3 and fig.4, respectively.

2. MATERIALS AND METHODS

2.1. *Materials*

Materials have been described previously [20]. aFGF and bFGF were purified from bovine brain according to the published procedure of Pettmann et al. [21].

2.2. *Cell culture*

Cultures of astroblasts were prepared by the method of Booher and Sensenbrenner [22] with slight modifications as described previously [20]. Culture medium was Waymouth's MD 705/1 medium supplemented with sodium pyruvate (110 mg/l), antibiotics, and 10% fetal calf serum. After 20 days in culture, when rat astroglial cells were mostly quiescent, they were switched to a serum-free chemically defined medium containing Waymouth's medium, 5 μg/ml insulin, 0.5 mg/ml bovine serum albumin, antibiotics and sodium pyruvate [23].

2.3. *Cell treatments*

For all the experiments shown, cells were treated with aFGF and bFGF for 18 h, 48 h or 5 days from day 20 onwards, just after the culture medium change. Eighteen hours before harvesting the culture medium was changed and a special medium containing only 1 mg/l methionine, instead of 50 mg/l in the normal medium, was used and eventually a labeled precursor, [^{125}I]dUrd or [^{35}S]methionine, and FGFs were added. Cells treated for only 18 h were switched to this special medium at the beginning of their treatment. Cells treated for 48 h stayed for 30 h in the normal defined medium and for 18 h in the special medium. Cells treated for 5 days were submitted to one more medium change at 72 h and switched to the methionine-poor medium 18 h before harvesting. For treated cultures, FGFs were added at each medium change.

2.4. *Glutamine synthetase (GS) determination*

Culture dishes were put on ice. and rinsed three times with cold isotonic NaCl. Cells were scraped off with a rubber policeman, sedimented and the pellets were kept frozen at -20°C until use. For the determinations, the method of Miller et al. [24] was used with slight modifications.

2.5. *[^{125}I]dUrd incorporation*

Cells were treated with 0.5 μCi [^{125}I]dUrd in 1 ml culture medium for 18 h, then they were harvested as described for GS determination. Radioactivity of the cell pellets was counted in a gamma-counter.

2.6. *[^{35}S]Methionine labeling*

Cells were treated with 50 μCi of [^{35}S]methionine in 1 ml culture medium for 18 h and harvested as described for GS determination. Cell pellets were kept frozen at -80°C.

2.7. *Two-dimensional polyacrylamide gel electrophoresis*

Samples were prepared as described by Garrels [25] and 2D-PAGE was performed as described by O'Farrel [26] with some modifications [20,27]. First dimension was isoelectric focusing and second dimension LDS-PAGE. Gels were then incubated in 50% methanol for 16 h, dried under vacuum and exposed to Kodak film X AR5 at 4°C for 15 days and eventually for 24 h and for 4 days to allow comparison of the most intense spots.

3. RESULTS

The effects of the two FGFs on proliferation and on GS activity were investigated on rat astroblasts grown for 20 days in culture (fig.1). A transient increase in [^{125}I]dUrd incorporation was observed after 48 h of treatment (fig.1A) while GS activity was not significantly affected at that delay. In an opposite way to proliferation, GS activity increased from 48 h to 5 days after the beginning of the treatment (fig.1B). For studies on the regulation of protein synthesis, cells were treated with each FGF for 5 days since at that time the effect on proliferation is finished and the effect on

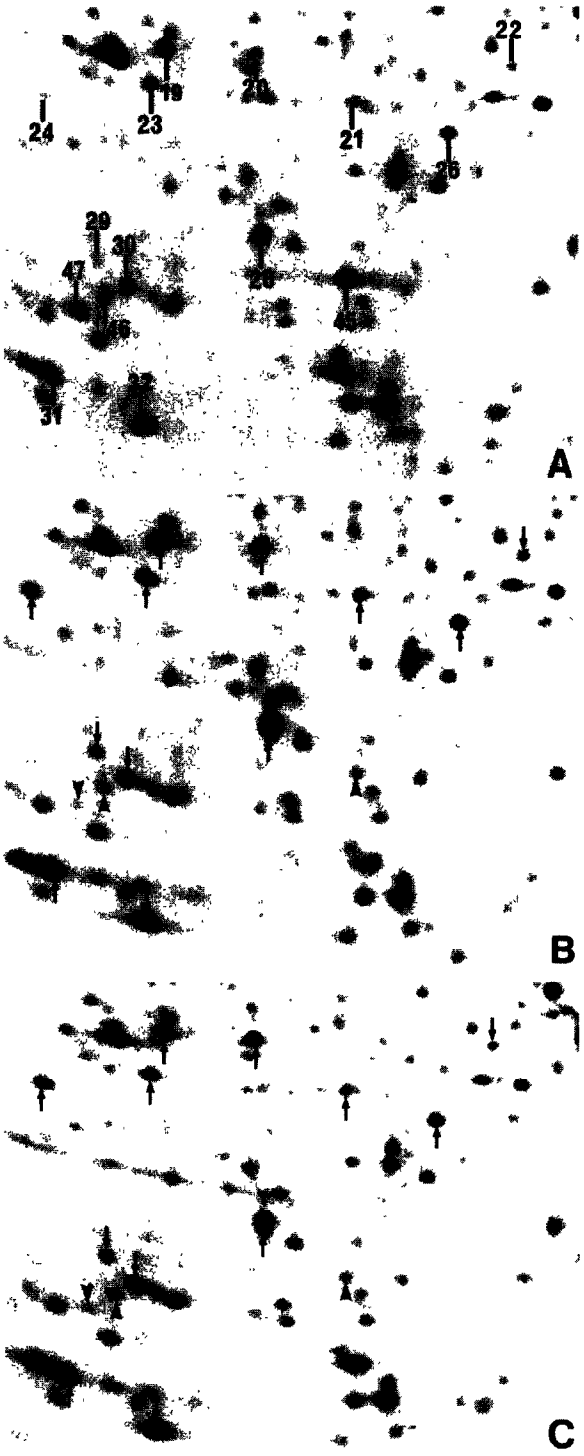

Fig.3. Comparison of the effects of aFGF and bFGF on protein synthesis. (A) Control cells. (B) Cells treated for 5 days with aFGF and (C) bFGF. Arrows show spots which are intensified by treatments. Arrowheads indicate spots whose intensity decreased after treatments.

maturation is prevailling. A dose-response effect of each FGF on protein synthesis was also investigated in order to prevent possible differences due to this parameter.

For two-dimensional polyacrylamide gel electrophoresis, three independent experiments were done and, for each one, duplicate gels were run. An autoradiogram of the proteins of cells treated with aFGF is shown in fig.2. The darkness and the size of homologous spots were compared visually between gels from control and from treated cultures. Out of about 600 spots which were compared, 47 were found to be reproducibly and obviously altered by the FGFs. They are numbered on fig.2. The synthesis of 32 proteins was enhanced, they are circled; that of 15 proteins was diminished, they are shown in squares. The larger delimited area is shown in fig.3 to allow comparison between control cells and cells treated with aFGF and bFGF. No significant difference was observed between the effects of the two FGFs. The same results were also seen on the whole gel.

The smaller delimited area of fig.2 is shown after treatments with increasing doses of bFGF (fig.4). For the most intense spots, a modulated progressive variation of intensity can be observed, for instance with spots 17, 18 and 26.

4. DISCUSSION

On rat astroblasts grown in primary culture, acidic and basic FGFs elicit similar effects on proliferation, on glutamine synthetase activity and on the rate of synthesis of the proteins. These results corroborate some of our previous results on astroblast proliferation and maturation obtained in various conditions [21,28]. Although the analyses of the autoradiograms have been done visually, the main result which is a similarity between the effects of aFGF and bFGF cannot be disputed since, with the same technical approach, in a previous work we have shown that epidermal growth factor and thrombin modulated the synthesis of many proteins differently than aFGF [20].

Our present results bring indirect indication that, like on other cells, both FGFs are acting on rat astroblasts probably through the same membrane receptors. If the physiological effects of FGFs are not all mediated by specific membrane receptors, but also result from a direct action of the factors inside the cell nucleus as it has been suggested [29], it is likely that the effects due to this latter intracellular action of FGFs molecules are also similar.

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REFERENCES

[1] Gospodarowicz, D. and Cheng, J. (1986) J. Cell Physiol. 128, 475–484.
[2] Mormede, P., Baird, A. and Pigeon, P. (1985) Biochem. Biophys. Res. Commun. 128, 1108–1113.
[3] Schweigerer, L., Neufeld, G., Friedman, J., Abraham, J., Fiddes, J.C. and Gospodarowicz, D. (1987) Nature 325, 257–259.
[4] Miyagawa, K., Sakamoto, H., Yoshida, Y., Yamashita, Y., Mitsui, Y., Furusawa, M., Maeda, S., Takaku, F., Sugimura, T. and Terada, M. (1988) Oncogene 3, 383–389.
[5] Bovi, P.D., Curatola, A.M., Kern, F.G., Greco, A., Ittmann, M. and Basilico, C. (1987) Cell 50, 729–737.
[6] Dickson, C. and Peters, G. (1987) Nature 326, 833.

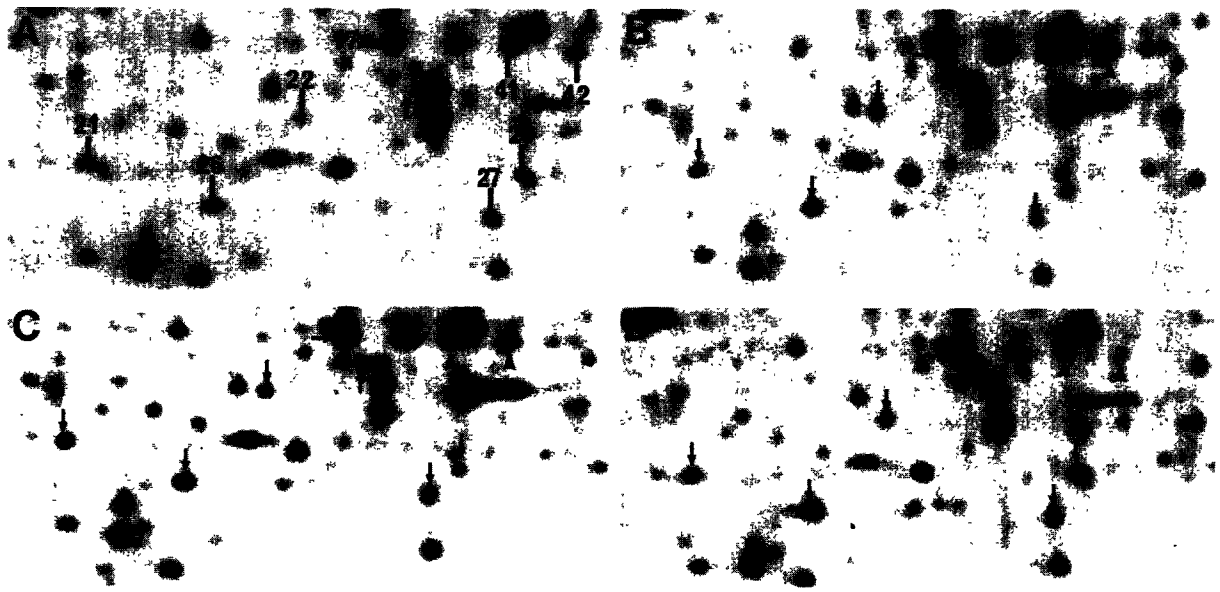

Fig.4. Dose-response effect of bFGF on protein synthesis analyzed by two-dimensional polyacrylamide gel electrophoresis. (A) Control cells. Cells treated for 5 days with bFGF. (B) 0.25 ng/ml, (C) 2.5 ng/ml, (D) 5 ng/ml. Other specifications as for fig.3.

[7] Thomas, K.A., Rios Candelore, M. and Fitzpatrick, S. (1984) Proc. Natl. Acad. Sci. USA 81, 357-361.
[8] Gautschi-Sova, P., Jiang, Z., Frater-Schroder, M. and Bohlen, P. (1987) Biochemistry 26, 5844-5847.
[9] Rodan, S.B., Weslowski, G., Thomas, K. and Rodan, G.A. (1987) Endocrinology 121, 1917-1923.
[10] Morrison, R.S., Sharma, A., DeVellis, J. and Bradshaw, R.A. (1986) Proc. Natl. Acad. Sci. 83, 7537-7541.
[11] Unsicker, K., Reichert-Preibsch, H., Schmidt, J.R., Pettmann, B., Labourdette, G. and Sensenbrenner, M. (1987) Proc. Natl. Acad. Sci. USA 84, 5459-5463.
[12] Baird, A., Mormede, P., Ying, S., Wehrenberg, W.B., Ueno, N., Ling, N. and Guillemin, R. (1985) Proc. Natl. Acad. Sci. USA 82, 5545-5549.
[13] Conn, G. and Hatcher, V.B. (1984) Biochem. Biophys. Res. Commun. 124, 262-268.
[14] Courty, J., Loret, C., Chevallier, B., Moenner, M. and Barritault, D. (1987) Biochimie 69, 511-516.
[15] Lobb, R.R., Harper, J.W. and Fett, J.W. (1986) Anal. Biochem. 154, 1-14.
[16] Esch, F., Ueno, N., Baird, A., Hill, F., Denoroy, L., Ling, N., Gospodarowicz, D. and Guillemin, R. (1985) Biochem. Biophys. Res. Commun. 133, 554-562.
[17] Neufeld, G. and Gospodarowicz, D. (1986) J. Biol. Chem. 261, 5631-5637.
[18] Neufeld, G., Gospodarowicz, D., Dodge, L. and Fujii, D.K. (1987) J. Cell Physiol. 131, 131-140.
[19] Perraud, F., Labourdette, G., Miehe, M., Loret, C. and Sensenbrenner, M. (1988) J. Neurosci. Res. 20, 1-11.
[20] Loret, C., Sensenbrenner, M. and Labourdette, G. (1989) J. Biol. Chem. 264, 8319-8327.
[21] Pettmann, B., Weibel, M., Sensenbrenner, M. and Labourdette, G. (1985) FEBS Lett. 189, 102-108.
[22] Booher, J. and Sensenbrenner, M. (1972) Neurobiology 2, 97-105.
[23] Weibel, M., Pettmann, B., Daune, G., Labourdette, G. and Sensenbrenner, M. (1984) Int. J. Dev. Neurosci. 4, 355-366.
[24] Miller, R.E., Hackenberg, R. and Gershman, H. (1978) Proc. Natl. Acad. Sci. USA 75, 1418-1422.
[25] Garrels, J.I. (1979) J. Biol. Chem. 254, 7961-7977.
[26] O'Farrell, P.H. (1975) J. Biol. Chem. 250, 4007-4021.
[27] Loret, C., Sensenbrenner, M. and Labourdette, G. (1988) Cell Differ. Dev. 25, 37-46.
[28] Perraud, F., Besnard, F., Pettmann, B., Sensenbrenner, M. and Labourdette, G. (1988) Glia 1, 124-131.
[29] Bouche, G., Gas, N., Prats, H., Baldia, V., Tauber, J.P., Teissié, J. and Amalric, F. (1987) Proc. Natl. Acad. Sci. USA 84, 6770-6774.