Regulation of protein metabolism and DNA synthesis by fibroblast growth factor in BHK-21 cells

Marina ROSS and F. John BALLARD

CSIRO Division of Human Nutrition, Kintore Avenue, Adelaide, S.A. 5000, Australia

1. Acidic and basic fibroblast growth factors (aFGF, bFGF) were shown to inhibit protein breakdown in BHK-21 cells, with the latter exhibiting approx. 6-fold greater sensitivity. 2. The maximum response achieved was less than observed with insulin or insulin-like growth factor-1 (IGF-1), and was not additive with those growth factors over the 4 h measurement period. The inhibition of protein breakdown followed the same time course with all the growth factors tested, and was enhanced equally by NH_4^+ ions. Taken together, these results suggest similar mechanisms for effects of the different growth factors on protein breakdown. 3. Protein synthesis was stimulated by bFGF, insulin and IGF-1, with partial additivity evident between bFGF and the other peptides. Increases in cell-culture protein content paralleled the changes in the rate of protein synthesis. 4. DNA synthesis was stimulated more effectively in BHK-21 cells by aFGF or bFGF than by insulin or IGF-1, with partial additivity between the two groups of growth factors. 5. Since each of the growth factors independently produced both relatively rapid effects on protein metabolism and more prolonged increases in DNA synthesis, some caution is warranted before classifying them into truly distinct groups as either competence or progression factors.

INTRODUCTION

Protein accumulation of cell cultures maintained in serum-free media is stimulated by insulin or IGF-1 and is a consequence of both an increase in protein synthesis and a decrease in protein breakdown (Ballard, 1982; Ballard & Gunn, 1985; Ballard & Read, 1985; Ballard *et al.*, 1986). Although the response to such peptides occurs rapidly and is typically as large as observed with fetal bovine serum, the inhibition of protein breakdown by insulin or IGF-1 may decrease after some hours, so that serum can eventually produce greater increases in protein content (Ballard, 1982; F. J. Ballard, unpublished work).

An explanation for the sometimes transient effects of insulin and IGF-1 is the concomitant requirement for other factors normally present in serum to sustain the increased rate of protein accumulation. Thus research by Stiles, Pledger and co-workers (Scher *et al.*, 1979; O'Keefe & Pledger, 1983; Stiles, 1985) demonstrated the need for a competence factor such as platelet-derived growth factor or FGF as well as IGF-1 or another progression factor for the cell cycle to be completed. In the present report we have examined the effects of FGF and the insulin-like growth factors on protein synthesis and breakdown in BHK-21 cells, with particular emphasis on the identification of interactions between the different growth factors that could lead to enhanced cell growth.

MATERIALS AND METHODS

Materials

Acidic and basic FGF purified from bovine brain were kindly provided by Dr. Peter Böhlen and Dr. Peter Gautschi, Biochemisches Institut, Universität Zurich.

Bovine IGF-1 was purified from bovine colostrum (Francis *et al.*, 1986). Insulin was Actrapid from Novo Industri A/S, Copenhagen, Denmark. Growth factors were diluted in Hanks salts containing 0.1% bovine serum albumin. Fetal bovine serum (batch 29101780), Waymouth's MB 752/1 medium and Dulbecco's modification of Eagle's medium were obtained from Flow Laboratories, North Ryde, Australia; bovine serum albumin (radioimmunoassay grade), thymidine, amino acids and vitamins for the preparation of media were from Sigma Chemical Co., St. Louis, MO, U.S.A.; L-[4,5- ^3H]leucine (40–60 Ci/mmol) and [methyl- ^3H]thymidine (20 Ci/mmol) were from New England Nuclear, Boston, MA, U.S.A. Human lung fibroblasts [HE(39)L], Balb/c3T3, EBTr and BHK-21 cells were obtained from Commonwealth Serum Laboratories, Melbourne, Australia; Swiss 3T3 fibroblasts were generously provided by Dr. H. Herschman, Department of Biological Chemistry, UCLA, L6 myoblasts by Dr. J. M. Gunn, Department of Biochemistry and Biophysics, Texas A & M University, SD8 ArA cells by Dr. J. Funder, Prince Henry's Hospital, Melbourne, and human skin fibroblasts (SF 1972) by Dr. W. Carey, Adelaide Children's Hospital. Cultures of chick-embryo fibroblasts (Hauschka & Konigsberg, 1966) were used between the third and sixth passage.

Measurement of protein breakdown

Cells were grown to confluence in 24-place multiwell dishes, labelled for 18 h with [^3H]leucine and chased for 3 h as described previously (Ballard *et al.*, 1986). The measurement was carried out in the presence of added growth factors, by using a 1:1 mixture of Dulbecco's modification of Eagle's medium and Waymouth's MB 752/1 medium with the leucine concentration adjusted to 2 mM. The percentage of protein degraded during the

Table 1. Protein-breakdown inhibition by growth factors in several cell lines

Protein breakdown measured over a 4 h period immediately after addition of the respective growth factor was expressed as the percentage inhibition compared with serum-free medium. Values are mean effects $\pm$ S.E.M., with the numbers of determinations given in parentheses.

Cell line	Inhibition of protein breakdown (%)			
	aFGF (1 nM)	bFGF (1 nM)	Insulin (1 μ M)	IGF-1 (10 nM)
BHK-21 (12)	21 $\pm$ 1	20 $\pm$ 1	34 $\pm$ 1	30 $\pm$ 1
Swiss 3T3 (8)	16 $\pm$ 3	14 $\pm$ 3	33 $\pm$ 1	33 $\pm$ 1
Chick-embryo fibroblasts (4)	2 $\pm$ 1	2 $\pm$ 2	31 $\pm$ 1	32 $\pm$ 1
EBTr (4)	-2 $\pm$ 1	-2 $\pm$ 1	21 $\pm$ 1	22 $\pm$ 1
SD8ArA (4)	1 $\pm$ 1	-4 $\pm$ 2	16 $\pm$ 1	13 $\pm$ 2
L6 (4)	6 $\pm$ 1	4 $\pm$ 2	22 $\pm$ 1	23 $\pm$ 1
Human skin fibroblasts (4)	1 $\pm$ 1	5 $\pm$ 1	23 $\pm$ 2	21 $\pm$ 2
Balb/c 3T3 (4)	12 $\pm$ 1	7 $\pm$ 1	23 $\pm$ 1	24 $\pm$ 1
Human lung fibroblasts (4)	4 $\pm$ 1	1 $\pm$ 1	22 $\pm$ 1	20 $\pm$ 1

measurement period was calculated as 100 times the trichloroacetic acid-soluble radioactivity divided by the total radioactivity in the monolayer (Ballard *et al.*, 1980b, 1986).

Measurement of protein synthesis

Cells grown to near confluence in 24-place multiwell dishes were washed twice with Eagle's Minimal Essential Medium before the measurement period. Protein-

synthesis rates were determined in the presence of added growth factors by the incorporation of [3 H]leucine into cell monolayer protein as described previously (Ballard *et al.*, 1986), but by using a 1:1 mixture of Dulbecco's modification of Eagle's medium and Waymouth's MB 752/1 medium with the leucine concentration adjusted to 2 mM. Rates were expressed either as radioactivity incorporated per well or per mg of cell protein after measuring protein as described by Dulley & Grieve (1975).

Measurement of DNA synthesis

Confluent monolayers in 24-place multiwell dishes were washed twice with Eagle's Minimal Essential Medium over a 2 h period and exposed to growth factors for 18 h in 1 ml of a 1:1 mixture of Dulbecco's modification of Eagle's medium and Waymouth's MB 752/1 medium. At the end of this period, 5 nmol of thymidine containing 1 μ Ci of [3 H]thymidine was added for a further 6 h. Harvesting of monolayers was carried out as described previously (Ballard *et al.*, 1986). Cell protein was measured (Dulley & Grieve, 1975) to determine the differences in protein content.

RESULTS

Cells screened for the ability of both aFGF and bFGF to inhibit protein breakdown that showed negligible effects included chick-embryo fibroblasts, embryonic bovine tracheal (EBTr) cells, rat aortic smooth-muscle (SD8 ArA) cells, rat L6 myoblasts, human skin fibroblasts and human lung fibroblasts (Table 1). Insulin (1 μ M) and bovine IGF-1 (10 nM) produced substantial inhibitory effects in all these cell lines. Significant responses of FGF on protein breakdown were obtained with Balb/c3T3, Swiss 3T3 and especially BHK-21 cell lines (Table 1). BHK-21 cells have been used for the remaining experiments in this paper.

The concentration-dependence of FGF effects on protein breakdown is illustrated in Fig. 1. Both aFGF and bFGF produce a similar maximum response of 22% at 1 nM, but half-maximum inhibitions occur at 0.02 nM-bFGF and 0.12 nM-aFGF. Insulin and IGF-1 also inhibit protein breakdown in BHK-21 cells, the former with a

Fig. 1. Inhibition of protein breakdown in BHK-21 cells

Growth factors were added at the indicated concentrations, and the percentage inhibition of protein breakdown in relation to the rate in serum-free medium was measured over a 4 h period. Values are means of eight determinations, with S.E.M. indicated where sufficiently large. C, control medium; ○, aFGF; ●, bFGF; △, insulin; ■, IGF-1.

Table 2. Additivity of effects on protein breakdown

Measurements were made over a 4 h period and are expressed as the percentage of labelled protein degraded, $\pm$ S.E.M. for four determinations. The percentage inhibition of breakdown is expressed relative to the rate in medium alone, except where indicated by superscripts a, b and c, where they are relative to the rate in medium containing IGF-1, insulin or bFGF respectively.

Additions	Protein degraded (%)	Inhibition (%)
None	9.58 $\pm$ 0.07	—
Fetal bovine serum (10 %)	6.81 $\pm$ 0.06	29
IGF-1 (10 nM)	6.94 $\pm$ 0.03	28
Insulin (100 nM)	6.70 $\pm$ 0.17	30
bFGF (1 nM)	8.13 $\pm$ 0.09	15
IGF-1 + insulin	6.81 $\pm$ 0.13	29
IGF-1 + bFGF	6.66 $\pm$ 0.15	30
Insulin + bFGF	6.64 $\pm$ 0.09	30
NH ₄ Cl (5 mM)	7.07 $\pm$ 0.02	26
IGF-1 + NH ₄ Cl	5.30 $\pm$ 0.06	24 ^a
Insulin + NH ₄ Cl	5.14 $\pm$ 0.11	23 ^b
bFGF + NH ₄ Cl	6.21 $\pm$ 0.09	24 ^c

sensitivity comparable with that of bFGF and the latter showing a half-maximum effect at approx. 1.5 nM (Fig. 1). The greater maximum response with insulin or IGF-1 than that observed with aFGF or bFGF (Fig. 1) is striking, and suggests that the two groups of growth factors may produce their effects via different mechanisms. The following two experiments have been carried out to test such a hypothesis.

Firstly, the different growth factors were added in combination at concentrations that individually produce maximum effects. Table 2 shows that the addition of 1 nM-bFGF together with either 10 nM-IGF-1 or 100 nM-insulin produces no further inhibition of protein breakdown than with either IGF-1 or insulin alone. It should be noted that the rate of protein breakdown can be decreased below that measured in the presence of insulin or IGF-1. Thus 5 mM-NH₄Cl added together with insulin, IGF-1 or bFGF leads to a further 23–24% inhibition over that observed with the growth factors alone, very close to the 26% inhibition found when NH₄Cl is added without growth factors (Table 2).

Secondly, the time course of inhibitory effects of the different growth factors on protein breakdown has been examined to determine whether the lower response to FGF over the normal 4 h measurement period can be explained by a longer delay before inhibition is detected. This experiment (Fig. 2) indicates that insulin, IGF-1 and bFGF each have a lag period of 1–1.5 h before an inhibition of protein breakdown can be measured. After that delay, however, the observed inhibition is characteristic of the individual growth factor, at least up to 4.5 h. Clearly the decreased response to bFGF cannot be accounted for by a longer lag time.

The increase in protein content produced by growth factors in several cell lines is a consequence of a co-ordinate inhibition of protein breakdown and a stimulation of protein synthesis (Ballard & Gunn, 1985; Ballard *et al.*, 1986). A similar situation occurs in BHK-21 cells, where serum, bFGF, IGF-1 and insulin all lead

Fig. 2. Time course of inhibitory responses produced by bFGF (●), insulin (△) or IGF-1 (■) on protein breakdown

Values are expressed as percentages of labelled protein degraded and are plotted as means $\pm$ S.E.M. for four determinations.

Table 3. Additivity of effects of growth factors on protein synthesis

Measurements were made over a 4 h period and are expressed as radioactivity incorporated from 2 mM-leucine into protein. Values are means $\pm$ S.E.M. for data obtained from 10 wells per treatment. The numbers in parentheses represent percentage increases over the control value. The average protein content in control wells was 170 μ g.

Treatment	10 ⁻³ $\times$ Radioactivity incorporated (c.p.m./well)
None	14.8 $\pm$ 0.3
Fetal bovine serum (10 %)	21.5 $\pm$ 0.6 (46)
bFGF (1 nM)	19.1 $\pm$ 0.2 (29)
IGF-1 (10 nM)	20.4 $\pm$ 0.6 (38)
Insulin (1 μ M)	20.7 $\pm$ 0.4 (40)
IGF-1 + insulin	21.3 $\pm$ 0.5 (44)
IGF-1 + bFGF	22.2 $\pm$ 0.6 (50)
bFGF + insulin	22.4 $\pm$ 0.6 (51)

to a stimulation of protein synthesis when measured over a 4 h period (Table 3). As with protein breakdown, the response to bFGF is less than obtained with insulin or IGF-1. However, the inclusion of bFGF with either insulin or IGF-1 leads to a significantly ($P < 0.05$)

Table 4. Measurements over 24 h of protein synthesis and protein breakdown

Values are means $\pm$ S.E.M. for six determinations, with the percentage increases over the control wells given in parentheses.

Treatment	$10^{-3} \times$ Protein synthesis (c.p.m./well)	Protein breakdown (%)	Protein content (μ g/well)
None	22.1 ± 0.7	35.9 ± 0.4	75.5 ± 2.1
Fetal bovine serum (10%)	75.6 ± 2.5 (243)	25.5 ± 0.3 (-29)	176.1 ± 3.8 (133)
bFGF (1 nM)	29.7 ± 1.2 (35)	35.9 ± 0.6 (0)	95.8 ± 1.8 (27)
IGF-1 (10 nM)	30.1 ± 1.0 (36)	31.9 ± 0.2 (-11)	95.1 ± 2.4 (26)
Insulin (1 μ M)	31.0 ± 0.8 (41)	31.0 ± 0.2 (-14)	98.8 ± 2.1 (31)
IGF-1 + insulin	33.4 ± 1.0 (51)	30.7 ± 0.2 (-15)	103.7 ± 3.2 (37)
IGF-1 + bFGF	36.4 ± 0.7 (65)	29.3 ± 0.3 (-19)	109.4 ± 2.5 (45)
bFGF + insulin	37.6 ± 1.0 (71)	27.9 ± 0.2 (-22)	111.9 ± 2.8 (48)

Fig. 3. Effects of bFGF (●), aFGF (○), insulin (△) or IGF-1 (■) on DNA labelling in BHK-21 cells

Cells were exposed to the growth factors or control (C) serum-free medium for 24 h with [3 H]thymidine added for the last 6 h. Values are means $\pm$ S.E.M. for eight determinations.

greater stimulation of protein synthesis than noted with insulin or IGF-1 alone.

The effects of growth factors on protein breakdown and protein synthesis reported in Tables 2 and 3 were evaluated over 4 h measurement periods, substantially less than the time required for mammalian cells to complete a cycle of cell division. When the measurements are extended from 4 h to 24 h, the pattern of response of the protein-synthesis pathway to individual growth factors is essentially unchanged (compare Tables 3 and 4), with similar effects produced by bFGF, insulin or IGF-1 and partial additivity observed when bFGF is present together with insulin or IGF-1. On the other hand, a much smaller inhibition of protein breakdown is

noted over the longer measurement period (compare Tables 2 and 4), with the response to bFGF totally lost. Moreover, by 24 h the inclusion of bFGF with either IGF-1 or insulin leads to small but significant ($P < 0.01$) increases in the percentage inhibition of protein breakdown (Table 4). The changes in protein content in this experiment also show partial additivity between bFGF and insulin or IGF-1. It should be noted that, with the relatively long incubation periods used in the experiment in Table 4, any change in protein breakdown will contribute to the measured rate of protein synthesis, because the amount of protein per well will be altered.

Fetal bovine serum produces much greater increases in protein synthesis and in the protein content of the wells over the 24 h measurement period than was found with the individual growth factors alone or in combination (Table 4). A similar but less pronounced difference is evident in the protein-breakdown measurements. These greater relative effects of fetal bovine serum are not apparent in the 4 h studies of protein breakdown (Table 2) or protein synthesis (Table 3).

In addition to producing changes in rates of protein metabolism, the individual growth factors stimulate DNA synthesis in quiescent cultures of BHK-21 cells (Fig. 3). The aFGF and bFGF increased DNA labelling from radioactive thymidine in this experiment to approx. 70% of that found in the presence of 10% fetal bovine serum and gave half-maximum responses at 0.3 nM and 0.05 nM respectively. The maximum effects produced by insulin and IGF-1 were smaller than with FGF, and half-maximum increases in DNA labelling occurred at approx. 0.1 nM-insulin and 5 nM-IGF-1 respectively (Fig. 3).

The addition of either insulin or IGF-1 together with bFGF to quiescent cultures of BHK-21 cells led to an increase in the DNA labelling above that found with bFGF alone (Table 5). In this experiment the responses to combinations of growth factors were substantially less than noted with fetal bovine serum, although in other studies, where the average protein content of control wells was higher, bFGF plus insulin or IGF-1 produced DNA labelling comparable with that with fetal bovine serum.

DISCUSSION

Intracellular protein breakdown is inhibited by FGF in BHK-21, Swiss 3T3 and Balb/c3T3 cells, lines that have been shown previously to respond to FGF by

Table 5. Changes in protein content and rates of DNA synthesis in BHK-21 cells exposed to growth factors

Values are expressed as mean thymidine incorporation rates over the last 6 h of a 24 h exposure period to the indicated growth factors, or mean protein contents at the time of harvesting. The S.E.M. values are for 10 determinations. Numbers in parentheses represent the percentage increases over control wells.

Treatment	$10^{-3} \times$ DNA synthesis (c.p.m./well)	Protein content (μ g/well)
None	31.9 ± 0.9	125.2 ± 1.7
Fetal bovine serum (10 %)	225.5 ± 19.1 (607)	249.2 ± 4.1 (99)
bFGF (1 nM)	105.0 ± 7.1 (229)	183.5 ± 11.0 (47)
IGF-1 (10 nM)	64.4 ± 3.3 (102)	187.0 ± 4.4 (49)
Insulin (1 μ M)	69.4 ± 4.4 (118)	196.9 ± 4.9 (57)
IGF-1 + insulin	77.7 ± 5.3 (144)	201.0 ± 4.1 (61)
IGF-1 + bFGF	129.5 ± 4.7 (306)	229.1 ± 9.7 (83)
bFGF + insulin	118.6 ± 2.9 (272)	230.8 ± 14.6 (84)

increases in DNA synthesis and cell number (Gospodarowicz *et al.*, 1978; Neufeld & Gospodarowicz, 1986). Moreover, the concentration-dependence of the different responses to bFGF and aFGF on BHK-21 cells is remarkably similar, with half-maximum effects evident on cell growth at 0.04 nM-bFGF and 0.6 nM-aFGF (Neufeld & Gospodarowicz, 1986), on DNA synthesis at 0.05 nM-bFGF and 0.3 nM-aFGF (Fig. 3) and on protein breakdown at 0.02 nM-bFGF and 0.12 nM-aFGF (Fig. 1) respectively. In each of the assays similar maximum effects occurred with aFGF and bFGF.

As reported here for both aFGF and bFGF and suggested in an earlier study, where commercial FGF was tested on human fibroblasts (Ballard *et al.*, 1980a), the maximum inhibitory response on protein breakdown is somewhat smaller than that observed with the insulin/IGF group of growth factors. One explanation of this difference could be the inhibition of distinct pathways of protein degradation by the two types of growth factors. Such an explanation seems unlikely, because additive effects could not be demonstrated, at least during the first 4 h of exposure. Moreover, the addition of NH_4^+ ions, known to decrease protein breakdown via alkalization of the lysosomal system (de Duve *et al.*, 1974; Ohkuma, 1987), produced equal and additive responses on protein breakdown in the presence of bFGF, insulin or IGF-1. These results, taken together with our finding of similar inhibitory time courses for the various growth factors, suggest that FGF is affecting protein breakdown by the same prevention of autophagy mechanism that has been established for insulin (Pfeifer, 1978, 1987). An explanation as to how autophagy can be inhibited more completely by either IGF-1 or insulin than by FGF must await clarification of the sequence of events between receptor occupancy and prevention of autophagy.

Although FGF is a less effective inhibitor of protein breakdown than insulin or IGF-1, the reverse situation holds for the stimulation of DNA synthesis. The difference is particularly striking for insulin, because this hormone produces its modest maximum response on DNA synthesis in BHK-21 cells at a lower concentration than found with aFGF (Fig. 3). But, unlike the effects on protein breakdown, a partial additivity of effects on DNA synthesis can be demonstrated between insulin (or IGF-1) and bFGF. A number of studies have reported either additivity or synergism on DNA synthesis between FGF and other growth factors such as insulin, IGF or

epidermal growth factor (Gospodarowicz *et al.*, 1978; Kato *et al.*, 1983). Moreover, in most assays used to screen FGF activity, plasma or serum is present as a source of IGF (Gospodarowicz *et al.*, 1985; Böhlen *et al.*, 1985).

A requirement for more than one growth factor for growth to be stimulated has led to the concept that an interplay is required between competence factors, such as FGF, and progression factors, including insulin and IGF-1 (Scher *et al.*, 1979; O'Keefe & Pledger, 1983; Stiles, 1985). Our results with BHK-21 cells do indeed show partial additivity between bFGF and the progression factors with respect to DNA synthesis, protein synthesis and long-term measurements of protein breakdown, but the additivity is slight when compared with the individual responses produced by bFGF, insulin and IGF-1. These findings do not argue against a need for more than one type of growth factor in order for cells to achieve a maximum growth rate, because the mixture of factors present in serum produced a more prolonged anabolic response. Rather, the classification of the growth factors is based on quantitative and not qualitative differences in growth effects.

We thank Dr. P. Böhlen and Dr. P. Gautschi for generously providing samples of aFGF and bFGF.

REFERENCES

- Ballard, F. J. (1982) *Biochem. J.* **208**, 275–287
- Ballard, F. J. & Gunn, J. M. (1985) *J. Nutr. Growth Cancer* **2**, 1–14
- Ballard, F. J. & Read, L. C. (1985) *Mech. Ageing Dev.* **80**, 11–22
- Ballard, F. J., Knowles, S. E., Wong, S. S. C., Bodner, J. B., Wood, C. M. & Gunn, J. M. (1980a) *FEBS Lett.* **114**, 209–212
- Ballard, F. J., Wong, S. S. C., Knowles, S. E., Partridge, N. C., Martin, P. J., Wood, C. M. & Gunn, J. M. (1980b) *J. Cell. Physiol.* **105**, 335–346
- Ballard, F. J., Read, L. C., Francis, G. L., Bagley, C. J. & Wallace, J. C. (1986) *Biochem. J.* **233**, 223–230
- Böhlen, P., Esch, F., Baird, A. & Gospodarowicz, D. (1985) *EMBO J.* **4**, 1951–1956
- de Duve, C., de Barsy, T., Poole, B., Trouet, A. & van Hoof, F. (1974) *Biochem. Pharmacol.* **23**, 2495–2531
- Dulley, J. R. & Grieve, P. A. (1975) *Anal. Biochem.* **64**, 136–141

- Francis, G. L., Read, L. C., Ballard, F. J., Bagley, C. J., Upton, F. M., Gravestock, P. M. & Wallace, J. C. (1986) *Biochem. J.* **233**, 207–213
- Gospodarowicz, D., Greenburg, G., Bialecki, H. & Zetter, B. R. (1978) *In Vitro* **14**, 85–118
- Gospodarowicz, D., Massoglia, S., Cheng, J., Lui, G.-M. & Böhlen, P. (1985) *J. Cell. Physiol.* **122**, 323–332
- Hauschka, S. D. & Konigsberg, I. R. (1966) *Proc. Natl. Acad. Sci. U.S.A.* **55**, 119–126
- Kato, Y., Hiraki, Y., Inoue, H., Kinoshita, M., Yutani, Y. & Suzuki, F. (1983) *Eur. J. Biochem.* **129**, 685–690
- Neufeld, G. & Gospodarowicz, D. (1986) *J. Biol. Chem.* **261**, 5631–5637
- Ohkuma, S. (1987) in *Lysosomes: Their Role in Protein Breakdown* (Glaumann, H. & Ballard, F. J., eds.), pp. 115–148, Academic Press, London
- O'Keefe, E. J. & Pledger, W. J. (1983) *Mol. Cell. Endocrinol.* **31**, 167–186
- Pfeifer, U. (1978) *J. Cell Biol.* **78**, 152–167
- Pfeifer, U. (1987) in *Lysosomes: Their Role in Protein Breakdown* (Glaumann, H. & Ballard, F. J., eds.), pp. 3–59, Academic Press, London
- Scher, C. D., Shepard, R. C., Antoniades, H. N. & Stiles, C. D. (1979) *Biochim. Biophys. Acta* **560**, 217–241
- Stiles, C. D. (1985) *Cancer Res.* **45**, 5215–5218

Received 7 May 1987/17 August 1987; accepted 18 September 1987