Inhibition of protein breakdown by epidermal growth factor in IMR90 human fibroblasts and other mammalian cell lines

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1. Epidermal growth factor (EGF) inhibits intracellular protein breakdown in IMR90 human fibroblasts and other cell lines having EGF receptors. 2. Inhibition is achieved within 1 h of exposure to the growth factor and is reversed equally rapidly upon removal of EGF. 3. EGF inhibits protein breakdown and stimulates protein and DNA labelling with similar dependency on concentration. Half-maximal effects for all processes with IMR90 and AG2804 cell lines occur at 0.2 nM- and 0.05 nM-EGF respectively. 4. EGF and insulin effects on protein breakdown are additive only when the factors are included at suboptimal concentrations. 5. The apparent K_d for EGF binding in several cell lines is approximately 10-fold higher than the concentration needed for half-maximal inhibition of protein breakdown. 6. Down-regulation of EGF receptors in IMR90 cells produced a 60% decrease in the binding of ^{125}I -labelled EGF. This was accompanied by a displacement of the concentration curve for EGF inhibition of protein breakdown by approximately two orders of magnitude, suggesting that protein breakdown can no longer respond to the down-regulated receptor–growth-factor complex. 7. Phorbol esters decrease the inhibitory effect of EGF, but not of insulin, on protein breakdown in IMR90 cells.

EGF produces a wide range of biological responses in cultured cells, including increased protein, RNA and DNA synthesis, stimulation of amino acid and uridine transport and the transient induction of ornithine decarboxylase (Carpenter & Cohen, 1979; Hollenberg, 1979). These effects are part of a set of processes involved in the stimulation of cell growth that have been termed the pleiotypic response (Hershko *et al.*, 1971). We have previously shown that various serum factors which initiated cell growth also lead to the inhibition of intracellular protein breakdown (Ballard *et al.*, 1980a). EGF was shown to decrease rates of protein breakdown in many cell lines, with IMR90 human fibroblasts being particularly responsive (Ballard *et al.*, 1980a). Here we report information on the mechanism by which EGF acts to decrease protein breakdown in cultured cells.

Abbreviations used: EGF, epidermal growth factor; TPA, 12-*O*-tetradecanoylphorbol 13-acetate.

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Experimental

Materials

EGF was either purchased from Collaborative Research Inc., Waltham, MA, U.S.A., or was a gift from Dr. J. B. Moore, Jr., Texas A & M University. The insulin used was Actrapid from Novo Industri A/S, Copenhagen, Denmark. Crystalline bovine serum albumin was purchased from Calbiochem–Behring Corp., La Jolla, CA, U.S.A. Phorbol and TPA were obtained from P-L Biochemicals, Milwaukee, WI, U.S.A. Iodogen was purchased from Pierce Chemical Co., Rockford, IL, U.S.A. Na^{125}I (carrier free), 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. Sources for the IMR90 fibroblasts and other cell lines used in this study have been described previously (Ballard *et al.*, 1980a). The epithelial cell line referred to previously as liver line (Ballard *et al.*, 1980a) is here termed Hep10C.

Measurements of protein breakdown

Rates of intracellular protein degradation in confluent monolayers were measured as the release of trichloroacetic acid-soluble radioactivity into the culture medium after labelling cells with [^3H]leucine for 18 h followed by a 3 h chase period (Ballard *et al.*, 1980b). The control medium used was Eagle's Minimal Essential Medium containing antibiotics and 2 mM-leucine unless stated otherwise. Protein breakdown was expressed as the percentage of labelled protein degraded over the 4 h incubation period. In all experiments effectors were added at the beginning of the degradation period.

Binding of ^{125}I -labelled EGF to cell monolayers

Labelled EGF was prepared by using Iodogen and Na^{125}I as described by Fraker & Speck (1978) and stored at -20°C . The specific radioactivity was approx. $100\mu\text{Ci}/\mu\text{g}$. Binding of iodinated EGF in the presence of different concentrations of unlabelled EGF was measured by using similar techniques to those described for insulin (Ballard *et al.*, 1980b). Briefly, cells grown in six-place Costar multiwell plates were washed with Hanks salts to remove serum and incubated in Hanks salts containing 100 mM-Tes (2-[[2-hydroxy-1,1-bis-(hydroxymethyl)ethyl]amino]ethanesulphonic acid) buffer, pH 7.6, 0.1% bovine serum albumin, 0.05 nM- ^{125}I -labelled EGF and unlabelled EGF at concentrations between 0.01 and 100 nM. After 2 h incubation at 15°C with gentle shaking, the monolayer was washed with cold Hanks salts and the percentage binding measured. This binding protocol was varied as indicated in the appropriate Figure legends.

Measurements of protein labelling

Confluent monolayers of cells in 24-place dishes were incubated overnight in Eagle's Minimal Essential Medium containing 0.5% calf serum to deplete the culture of serum factors. The monolayers were washed once with Earle's salts and incubated in Eagle's Minimal Essential Medium containing 20 mM-Tes, pH 7.3, and 0.1% crystalline bovine serum albumin with the leucine concentration decreased to $40\mu\text{M}$. Then 1 h later the medium in each well was replaced with 1 ml of a similar medium but containing $1\mu\text{Ci}$ of [^3H]leucine and other additions as indicated for each experiment. After a 6 h labelling period the medium was removed and each monolayer washed twice with Hanks salts, pH 7.5, and dissolved in 0.1 M-NaOH containing 0.1% Triton X-100. The dissolved protein was precipitated by the addition of trichloroacetic acid to give a final concentration of 10% (w/v) and kept at 0°C overnight. After centrifugation at 1500 g for 10 min the supernatant was discarded and the pellet dissolved in 0.5 M-NaOH for the determination of radioactivity (Knowles *et al.*, 1975) and protein

(Dulley & Grieve, 1975). Rates of protein labelling are expressed as radioactivity (c.p.m.) incorporated per mg of protein over the 6 h period.

Measurements of DNA labelling

Confluent monolayers were treated as described above for the measurement of protein synthesis, except that [^3H]leucine was omitted, the leucine concentration was increased to 0.4 mM, the incubation period in which effectors were present before the addition of label was extended to 24 h and $2\mu\text{Ci}$ of [^3H]thymidine was added for 2 h. DNA labelling is expressed as c.p.m. incorporated per mg of protein.

Results

Rates of protein and DNA labelling are stimulated and rates of protein breakdown are inhibited by

Fig. 1. Effects of EGF on (a) protein breakdown, (b) protein labelling and (c) DNA labelling in IMR90 fibroblasts (O) and AG2804 transformed fibroblasts (●). Values are means with the S.E.M. indicated when it is sufficiently large. The numbers of determinations at each concentration were: IMR90, protein breakdown, 9; protein labelling, 6; DNA labelling, 12; AG2804, protein breakdown, 12; protein and DNA labelling, 6. Other details are given in the Experimental section.

EGF in IMR90 human fibroblasts. Half-maximal responses for all three processes effects occurred at approx. 0.2nM (Fig. 1). The magnitude of the EGF effects on DNA labelling is decreased in AG2804 cells, a cell line derived by transformation of IMR90 cells by simian virus 40. However, the transformed line is somewhat more sensitive to EGF, showing half-maximal effects at 0.05nM (Fig. 1).

Addition of 10nM-EGF to monolayers of IMR90 fibroblasts or Hep10C epithelial cells produced a rapid inhibition of protein breakdown (Fig. 2). The magnitude of the response did not increase progressively with time. Removal of EGF-containing medium, rinsing the monolayer in Eagle's Minimal Essential Medium and replacement with a second portion of this EGF-free medium lead to a rapid increase in the breakdown rate to that observed in cells never exposed to the growth factor. Clearly the continual presence of EGF is required for the inhibition of protein breakdown to be sustained.

Protein breakdown in IMR90 cells is inhibited by insulin as well as by EGF (Ballard *et al.*, 1980a,b). An approximately additive response occurs when 0.1nM-EGF and 1nM-insulin are present (Table 1). The inhibitory responses produced by high concentrations of insulin and EGF, on the other hand, are only partially additive. We have also obtained

results indicating lack of additivity at high EGF and insulin concentrations with the AG2804 line. Thus protein breakdown was inhibited by 19% by 1μM-insulin and by 9% by 0.1μM-EGF, but only by 17% when both growth factors were present.

The relationship between EGF binding and the

Table 1. Additivity of insulin and EGF effects on protein breakdown in IMR90 fibroblasts

Growth factors were added at the beginning of the 4 h measurement period. Values are means ± s.e.m. for the numbers of determinations given after each addition. The percentage inhibition of protein breakdown with respect to Eagle's Minimal Essential Medium is given in parentheses after the degradation rate.

Additions	Degradation of labelled protein (%)
None (14)	8.50 ± 0.17
EGF, 0.1 nM (14)	7.97 ± 0.14 (6)
Insulin, 1 nM (14)	8.05 ± 0.17 (5)
EGF, 0.1 nM, +insulin, 1 nM (21)	7.57 ± 0.18 (11)
EGF, 0.1 μM (14)	7.34 ± 0.13 (14)
Insulin, 1 μM (14)	6.82 ± 0.08 (20)
EGF, 0.1 μM, +insulin, 1 μM (13)	6.46 ± 0.15 (24)

Fig. 2. Time course of EGF effects on protein breakdown in (a) Hep10C epithelial cells and (b) IMR90 fibroblasts. After an 18 h labelling period and a 3 h chase period, protein breakdown in Eagle's Minimal Essential Medium was followed by harvesting wells at 30 min intervals. The medium in all wells not harvested by 2 h was removed and replaced by either similar medium (O) or medium containing 1 nM-EGF (●). After 4 h the remaining cell monolayers were washed twice with Earle's salts and the medium was replaced with Eagle's Minimal Essential Medium (O, Δ) or medium containing 1 nM-EGF (●). Values are means + s.e.m. (where sufficiently large) for six determinations at each time point.

Table 2. Comparison between rates of protein breakdown and binding of ^{125}I -labelled EGF

Measurements of the percentage of protein degraded over a 4 h period in Eagle's Minimal Essential Medium (control) and the binding of ^{125}I -labelled EGF (0.05 nM) to cell monolayers are described in the Experimental section. Values are means $\pm$ S.E.M. with the numbers of determinations given in parentheses. Abbreviation: N.D., not determined.

Cell line	Protein breakdown		% inhibition by EGF	EGF binding (% per mg of protein)
	Control	+ 10 nM-EGF		
H35	7.84 $\pm$ 0.03 (9)	7.85 $\pm$ 0.08 (14)	0	<1 (8)
HTC	7.27 $\pm$ 0.09 (6)	7.51 $\pm$ 0.10 (11)	-3	1.23 $\pm$ 0.10 (8)
MH ₁ C ₁	8.14 $\pm$ 0.09 (13)	7.94 $\pm$ 0.06 (20)	3	4.81 $\pm$ 0.56 (10)
Hep10C	10.40 $\pm$ 0.06 (37)	8.22 $\pm$ 0.03 (31)	21	55.1 $\pm$ 4.2 (17)
Hepatocytes	9.91 $\pm$ 0.12 (11)	9.34 $\pm$ 0.11 (9)	6	32.5 $\pm$ 0.6 (7)
Calvarial osteoblasts	12.53 $\pm$ 0.12 (10)	11.00 $\pm$ 0.12 (6)	12	7.50 $\pm$ 1.08 (4)
OS104 osteogenic sarcoma	8.00 $\pm$ 0.07 (12)	7.00 $\pm$ 0.08 (13)	12	34.9 $\pm$ 2.5 (13)
BEN	6.61 $\pm$ 0.01 (9)	6.69 $\pm$ 0.05 (15)	-1	<1 (4)
Swiss 3T3	10.33 $\pm$ 0.15 (12)	9.63 $\pm$ 0.38 (12)	7	11.4 $\pm$ 0.6 (4)
Balb/c 3T3	8.71 $\pm$ 0.18 (16)	7.33 $\pm$ 0.10 (16)	16	N.D.
3T12	7.49 $\pm$ 0.16 (12)	6.73 $\pm$ 0.09 (12)	10	N.D.
Balb/c 3T3-SV40	5.20 $\pm$ 0.08 (15)	4.49 $\pm$ 0.06 (15)	14	N.D.
IMR90	8.56 $\pm$ 0.06 (9)	6.76 $\pm$ 0.11 (9)	21	7.66 $\pm$ 0.19 (21)
AG2804	6.23 $\pm$ 0.05 (22)	5.24 $\pm$ 0.11 (12)	16	25.2 $\pm$ 0.4 (40)

inhibitory response on protein breakdown has been examined in a range of cultured cells (Table 2). Marked effects of the growth factor on protein breakdown are seen in primary rat calvarial osteoblasts, the rat osteogenic sarcoma line OS104, Balb/c 3T3 and simian-virus-40-transformed Balb/c 3T3 cells as well as the three lines discussed above, IMR90, AG2804 and Hep10C cells. Swiss 3T3 mouse fibroblasts and rat hepatocytes in primary culture show a slight but measurable inhibition of protein breakdown. No EGF responses could be demonstrated with H35, HTC or MH₁C₁ rat hepatomas or the BEN human bronchial carcinoma line (Table 2). Binding measured at 0.05 nM labelled hormone could not be detected in either H35 or BEN lines, but occurs to a substantial extent in hepatocytes, OS104 and AG2804 cells, and especially in Hep10C cells. The binding data are generally in accord with the biological responses to EGF, although the similar inhibition of protein breakdown observed in IMR90 and Hep10C cells or in calvarial osteoblasts and the osteogenic sarcoma line OS104 is accompanied by very different amounts of binding. Also the substantial binding of EGF to hepatocytes leads to only a small inhibition of protein breakdown.

The concentration-dependence of EGF effects on protein breakdown and the ability of non-radioactive EGF to compete with ^{125}I -labelled EGF for surface binding sites have been compared in IMR90 and OS104 cells and the Hep10C line (Fig. 3). Half-maximal responses on protein breakdown occurred at about 0.02 nM in Hep10C cells, and

between 0.1 and 0.2 nM in IMR90 fibroblasts and the OS104 sarcoma. Half-maximal competition for the binding of ^{125}I -labelled EGF occurred at higher concentrations, 0.5 nM in the OS104 cells and 4 nM in IMR90 fibroblasts. Calculation of the concentration at which binding is half-maximally decreased is not possible for Hep10C cells, because of the shape of the binding curve, but the EGF concentration needed to prevent half the binding of 0.01 nM- ^{125}I -labelled EGF was no greater than 0.2 nM. If the assumption is made that the measured binding of ^{125}I -labelled EGF that occurs at concentrations of non-radioactive EGF greater than 10 nM represents non-specific binding, the fraction of cell receptors occupied at EGF concentrations that produce half-maximal effects on protein breakdown can be calculated. Such calculations indicate that between 5% and 10% of receptors need to be occupied in each cell line to produce the half-maximal biological response. The values would be lower if some EGF binding at 10 nM is considered specific.

Exposure of IMR90 fibroblasts to 10 nM-EGF for 18 h before the measurement of binding of ^{125}I -labelled EGF leads to a 60% diminution in the amount of labelled hormone bound to surface receptors (Fig. 4). This decreased binding is evident at all concentrations of added non-radioactive EGF. When protein breakdown is measured under similar down-regulating conditions, the EGF response curve is displaced so that an approx. 100-fold higher concentration is needed to produce a 10% inhibition of degradation as compared with cells not previously exposed to EGF (Fig. 4). Moreover, the rate

Fig. 3. Comparison between the concentration-dependence of the effect of EGF on (a) protein breakdown and (b) the binding of ^{125}I -labelled EGF

Details of protein-breakdown measurements over 4 h and binding studies over 2 h at 15°C are given in the Experimental section. The concentration of ^{125}I -labelled EGF was 0.01 nM . Values are means for three determinations of binding and means $\pm$ s.e.m. for 9, 12 and 31 measurements of protein breakdown in IMR90 (●), OS104 (■) and Hep10C (▲) cells respectively.

Fig. 4. Down-regulation of EGF-receptor binding and EGF effects on protein breakdown in IMR90 cells

Confluent monolayers were exposed to Eagle's Minimal Essential Medium containing 0.5% newborn-calf serum for 18 h in the presence (▲, ■) or absence (●) of 10 nM -EGF. (a) For the measurement of protein breakdown, $1\mu\text{Ci}$ of $[^3\text{H}]$ leucine/ml was included. At the completion of the 18 h period the monolayers for protein-breakdown studies were washed twice with Earle's salts and incubated for a further 2 h in Eagle's Minimal Essential Medium plus 2 mM -leucine in the presence (▲) or absence (●, ■) of 10 nM -EGF. Protein breakdown was subsequently measured over a 4 h period after washing monolayers twice with Earle's salts to remove medium EGF. The indicated concentrations of EGF were added at the beginning of the degradation period. (b) To quantify EGF binding, the monolayers were washed twice with Earle's salts and incubated for 40 min at 37°C in Eagle's Minimal Essential Medium containing 0.05 nM - ^{125}I -labelled EGF and unlabelled EGF as indicated. Values are means $\pm$ s.e.m. for five binding measurements and six degradation measurements at each concentration.

Table 3. *Interaction of phorbol esters and EGF in IMR90 fibroblasts*

Protein breakdown was measured as the percentage degraded over 4 h. Hormones and phorbol esters were added at the beginning of the degradation period. Values are means $\pm$ s.e.m. for six determinations. The percentage inhibitory effects from the respective control rates in Eagle's Minimal Essential Medium are given in parentheses. α -Phorbol and TPA were each added at 1 μ M.

Additions	Protein degraded (%)		
	Control	α -Phorbol	TPA
None	8.61 $\pm$ 0.12	8.60 $\pm$ 0.13	9.25 $\pm$ 0.22
EGF (0.1 nM)	7.82 $\pm$ 0.13 (9)	7.88 $\pm$ 0.15 (8)	8.84 $\pm$ 0.22 (4)
EGF (1 nM)	7.08 $\pm$ 0.13 (18)	7.28 $\pm$ 0.13 (15)	8.64 $\pm$ 0.18 (7)
Insulin (1 nM)	7.93 $\pm$ 0.23 (8)	7.87 $\pm$ 0.27 (9)	8.25 $\pm$ 0.19 (11)
Insulin (1 μ M)	6.90 $\pm$ 0.19 (20)	7.03 $\pm$ 0.18 (18)	7.62 $\pm$ 0.11 (18)

of protein breakdown in the absence of EGF is also increased. If preincubation of the fibroblasts with EGF is terminated 3 h before commencing the degradation measurement, an intermediate response curve is obtained.

Phorbol esters have been reported to decrease EGF binding in several cell lines and either to enhance or to inhibit the biological response to the growth factor, depending on the cell line and property tested (Lee & Weinstein, 1978; Frantz *et al.*, 1979; Brown *et al.*, 1979; Lechner & Kaighn, 1980). Rates of protein degradation in IMR90 fibroblasts are increased in the presence of the tumour promoter TPA (Table 3), but not when the related but inactive compound α -phorbol is added. Furthermore, effects of 0.1 or 1 nM-EGF on protein degradation are decreased when the medium contains TPA but not phorbol. Control experiments with 1 nM- or 1 μ M-insulin show no difference in the relative inhibition of protein breakdown when either TPA or phorbol is added.

Discussion

Control of the rate of protein breakdown provides an important means by which the amounts of individual or total proteins can be regulated in mammalian cells. The mechanisms responsible remain to be elucidated, although considerable evidence points to the modification of the rate of autophagic sequestration as a key process in the regulation of total protein turnover (Mortimore, 1982; Ballard & Gunn, 1982). Such evidence especially applies to the inhibition of protein breakdown by insulin, a hormone that has been shown to decrease the number of autophagic vacuoles in liver with a half-time of about 10 min (Pfeifer, 1978). Also close correlations between the numbers of autophagic vacuoles and the overall rates of protein breakdown have been established in regenerating liver, in hepatocytes during the diurnal starvation-refeeding cycle of rats and in liver perfused with

different concentrations of amino acids (Mortimore & Hutson, 1981; Pfeifer, 1981; Hundt *et al.*, 1980). Although there is no direct evidence that EGF inhibits protein breakdown by decreasing autophagy, the similar rapid time course of EGF action as well as the lack of additivity of EGF and insulin when present at saturating concentrations is consistent with EGF affecting the same intracellular process as insulin. Not only is the protein-breakdown rate depressed quickly after addition of EGF to cultured cells (Fig. 2), but the response disappears equally rapidly upon removal of the growth factor. Accordingly, the continual presence of EGF is necessary in order for the biological response to be sustained, a conclusion reached by Carpenter & Cohen (1979) and Shechter *et al.* (1978) for the EGF effect on DNA labelling.

Ammonia and other weak bases decrease the rate of protein breakdown in cultured cells by increasing the intralysosomal pH beyond that at which the lysosomal proteinases are active (Seglen, 1975; Knowles & Ballard, 1976; Amenta *et al.*, 1978; Ohkuma & Poole, 1978). Removal of ammonia is accompanied by a transient increase in protein degradation to a rate even greater than observed in cells never exposed to the weak base. This result probably indicates that lysosomal sequestration continues while ammonia is present, so that the lysosomal proteinases have a greater pool of substrate protein available for catabolism once their inhibition is alleviated (Amenta & Brocher, 1980). However, no catch-up was seen when EGF was removed from the medium (Fig. 2). A similar result was obtained in experiments where insulin-like growth factor-1 was added and subsequently removed from HTC cells (Ballard *et al.*, 1981), and where insulin was added and removed from H35 cells (J. M. Gunn, C. M. Wood, S. E. Knowles & F. J. Ballard, unpublished work). The lack of catch-up in the degradation rate upon removal of each of these three growth factors implies an inhibition of protein breakdown at an earlier, rate-limiting, step

in the overall process than lysosomal proteolysis. Inhibition of autophagocytosis would be consistent with the experimental findings.

The concentration-dependence of EGF effects on protein breakdown indicates maximal inhibition at 1 nM for all the cell lines tested herein (see Figs. 1 and 3) and half-maximal responses between 0.05 and 0.2 nM. Since EGF concentrations of 0.02 and 0.2 nM have been reported in human (Hirata *et al.*, 1980) and mouse (Byyny *et al.*, 1974) plasma respectively, it is clear that protein breakdown in susceptible cells will respond over the range of circulating EGF concentrations. Moreover, the experiment illustrated in Fig. 4 indicates that chronic exposure of cells to high EGF concentrations markedly desensitizes the effect on protein breakdown. We are not aware of other biological responses to EGF in isolated cells that are blunted after receptor down-regulation, although the inhibition of protein synthesis noted at very high EGF concentrations (Fig. 1) could be interpreted in this context. It should be emphasized that down-regulation of the EGF receptor in the IMR90 cells is not accompanied by any change in the apparent K_d for EGF-receptor binding. Thus the receptor numbers are decreased, but not the binding properties. A major problem is how to reconcile a modest fall in receptor numbers with a very large, perhaps 100-fold, displacement of the concentration curve for EGF inhibition of protein breakdown (Fig. 4). One possibility to explain why the down-regulated receptor is non-functional is that binding may be dissociated from the post-receptor event responsible for the inhibition of protein breakdown. We cannot provide any direct evidence on this point, although Knutson *et al.* (1982) have demonstrated that labilization of the insulin receptor accompanies down-regulation. Perhaps the EGF-receptor complex needs to be internalized and remain undegraded for a time in order for protein breakdown to be inhibited.

Tumour-promoting phorbol esters and related compounds decrease the binding of 125 I-labelled EGF to receptors on several cell lines (Lee & Weinstein, 1978; Brown *et al.*, 1979; Shoyab *et al.*, 1979), probably by decreasing the affinity of the receptor towards EGF (Brown *et al.*, 1979). Although active tumour promoters such as TPA stimulate DNA synthesis and produce similar biological effects to those initiated by EGF, when the two factors are added together the growth response can either be synergistic (Brown *et al.*, 1979; Frantz *et al.*, 1979) or antagonistic (Lechner & Kaighn, 1980). Added by itself to IMR90 fibroblasts, TPA gives rise to a slight increase in the protein-breakdown rate, the opposite response to that found with EGF (Table 3). Moreover, inclusion of TPA together with EGF almost completely prevents the inhibitory effect of EGF on protein breakdown.

Specificity towards active tumour promoters and EGF is indicated by the findings that the inactive analogue α -phorbol does not affect protein breakdown and TPA does not modify the inhibition of protein breakdown that is produced by insulin. These results suggest that the response in IMR90 cells may be similar to that found by Lechner & Kaighn (1980) in NF2 human fibroblasts, where TPA neutralized the growth effect of EGF.

It is interesting to compare the effects of TPA on protein breakdown in IMR90 cells with those on down-regulation. Both treatments result in an increased rate of protein degradation together with a decreased sensitivity to EGF. Moreover, protein breakdown in senescent IMR90 cells is also less responsive to EGF (Ballard *et al.*, 1981). The comparisons are striking and suggest that in all three instances a similar mechanism or defect may be responsible for the altered EGF effect on protein degradation.

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