Altered epidermal growth factor (EGF)-stimulated protein kinase activity in variant A431 cells with altered growth responses to EGF

(tyrosine phosphorylation/cell proliferation)

JANICE E. BUSS*, JEFFREY E. KUDLOW†, CHERI S. LAZAR*, AND GORDON N. GILL*‡

*Department of Medicine, Division of Endocrinology, University of California, San Diego, School of Medicine, La Jolla, California 92093; and †University of Toronto, Banting Institute, Toronto, Ontario M5G 1L5, Canada

Communicated by Robert W. Holley, December 30, 1981

ABSTRACT To determine the role of epidermal growth factor (EGF)-stimulated protein kinase in the biological effects caused by EGF, tyrosine-specific kinase activity has been quantitated in A431 human epidermoid carcinoma cells and six variant cell lines. Because EGF inhibited proliferation of A431 cells, variants resistant to this inhibition were selected by treatment with mutagen and maintenance for 1 month in 0.1 μ M EGF. After cloning and growth for 6–20 generations without EGF, the resistance of the variants to the growth-inhibitory effect of EGF was confirmed. Whereas EGF increased cellular phosphotyrosine content $\approx$ 10-fold in parental A431 cells, EGF caused smaller or undetectable increases in the six variant cell lines. Solubilized membranes from the six variants displayed diminished EGF-stimulated phosphorylation of the EGF receptor and of antibodies to p60^{src} (the product of the Rous sarcoma virus transforming gene), which act as an exogenous substrate. The decrease in EGF-stimulated tyrosine-specific protein kinase activity varied from $\approx$ 40% (clone 16) to $\approx$ 8% (clone 18) of parental A431 activity. Phosphorylated EGF receptors from parental and variant cells migrated identically on sodium dodecyl sulfate/polyacrylamide gels. The number of EGF receptors in variant cells decreased in parallel with EGF-stimulated protein kinase activity, so that the specific activity of EGF-stimulated protein kinase per EGF receptor remained constant in the six variant cell lines with reductions in both activities to as low as 10%. These results suggest that this tyrosine-specific protein kinase activity mediates the growth-inhibitory effect of EGF on A431 cells and that both EGF binding and kinase activities reside in the same or tightly associated molecules.

Epidermal growth factor (EGF), which binds to specific high-affinity cell surface receptors, stimulates replication of a number of cell types (1, 2). *In vitro*, EGF stimulates a membrane-associated protein kinase that catalyzes phosphorylation at tyrosine residues of a 150,000–170,000 M_r protein thought to be the EGF receptor (3, 4). EGF-stimulated protein kinase also catalyzes phosphorylation of tyrosine residues in anti-p60^{src} serum, a substrate for p60^{src}, the transforming protein of Rous sarcoma virus (RSV), but, like p60^{src}, does not phosphorylate control serum (5, 6). This suggests that both EGF-stimulated and p60^{src} tyrosine-specific protein kinases recognize some of the same substrate determinants. Transformation of cells by several retroviruses results in an increase in cellular phosphotyrosine content (7–10). Recent studies indicate that EGF also increases cellular phosphotyrosine content in intact human epidermoid carcinoma A431 cells (11, 12). It has been tempting to speculate that the growth-promoting factor EGF stimulates mitogenesis via changes in covalent modification of proteins by phosphorylation at tyrosine residues analogous to those induced by the transforming proteins of RNA tumor viruses. However, in contrast to the mitogenic effect of EGF on many cell types,

EGF inhibits proliferation of A431 cells (12, 13). EGF also inhibits growth of rat GH₄C₁ and certain human breast cancer cells (14, 15).

To determine whether EGF-stimulated protein kinase activity plays a role in the inhibition of A431 cell growth by EGF, we have isolated variant A431 cells that are resistant to the growth-inhibitory effects of EGF. Six of these clones have been analyzed in detail and have been found to contain decreased EGF-stimulated protein kinase activity both *in vivo* and *in vitro*.

METHODS

Variant Selection and Cell Growth. Parental A431 human epidermoid carcinoma cells were treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine at 1.25 μ g/ml for 2 hr and subsequently grown in Dulbecco's modified Eagle's medium with 10% calf serum containing 0.1 μ M EGF for 3 weeks. This concentration of the mutagen allowed survival of 30% of cells cultured in the absence of EGF. Cells were then subcultured and maintained with 0.1 μ M EGF until visible colonies appeared. From 6×10^7 cells treated with mutagen and continuously maintained with EGF, $\approx$ 300 survived, of which 40 produced large colonies. Six of these colonies were chosen for further study and grown to confluence in the presence of 0.1 μ M EGF. Each clone was then grown for 6–20 population doublings in the absence of EGF prior to experimental use. Because a separate selection was not carried out in the absence of mutagen, it is possible that resistant cells were already present in the A431 cell population. Resistant clones are therefore referred to as variants.

¹²⁵I-Labeled EGF (¹²⁵I-EGF) Binding. Binding of ¹²⁵I-EGF to intact cells was performed exactly as described (12). Binding of ¹²⁵I-EGF to intact cell membranes was done in a strictly analogous manner, using 0.6 nM ¹²⁵I-EGF and increasing amounts of unlabeled EGF with incubation for 1 hr at 25°C. Membrane-bound EGF was isolated on Millipore Cellotape (EGWP 0025) filters. The values for mixtures containing 1 μ M unlabeled EGF have been subtracted from each data point. Data were plotted by the method of Scatchard and apparent dissociation constant K_d and maximal binding B were determined by linear regression analysis, using a computer program. All lines gave $r^2 > 0.9$ and exhibited a single class of affinity sites.

Cellular Phosphotyrosine Content. Exponentially growing A431 parental and variant cell lines at $\approx 2 \times 10^4$ cells per cm² were labeled with [³²P]orthophosphate (1 mCi/ml; 1 Ci = 3.7 $\times 10^{10}$ becquerels) for 18 hr to equilibrium (12). EGF (0.1 μ M) was then added to some dishes for 2 hr. Total cell protein was extracted and hydrolyzed in 6 M HCl at 110°C for 2 hr (7). Au-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: EGF, epidermal growth factor; p60^{src}, the 60,000 M_r protein product of the transforming gene of Rous sarcoma virus; RSV, Rous sarcoma virus.

‡ To whom reprint requests should be addressed.

thetic standards were added and *O*-phosphorylated amino acids were separated by electrophoresis at pH 1.9 and pH 3.5 (7). After autoradiography *O*-phosphorylated amino acids were carefully scraped from the plate and their radioactivities were measured. A separate area of each plate was removed, and its radioactivity was measured and subtracted as a background from each *O*-phosphorylated amino acid. Phosphotyrosine content was calculated as a percentage of the total *O*-phosphorylated amino acids present (7).

EGF-Stimulated Protein Kinase Activity. Membranes were prepared from parental A431 and variant cell lines growing at a density of $\approx 5 \times 10^4$ cells per cm^2 by the procedure of Thom *et al.* (16). Membranes (2–4 mg of protein per ml) were solubilized with 1% Triton X-100/10% (vol/vol) glycerol (3) and incubated without or with 0.3 μM EGF for 10 min at 23°C. Kinase reaction mixtures, which contained 20 mM Hepes at pH 7.2, 1 mM MnCl_2 , 2 μl of anti-p60^{src} serum as an exogenous substrate, 0.5 μM ATP (2 μCi of [γ - ^{32}P]ATP, 3,000 Ci/mmol), and 0.15% Triton X-100 in a final volume of 33 μl , were incubated for 5 min at 4°C. The amount of anti-p60^{src} serum added was saturating for the enzyme present. Reactions were terminated by the addition of EDTA to 30 mM followed immediately by dilution with 150 μl of RIPA buffer (17), which contains 0.1% NaDodSO₄. Formalin-fixed protein A-containing *Staphylococcus aureus* (Pansorbin, Calbiochem) was added for 1 hr at 4°C to adsorb IgG, and the mixture was centrifuged to separate the immune complex from the EGF receptor-containing supernatant. The supernatant and pellet were individually collected on filter papers by precipitation with trichloroacetic acid (5). After separation on NaDodSO₄/10% polyacrylamide gels (18), phosphorylated proteins were visualized by autoradiography.

Protein concentrations were determined by the fluorescamine assay (19), using bovine serum albumin in the 0.15% Triton X-100 buffer of the kinase reaction as standard.

RESULTS

Selection of Variant Cells. Because EGF inhibits proliferation of A431 cells (12), it appeared possible to select for variants that would grow in the presence of saturating concentrations of EGF. Previous attempts to generate variants of 3T3 cells, for which EGF is a mitogen, yielded cells that lacked functional EGF receptors (20). Because A431 cells have an unusually high density of EGF receptors, 10- to 30-fold greater than most other cells types (21), a complete loss of EGF receptors was considered unlikely. When exposed to EGF during logarithmic growth, none of the six variants selected for study showed the decreased growth rate characteristic of parental A431 cells (see ref. 12) (Fig. 1). The ability of variant clones to grow in the presence of EGF was maintained when cells were retested after subsequent passages and thus appeared to be a stable change. As shown in Fig. 1C, a slight inhibitory effect of EGF remained in clone 16 and a slight stimulatory effect was evident in clone 4. Although EGF does not affect logarithmic growth rates of variant cells, it may still affect an individual variant's morphology, cloning efficiency, or saturation density (unpublished data).

Binding of EGF to Variant Cells. Each of the six variant cell lines had a reduced number of EGF receptors (Table 1). As measured in intact cells, the variants retained 8–40% of the number of receptors per cell of parental A431 cells. There was no apparent change in the binding affinity. The reduction in EGF receptors without an apparent change in affinity for EGF was also evident in plasma membranes isolated from the six variant cell lines (Table 1). In each assay EGF receptors were decreased, the greatest decrease, to <10%, being observed in

FIG. 1. Effect of EGF on growth of variants derived from A431 cells. Variant cell lines were subcultured into 35-mm plates and allowed to establish logarithmic growth before addition of EGF (arrow). Medium was then replenished without ($\circ$, $\square$) or with ($\bullet$, $\blacksquare$) 0.1 μM EGF and was subsequently replenished every 2 days. Each point represents the mean $\pm$ SD of triplicate cultures.

variant number 18. Because parental A431 cells contain such a large number of EGF receptors, the variant cells still possess a complement of high-affinity EGF receptors equal to or greater than that of other cell types.

The decrease in EGF receptors as measured by ligand binding was confirmed in clone 29 by immunoprecipitation of EGF receptors from cells that were labeled to equilibrium with [^{35}S]methionine. As determined by using a monoclonal anti-

Table 1. EGF binding to intact A431 and derived variant cells and to isolated membranes prepared from these cells

Cell line	Intact cells*		Membranes†	
	Receptor sites, pmol/10 ⁶ cells	Apparent K _d , nM	Receptor sites, pmol/mg protein	Apparent K _d , nM
Parental A431	2.4 ± 0.7	3.5 ± 0.8	19.7 ± 1.3	1.9 ± 0.6
Clone 16	0.8 ± 0.04	3.2 ± 0.7	10.8 ± 2.5	6.2 ± 2.6
29	0.9 ± 0.2	1.9 ± 0.5	7.1 ± 1.7	3.6 ± 2.5
24	1.0 ± 0.3	2.5 ± 0.7	5.3 ± 2.6	4.6 ± 1.9
33	0.4 ± 0.1	3.8 ± 0.5	5.7 ± 0.8	8.0 ± 4.9
4	0.2 ± 0.05	2.3 ± 0.7	3.9 ± 1.3	3.5 ± 2.4
18	0.2 ± 0.05	2.0 ± 0.2	1.8 ± 0.7	8.3 ± 3.9

* Cells grown in the absence of EGF were subcultured 24 hr prior to use. Because cell density affects EGF receptors in the parental A431 cells (12), binding studies were performed using cultures with 1–2 × 10⁴ cells per cm². Mean ± SD of three to five separate experiments is shown.

† Membranes were prepared from cells growing at a density of ≈ 5 × 10⁴ per cm². Mean ± SD of four to six separate experiments is shown.

body directed against the EGF receptor, there was reduction of EGF receptors in clone 29 by an average of 53% compared to parental A431 cells. This compares favorably with the 63% reduction indicated by ligand binding (Table 1).

Effect of EGF on Cellular Phosphotyrosine Content. In parental A431 cells, EGF increased the cellular content of phosphotyrosine (Fig. 2 A and B). This EGF-dependent increase ranged between 5- and 10-fold and resulted in an increase in phosphotyrosine content from 0.02% to 0.22% of the acid-stable phosphorylated amino acids in the cell (12) (Table 2). In the EGF-resistant variant cell lines, EGF either failed to detectably increase total cellular phosphotyrosine content (Fig. 2 C and D) or increased it to a lesser extent (Fig. 2 E and F). The decrement in EGF stimulation of cellular phosphotyrosine content of each variant compared to parent A431 cells is summarized in Table 2. This method of quantitation does not permit detection of increases in phosphotyrosine of less than 2-fold.

EGF-Stimulated Protein Kinase Activity in Solubilized Membranes. Because the sensitivity of detection of reduced levels of total cellular phosphotyrosine was limited, a more sensitive *in vitro* protein kinase assay was used to determine the extent of reduction in EGF-stimulated protein kinase activity in variant cells. Membranes from A431 cells contain an EGF-stimulated protein kinase activity that catalyzes phosphorylation at tyrosine residues of a 170,000 *M_r* protein thought to be the EGF receptor (3–6). Because EGF receptors are reduced in variant cells, decreased phosphorylation of putative EGF receptors could reflect either reduced enzyme activity or reduced substrate. Anti-p60^{src} IgG, an exogenous substrate that is phosphorylated at tyrosine residues by EGF-dependent pro-

tein kinase (5, 6), was therefore included, and phosphorylation of both the EGF receptor and added IgG was determined. As shown in Table 3, EGF-stimulated protein kinase activity was decreased in each of the variant cells compared to parental A431 cells. The decrease was apparent when phosphorylation of either the EGF receptor or exogenously added anti-p60^{src} IgG was examined (Table 3 and Fig. 3). The extent of reduction of EGF-stimulated protein kinase activity varied among the six resistant lines studied, from ≈ 50% of parental levels in clone 16 to ≈ 10% of parental levels in clone 18. The parallel reduction in phosphorylation of both the EGF receptor and heavy chain of anti-p60^{src} IgG can be used to argue that kinase activity itself was decreased, not just the availability of the EGF receptor substrate. The decreased phosphotyrosine response of intact cells to EGF was thus confirmed by the reduction in membrane-associated kinase activity.

The reduction in apparent kinase activity did not arise from an altered affinity for ATP (data not shown). There was also no apparent change in the EGF receptor. All variants displayed EGF-stimulated phosphorylation of proteins with the same *M_r* as in the parental A431 membrane (Fig. 3). The 170,000 *M_r* protein, presumed to represent the intact EGF receptor (3, 22), demonstrated a higher degree of phosphorylation in the absence of EGF than the 150,000 *M_r* protein, presumed to represent a proteolytic product of the EGF receptor. Phosphorylation of both was stimulated by EGF.

If the data of Tables 1 and 3 are combined, the apparent activity of each variant's EGF-stimulated kinase can be calculated as a function of that variant's remaining EGF receptors (Table 4). In the six resistant lines, the decreases in EGF receptors and

FIG. 2. Effect of EGF on phosphotyrosine content of parental A431 cells (A and B) and resistant clones 4 (C and D) and 29 (E and F). Cells were labeled with [³²P]orthophosphate for 18 hr prior to treatment without EGF (A, C, E) or with 0.1 μM EGF for 2 hr (B, D, F). Extraction and electrophoretic separation of O-phosphorylated amino acids were carried out as described in the text. Approximately 2 × 10⁶ cpm was applied to each plate and autoradiographic exposure was for 17 hr.

Table 2. Effect of EGF on phosphotyrosine content of A431 and variant cells

Cell line	Phosphotyrosine content			
	Without EGF		With EGF	
	cpm	%	cpm	%
Parental A431	154	0.020	1971	0.220
Clone 16	81	0.033	122	0.037
29	41	0.011	310	0.056
24	1	0.001	118	0.029
33	73	0.013	26	0.010
4	100	0.023	279	0.063
18	27	0.016	32	0.007

Autoradiograms as in Fig. 2 were exactly aligned with their counterpart ninhydrin-stained *O*-phosphorylated amino acid standards on the cellulose plates. Each phosphorylated amino acid was carefully removed and its radioactivity was measured, and phosphotyrosine content was calculated as a percentage of total radioactivity in all three phosphorylated amino acids. The percentage of radioactivity present in phosphoserine and phosphothreonine averaged 94% and 6%, respectively, and did not change with EGF treatment.

EGF-stimulated protein kinase activity were synchronous. Despite reductions in EGF-stimulated kinase activity per mg membrane protein to as low as 10%, variant cells retained the same relative activity per EGF binding site as the parental A431 cells. This was true when either EGF receptor or anti-p60^{src} IgG phosphorylation was examined.

DISCUSSION

The isolation of these variant cell lines has opened an avenue for investigation of the effects of EGF on cell proliferation. EGF

is presently the only regulator of phosphotyrosine-specific protein kinase activity described. Evidence has been presented that tyrosine-specific protein kinase activities associated with RNA tumor virus transforming proteins mediate transformation (23, 24) and that these viral transforming proteins have homologs in untransformed cells (23, 25). Whether these viral or cellular tyrosine-specific protein kinases can be regulated or if EGF-stimulated protein kinase is a member of any of these classes is not yet known. Although EGF receptors are clearly involved in the molecular mechanisms through which EGF affects cell proliferation, cell shape, and membrane transport (1), the role of protein phosphorylation in these processes is uncertain. The present studies suggest that EGF-stimulated protein kinase mediates inhibition of proliferation in A431 cells. EGF stimulates an increase in tyrosine-specific protein kinase activity and in cellular phosphotyrosine content in A431 cells in association with growth inhibition. Cells with decreased EGF-stimulated protein kinase activity are no longer growth inhibited by EGF. These data also imply that the EGF-stimulated protein kinase detected *in vitro* is a primary determinant of the phosphotyrosine content of A431 cells, because, when this kinase activity is decreased, *in vivo* stimulation of phosphorylation of cellular tyrosine residues by EGF is decreased. Because EGF receptors decreased in parallel with EGF-stimulated protein kinase activity, the possibility that growth inhibition results from an effect of EGF-receptor occupancy distinct from kinase activation cannot be excluded.

A431 cells have an unusually high density of EGF receptors [$0.5\text{--}3 \times 10^6$ per cell (12, 21)], so it is possible that an inhibitory pathway is activated because tyrosine kinase activity is excessively increased and that in other cells such a pathway is nor-

FIG. 3. EGF-dependent phosphorylation of the EGF receptor (Upper) or anti-p60^{src} IgG (Lower) in solubilized membranes prepared from parental A431 and variant cells (Cl, clone). After *in vitro* phosphorylation and separation of receptor-containing supernatant and IgG with *S. aureus*, proteins were displayed on 10% polyacrylamide gels containing NaDodSO₄ and autoradiographed. The amount of membrane protein loaded onto each channel was: A431, 5 µg; Cl 16, 5 µg; Cl 29, 5 µg; Cl 33, 7.5 µg; Cl 24, 8 µg; Cl 4, 8 µg; and Cl 18, 9 µg. The film is overexposed for certain cells to display clones with lower activity.

Table 3. EGF-dependent protein kinase activities in solubilized membranes prepared from A431 and variant cells

Cell line	Membrane protein per assay, μ g	32 P incorporated, pmol/min per mg protein			
		EGF receptor phosphorylation		Anti-p60 ^{src} IgG phosphorylation	
		Without EGF	With EGF	Without EGF	With EGF
Parental A431	10	0.43 $\pm$ 0.12	1.80 $\pm$ 0.30	0.12 $\pm$ 0.01	0.96 $\pm$ 0.14
Clone 16	10	0.27 $\pm$ 0.04	0.82 $\pm$ 0.18	0.09 $\pm$ 0.01	0.43 $\pm$ 0.11
29	10	0.25 $\pm$ 0.01	0.77 $\pm$ 0.03	0.08 $\pm$ 0.02	0.42 $\pm$ 0.04
24	16	0.15 $\pm$ 0.03	0.34 $\pm$ 0.06	0.05 $\pm$ 0.02	0.17 $\pm$ 0.03
33	16	0.26 $\pm$ 0.08	0.48 $\pm$ 0.09	0.07 $\pm$ 0.03	0.21 $\pm$ 0.08
4	16	0.15 $\pm$ 0.05	0.32 $\pm$ 0.05	0.05 $\pm$ 0.03	0.17 $\pm$ 0.06
18	20	0.12 $\pm$ 0.05	0.14 $\pm$ 0.05	0.04 $\pm$ 0.01	0.065 $\pm$ 0.01

In vitro protein kinase assays were performed as described in the text. Data represent the mean $\pm$ SD of three to five separate assays using two different membrane preparations.

mally quiescent. p60^{src}, the transforming protein of RSV, is derived from a normal cellular protein, p60^{c-src} (26), and in RSV-transformed cells is present in much greater amounts ($\approx$ 50-fold) than the normal cell protein (27). It has therefore been proposed that RSV transformation results from a quantitative rather than a qualitative change in a cellular enzyme and its activity (28). Although EGF causes an increase in cellular phosphotyrosine in A431 cells similar in magnitude to that induced by retroviral transformation in other cells, the EGF-stimulated increases are associated with inhibition of A431 cell growth. Cooper and Hunter have successfully transformed A431 cells with RSV (29). These cells had a permanently increased phosphotyrosine content from the introduction of the viral protein p60^{v-src}, which produced no significant growth response in the carcinoma-derived A431 cells. However, a further EGF-mediated increase in phosphotyrosine was associated with characteristic growth inhibition (29).

Cohen *et al.* (3) have reported that EGF binding and EGF-stimulated protein kinase activities are enriched in parallel during purification, suggesting that both activities reside in the same molecule. In addition, EGF-dependent phosphorylation of the EGF receptor has been reported to decrease with receptor down-regulation (30). Study of A431 variants also provides evidence for the intimate association, perhaps identity, of the EGF receptor and EGF-stimulated protein kinase. The finding that the specific activity of EGF-stimulated protein kinase per EGF binding site remains constant in six variant cell lines with reductions to as low as 10% in both activities supports the idea that both EGF binding and tyrosine-specific protein kinase activities are located in the same molecule. Alternatively, the kinase may be highly dependent on interaction with the EGF receptor to be active.

Table 4. Specific activities of EGF-stimulated protein kinase in A431 and variant cells

Cell line	Phosphorylation, pmol 32 P incorporated/min per nmol 125 I-EGF bound	
	EGF receptor	Anti-p60 ^{src} IgG
Parental A431	91.3	48.7
Clone 16	75.9	39.8
29	108.4	59.2
24	71.7	35.8
33	84.2	36.8
4	82.1	43.6
18	77.8	36.1

Data from Tables 1 and 3 were combined to determine kinase activities associated with EGF binding sites. Data presented are for EGF-stimulated kinase activity.

The authors thank Drs. Jon Cooper and Tony Hunter for collaboration in experiments involving immunoprecipitation of metabolically labeled EGF receptors. These studies were supported by Research Grant BC-209 from the American Cancer Society and Research Grant AM13149 from the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases. J.E.K. was a Centennial Fellow of the Medical Research Council of Canada.

- Carpenter, G. & Cohen, S. (1979) *Annu. Rev. Biochem.* **48**, 193–216.
- Gospodarowicz, D., Greenburg, G., Bialecki, H. & Zetter, B. R. (1978) *In Vitro* **14**, 85–118.
- Cohen, S., Carpenter, G. & King, L., Jr. (1980) *J. Biol. Chem.* **255**, 4834–4842.
- Ushiro, H. & Cohen, S. (1980) *J. Biol. Chem.* **255**, 8363–8365.
- Kudlow, J. E., Buss, J. E. & Gill, G. N. (1981) *Nature (London)* **290**, 519–521.
- Chinkers, M. & Cohen, S. (1981) *Nature (London)* **290**, 516–519.
- Sefton, B. M., Hunter, T., Beemon, K. & Eckhart, W. (1980) *Cell* **20**, 807–816.
- Barbacid, M., Beemon, K. & Devare, S. G. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 5158–5162.
- Beemon, K. (1981) *Cell* **24**, 145–153.
- Sefton, B. M., Hunter, T. & Raschke, W. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1552–1556.
- Hunter, T. & Cooper, J. A. (1981) *Cell* **24**, 741–752.
- Gill, G. N. & Lazar, C. S. (1981) *Nature (London)* **293**, 305–307.
- Barnes, D. W. (1982) *J. Cell Biol.* **93**, 1–4.
- Schonbrunn, A., Krasnoff, M., Westendorf, J. M. & Tashjian, A. H., Jr. (1980) *J. Cell Biol.* **85**, 786–797.
- Imai, Y., Leung, C. K. H., Shiu, R. P. C. & Friesen, H. G. (1981) *J. Cell Biol.* **91**, 225a (abstr.).
- Thom, D., Powell, A. J., Lloyd, C. W. & Rees, D. A. (1977) *Biochem. J.* **168**, 187–194.
- Collett, M. S. & Erikson, R. L. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2021–2024.
- Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
- Udenfriend, S., Stein, S., Böhlen, P., Dairman, W., Leimgruber, W. & Weigeke, M. (1972) *Science* **178**, 871–872.
- Pruss, R. M. & Herschman, H. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 3918–3921.
- Fabricant, R. N., DeLarco, J. E. & Todaro, G. J. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 565–569.
- Wrann, M. M. & Fox, C. F. (1979) *J. Biol. Chem.* **254**, 8083–8086.
- Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
- Patchinsky, T. & Sefton, B. M. (1981) *J. Virol.* **39**, 104–114.
- Bishop, J. M. (1980) *N. Engl. J. Med.* **303**, 675–682.
- Karess, R. E., Hayward, W. S. & Hanafusa, H. (1979) *Proc. Natl. Acad. Sci. USA* **75**, 5812–5816.
- Collett, M. S., Brugge, J. S. & Erikson, R. L. (1978) *Cell* **15**, 1363–1369.
- Erikson, R. L., Purchio, A. F., Erikson, E., Collett, M. S. & Brugge, J. S. (1980) *J. Cell Biol.* **87**, 319–325.
- Cooper, J. A. & Hunter, T. (1981) *J. Cell Biol.* **91**, 878–883.
- Fernandez-Pol, J. A. (1981) *Biochemistry* **20**, 3907–3912.