Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 6451–6455, November 1983
Biochemistry

Monoclonal antibodies against epidermal growth factor receptor induce prolactin synthesis in cultured rat pituitary cells (GH_3)

(membrane receptor/gene expression/hormone internalization)

J. HAPGOOD*†, T. A. LIBERMANN*, I. LAX*, Y. YARDEN*, A. B. SCHREIBER*, Z. NAOR‡, AND J. SCHLESSINGER*

Departments of *Chemical Immunology and ‡Hormone Research, The Weizmann Institute of Science, Rehovot 76100, Israel

Communicated by Rita Levi-Montalcini, July 5, 1983

ABSTRACT **The addition of epidermal growth factor (EGF) to cultured rat pituitary cells (GH_3) leads to increased synthesis of prolactin and to partial inhibition of cell proliferation. Monoclonal antibodies generated against EGF receptor from human epidermoid carcinoma (A-431) cells were used to characterize the EGF receptor kinase system of GH_3 cells and to investigate the role of the hormone–receptor complex in the expression of the prolactin gene in these cells. The EGF receptor of GH_3 cells is a 170,000-dalton protein associated with a protein kinase. It is similar but not identical to the EGF receptor identified in other tissues. The immunoprecipitated membrane receptor is phosphorylated on both serine and tyrosine residues. The monoclonal antibody denoted 2G2-IgM binds to EGF receptor on GH_3 cells. Like EGF, the monoclonal antibody induced the synthesis of prolactin and morphological changes in these cells. Hence, EGF receptor in GH_3 cells, when properly triggered, contains all of the biological attributes necessary for the induction of EGF-induced gene expression and morphological changes in GH_3 cells.**

Epidermal Growth Factor (EGF) is a potent mitogen of various cells *in vitro* and *in vivo* (1). EGF binds to diffusely distributed, mobile (2–4) membrane receptors that rapidly cluster on the cell surface and become internalized and degraded by lysosomal enzymes (1–5).

Several studies indicate that EGF can regulate the expression of specific genes and thus cause cellular differentiation (6–8). The addition of EGF to cultured rat pituitary cells (GH_3) leads to increased synthesis of prolactin and inhibition of synthesis of growth hormone (8). EGF also induces morphological changes in GH_3 cells—i.e., the round cells become flat and elongated (8). After binding and internalization a small fraction of EGF molecules accumulates in the nucleus of GH_3 cells. The inhibition of hormone degradation by chloroquine increased the amount of hormone accumulated in the nucleus (9). These results together with the fact that EGF induced changes in the structure of chromatin in isolated nuclei of GH_3 cells suggest that the capacity of EGF to induce gene expression could be mediated via direct interactions between EGF and specific sites on the nucleus (10).

We have recently generated different monoclonal antibodies against various domains of EGF receptor (11–13). One monoclonal antibody denoted 2G2-IgM induces various early and delayed effects of EGF on cultured fibroblasts (11–13). We report here that 2G2-IgM binds to EGF receptors on GH_3 cells. Like EGF, the monoclonal antibody induced the synthesis of prolactin and morphological changes in these cells. Hence, EGF receptor contains all of the biological attributes necessary for the induction of EGF-induced gene expression and morphological changes in GH_3 cells.

MATERIALS AND METHODS

Materials. EGF was purified from the submaxillary glands of adult male mice by the method of Savage and Cohen (14). ^{125}I-Labeled EGF (^{125}I-EGF) was prepared by the chloramine-T method to a specific activity of 1.8×10^4 cpm/ng of EGF. Monoclonal antibodies against EGF receptor, 2G2-IgM and TL5-IgG, were prepared as described (11–13). The molecular masses of 2G2-IgM and TL5-IgG are 10^6 and 1.5×10^5 daltons, respectively. Goat anti-mouse IgG antibodies were iodinated by the chloramine-T method to a specific activity of 5×10^3 cpm/ng of protein.

Cells. Rat pituitary GH_3 cells were kindly provided by I. Vlodavsky. Human epidermoid carcinoma (A-431) cells were kindly provided by G. Todaro. Both cell lines were grown in culture in Dulbecco's modified Eagle's medium (DME medium; GIBCO) supplemented with 10% fetal calf serum.

Binding Assay of EGF. GH_3 cells were plated in 24-well Costar trays. Near confluency, the medium was replaced with DME medium with Hepes buffer (pH 7.4) containing 0.1% bovine serum albumin. The cells were incubated with increasing concentrations of EGF at either 4 or 37°C. Then, the cells were washed four times with cold buffer and lysed with 0.2 M NaOH, and the cell-associated radioactivity was determined.

Binding Assays of Monoclonal Antibodies. Nearly confluent GH_3 cells in 24-well Costar trays were maintained in DME medium containing Hepes buffer (pH 7.4) and 0.1% bovine serum albumin. The cells were incubated with increasing concentrations of either 2G2-IgM or TL5-IgG for 30 min at room temperature. The cells were washed two times with incubation medium and further incubated for 30 min at room temperature with ^{125}I-labeled goat anti-mouse Ig antibodies (^{125}I-GAM Ig antibodies). The cells were washed three times and lysed with 0.2 M NaOH for 30 min at 37°C, and the total cell-associated radioactivity was determined.

Immunoprecipitation. Aliquots of solubilized cells were diluted with phosphate-buffered saline/0.1% bovine serum albumin/10% glycerol/0.2% Triton X-100 and incubated with protein A-Sepharose beads coated with rabbit polyclonal anti-EGF-receptor antibodies (13) on an Eppendorf shaker for 2 hr at 4°C. After several washes the precipitated EGF–receptor kinase complex was mixed with 30 μl of 20 mM Hepes, pH 7.4/

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; HFF, human foreskin fibroblasts; ^{125}I-GAM Ig antibodies, ^{125}I-labeled goat anti-mouse Ig antibodies; TRH, thyrotropin-releasing hormone.

† Permanent address: Department of Biochemistry, University of Cape Town, South Africa.

150 mM NaCl/0.2% Triton X-100/10% glycerol/2 mM $MnCl_2$/0.03% NaN_3 and 1–2 μCi of [γ-^{32}P]ATP (1 Ci = 3.7×10^{10} Bq). The phosphorylation reaction was stopped after 10 min at 4°C by the addition of 30 μl of Laemmli buffer to the solution and by boiling it for 5 min.

Two-Dimensional Tryptic Maps. Two-dimensional tryptic maps were performed according to procedure described by Elder *et al.* (15).

Phosphoamino Acid Analysis. The phosphorylated EGF receptor was excised from the gel, washed for 4 hr in 10% methanol, lyophilized, and crushed in a glass homogenizer. The crushed gel was suspended in 50 mM NH_4HCO_3 (pH 8.0) containing 75 μg of L-1-tosylamido-2-phenylethyl chloromethyl ketone-treated trypsin (Worthington, 285 units/mg) per ml. After 12 hr at 35°C the suspension was filtered, lyophilized, hydrolyzed, and subjected to two-dimensional electrophoresis according to the procedure described by Hunter and Sefton (16).

Analysis of Prolactin Synthesis. GH_3 cells (3×10^4) were plated in Costar trays and maintained in culture medium for 3 days. Fresh culture medium containing either hormones or antibodies then was added: the cells were incubated for 4 days with fresh medium, and hormones and antibodies were added after 2 days. After 4 days of induction, fresh medium, hormones, and antibodies were added and the medium was harvested after 24 hr—i.e., for a total period of 96–110 hr of induction. The concentration of prolactin in the medium was determined by a sensitive radioimmunoassay for rat prolactin. Total protein was measured by the method of Lowery *et al.* (17). The rat prolactin radioimmunoassay kit was obtained from the National Institute of Arthritis, Metabolism and Digestive Diseases. The immunoreagents were created by A. F. Parlow, director of Pituitary Hormones and Antisera Center. The antiserum is specific for prolactin in the presence of large amounts of rat growth hormone, rat luteinizing hormone, rat follicle-stimulating hormone, and rat thyroid-stimulating hormone. Protein A was used to precipitate the prolactin–anti-prolactin complex.

RESULTS

EGF binds to specific membrane receptors on GH_3 cells (8, 9). Fig. 1 shows binding curves of ^{125}I-EGF to GH_3 cells at 4 and 37°C. Receptor saturation is accomplished with 30 ng of ^{125}I-EGF per ml when the binding experiment is performed at 4°C. However, at 37°C more ^{125}I-EGF becomes associated with the GH_3 cells as rapid internalization of the bound EGF occurs at this temperature (8, 9).

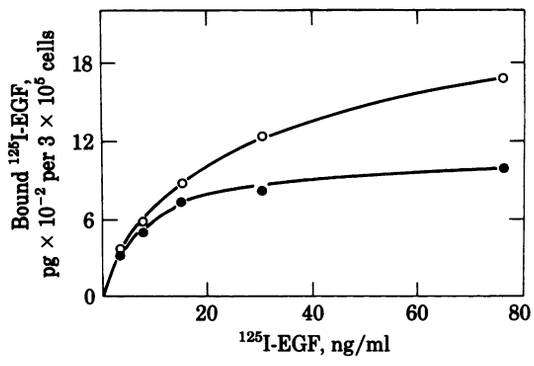

FIG. 1. Binding of ^{125}I-EGF to GH_3 cells. GH_3 cells were plated in 24-well Costar trays. Near confluency, the medium was replaced with DME medium with Hepes buffer (pH 7.4) containing 0.1% bovine serum albumin. The cells were incubated with increasing concentrations of EGF for either 45 min at 4°C (●) or for 45 min at 37°C (○). Then, the cells were washed and lysed with 0.2 M NaOH, and the cell-associated radioactivity was determined. Nonspecific binding was determined in the presence of a 100-fold excess of unlabeled EGF and did not exceed 15% of the total binding. Data are the average of duplicate measurements.

Monoclonal Antibodies Against EGF Receptor Bind to GH_3 Cells. We have used two types of monoclonal antibodies generated against the EGF receptor of human epidermoid carcinoma A-431 cells to characterize the EGF receptor from GH_3 cells. The monoclonal antibody 2G2-IgM binds to EGF receptor of human and mouse cells, inhibits the binding of ^{125}I-EGF, and induces various "EGF-like" effects, including stimulation of protein phosphorylation, induction of morphological changes, and cell proliferation (11–13, 18). The monoclonal antibody TL5-IgG binds to EGF receptor without interfering with the binding of EGF to the receptor molecule. TL5-IgG does not modulate the activity of EGF nor does it possess any EGF-like activity (11–13, 18).

Fig. 2 shows binding curves of 2G2-IgM and TL5-IgG to GH_3 cells. The monoclonal antibodies were incubated with the cells for 1 hr at 4°C. After several washes with cold buffer, the cells were incubated with ^{125}I-GAM Ig antibodies and the cell-associated radioactivity was determined. Half-saturation of EGF receptors with the antibodies is reached with about 0.02 μM 2G2-IgM antibody and 0.2 μM TL5-IgG antibody. However, both 2G2-IgM and TL5-IgG bind with similar affinities to EGF receptor of A-431 cells and to human foreskin fibroblasts (HFF), with half-saturation achieved at antibody concentrations of 0.02 μM (12, 13). The affinity of TL5-IgG towards EGF receptor of the rat GH_3 cells is ≈1/10th as much as its affinity towards EGF receptor of HFF and A-431 cells (13). Hence, TL5-IgG, although crossreactive, is somewhat species specific, whereas 2G2-IgM apparently is not.

2G2-IgM blocks the binding of ^{125}I-EGF to EGF receptor on GH_3 cells. The addition of 20 ng of ^{125}I-EGF per ml to 8×10^5 GH_3 cells in the presence or absence of 75 μg of 2G2-IgM per ml for 30 min at room temperature followed by several washes

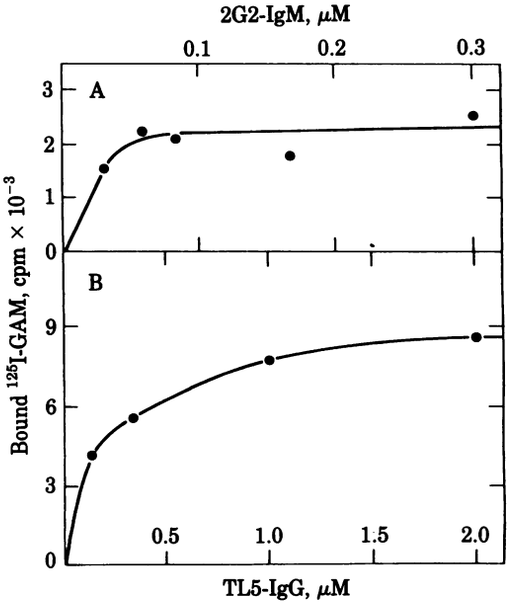

FIG. 2. Binding curves of monoclonal antibodies 2G2-IgM and TL5-IgG to GH_3 cells. GH_3 cells were incubated with increasing concentrations of either 2G2-IgM (*A*) or TL5-IgG (*B*) for 30 min at room temperature, washed, and further incubated with ^{125}I-GAM Ig antibodies. Nonspecific binding of ^{125}I-GAM Ig antibodies was determined in the absence of 2G2-IgM and it did not exceed 15% of the total bound antibody. Data are the average of duplicate measurements.

yielded cell-associated radioactivity of 3,000 cpm in the absence of 2G2-IgM and 40 cpm in the presence of 2G2-IgM. Hence, as in other cell types bearing EGF receptors, 2G2-IgM blocks the binding of ^{125}I-EGF to EGF receptors on GH_3 cells (11–13, 18).

Immunoprecipitation and Analysis of EGF Receptor from GH_3 Cells. The EGF–receptor–kinase system of GH_3 cells was immunoprecipitated with antireceptor antibodies and the phosphorylated receptor was compared to EGF receptor from A-431 cells. EGF receptor of GH_3 cells is a 170,000-dalton protein (Fig. 3*A*), which is phosphorylated at tyrosine and serine residues (Fig. 3*C*). However, EGF receptor from A-431 cells is phosphorylated mainly on tyrosine residues (Fig. 3*C*) (1, 19). These differences could be due to an additional serine kinase that is immunoprecipitated with the receptor molecule of GH_3 cells. Comparison of the phosphopeptide maps of EGF receptors from GH_3 and A-431 cells indicates that the two molecules are similar but not identical (Fig. 3*B*). Eight phosphopeptides derived from EGF receptor of GH_3 cells appear to be similar to phosphopeptides derived from EGF receptor of A-431 cells (Fig. 3*B*). Hence, EGF receptor from rat is similar to the human receptor.

Monoclonal Antibody 2G2-IgM Induces Prolactin Synthesis and Morphological Changes in GH_3 Cells. We have examined the capacity of 2G2-IgM to induce EGF-like effects in GH_3 cells. Fig. 4 summarizes the effect of EGF, thyrotropin-releasing hormone (TRH; Sigma), and 2G2-IgM on prolactin synthesis in GH_3 cells. The concentration of prolactin secreted into the culture medium of GH_3 cells was determined with a sensitive radioimmunoassay that detects prolactin at concentrations as low as 0.5 ng/ml. Prolactin production in the medium can be directly related to synthesis because storage of prolactin by GH_3 cells and degradation of prolactin in these cells and in the medium are negligible (20). Moreover, it was recently shown that the increase in prolactin synthesis induced by EGF is a result of an increase in prolactin mRNA (21).

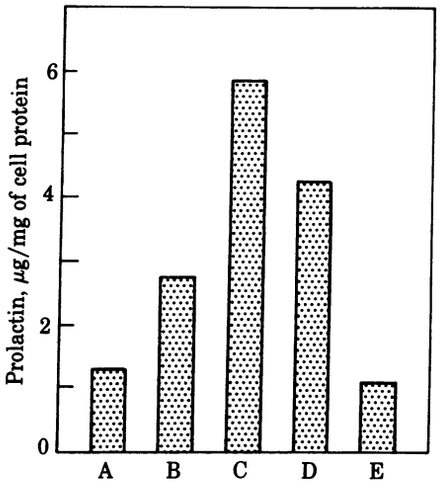

FIG. 4. Analysis of prolactin synthesis in GH_3 cells by radioimmunoassay. GH_3 cells were induced for 96–110 hr with the following hormones and antibodies: control medium (A); 100 ng of EGF per ml (B); 20 ng of TRH per ml (C); 27 μg of 2G2-IgM per ml (D); and 50 μg of control IgM per ml (E) (11).

The basal synthesis and secretion (mean ± SEM) of prolactin by GH_3 cells is 1.2 ± 0.25 μg/mg of cell protein. In the presence of EGF, the GH_3 cells produced 2.7 ± 0.30 μg of prolactin per mg of cell protein. TRH induced the synthesis of 6 ± 0.25 μg of prolactin per mg of cell protein and 2G2-IgM stimulated the synthesis of 4.2 ± 0.25 μg of prolactin per mg of cell protein. Control IgM antibody did not have any effect on prolactin synthesis. Interestingly, 2G2-IgM is more potent than EGF in inducing the synthesis of prolactin in GH_3 cells.

We also studied the morphological changes induced by 2G2-IgM on GH_3 cells (Fig. 5). In the absence of EGF, GH_3 cells appear as round cells (Fig. 5 *A* and *B*), whereas in the presence of either TRH (Fig. 5*C*) or EGF (Fig. 5*D*) the cells change their morphology and become flat and elongated. Similar changes were observed in GH_3 cells treated with 2G2-IgM (Fig. 5 *F* and *G*). Control IgM antibody did not change the morphology of the GH_3 cells (Fig. 5*E*). Hence, 2G2-IgM mimics EGF and TRH by inducing similar morphological changes in GH_3 cells.

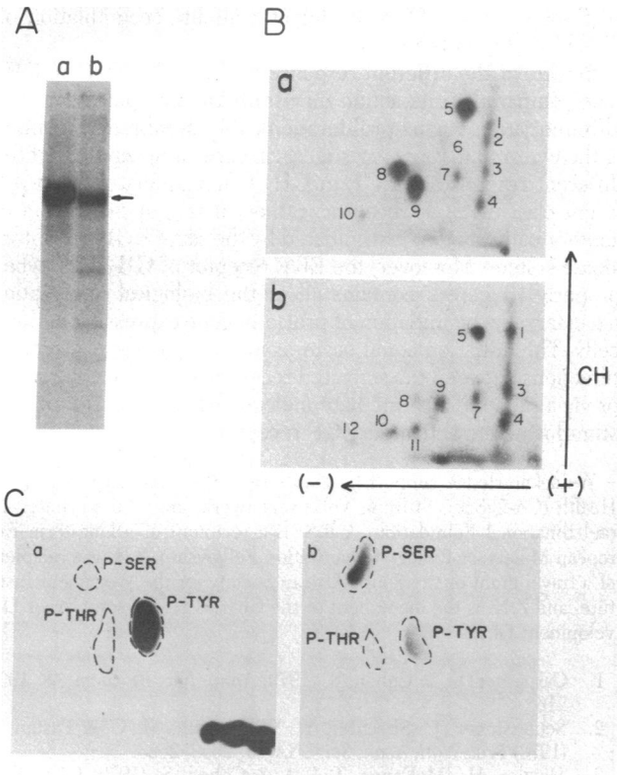

FIG. 3. Comparison of EGF receptors from GH_3 and human epidermoid carcinoma cells (A-431). (*A*) Autoradiograph of $NaDodSO_4$/polyacrylamide gel electrophoresis of immunoprecipitates of EGF–receptor–kinase system that were phosphorylated in the immunoprecipitate from either GH_3 (lane a) or A-431 (lane b) cells. The arrow denotes the 170,000-dalton polypeptide. (*B*) Two-dimensional tryptic maps of phosphopeptides derived from the 170,000-dalton EGF receptor bands, depicted in *A* of either GH_3 (a) or A-431 (b) cells. (*C*) Identification of the phosphorylated amino acid residues of EGF receptor from either GH_3 (a) or A-431 (b) cells.

DISCUSSION

EGF stimulates the proliferation of various cell types *in vitro* and *in vivo* (1). After binding to its membrane receptors EGF activates numerous early responses, including the activation of a tyrosine-specific cyclic nucleotide-independent protein kinase (18, 22–24), enhancement of the uptake of various nutrients (for review, see ref. 1), and induction of changes in cell morphology (25) and the organization of the cytoskeleton (26).

In rat pituitary GH_3 cells, EGF stimulates the transcription of prolactin gene, resulting in increased prolactin synthesis (21). Moreover, EGF partially inhibits the proliferation of the GH_3 cells (8). It has been reported that EGF accumulates in the nucleus of GH_3 cells (9, 10) and therefore has been postulated that direct interactions between EGF and nuclear receptors may mediate the expression of specific genes (10). Similar mecha-

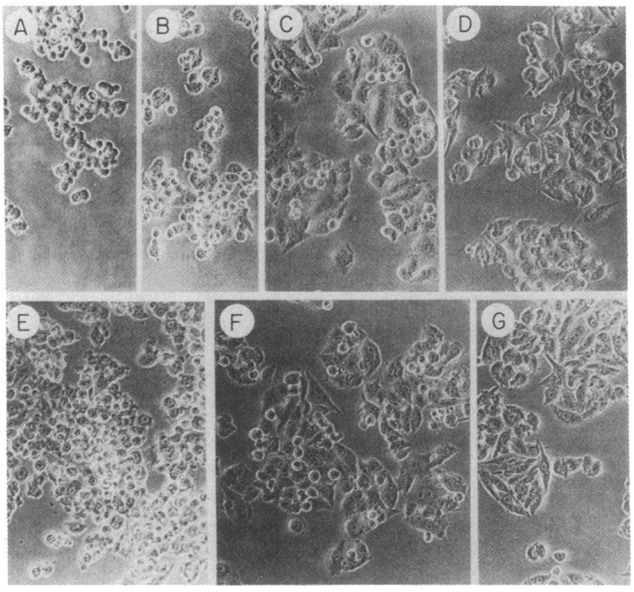

FIG. 5. The effect of hormones and antibodies on the morphology of GH_3 cells. Phase-contrast micrographs of GH_3 cells maintained in 35-mm dishes for 3 days in the presence of various hormones and antibodies. (*A* and *B*) Control medium; (*C*) 50 ng of EGF per ml; (*D*) 20 ng of TRH per ml; (*E*) 50 μg of control IgM per ml (11); (*F*) 15 μg of 2G2-IgM per ml; and (*F*) 37.5 μg of 2G2-IgM per ml. Photographs of phase-contrast images of the cells viewed with a Zeiss IM-35 inverted microscope were taken with Kodak Plus-X film. (×88.)

nisms were proposed for the initiation of the delayed effects mediated by insulin (27) and nerve growth factor (28, 29).

In this study we probed the EGF receptor of GH_3 cells with monoclonal antibodies directed against the EGF receptor of A-431 cells. Furthermore, we examined the capacity of the mitogenic monoclonal antibody 2G2-IgM (11–13, 18) to induce prolactin synthesis in GH_3 cells.

It is possible that the mechanism of EGF-induced differentiation response in GH_3 cells is different than the mitogenic response induced in fibroblasts by EGF and that the difference may lie in the receptor itself. However, the binding data with 2G2-IgM and TL5-IgG indicate that the antigenic determinant on EGF receptor that is recognized by 2G2-IgM is similar in the GH_3 cells, HFF, and A-431 cells. This result is consistent with the similar binding affinity determined for ^{125}I-EGF to EGF receptor of these cells (1, 8, 11). Thus, it further demonstrates that 2G2-IgM binds to a domain on EGF receptor that is part of, or close to, the combining site for EGF—a domain of the receptor molecule that is conserved along evolution. However, TL5-IgG binds to an antigenic determinant on EGF receptor that is remote from the combining site for EGF and is significantly different in GH_3 cells in comparison to a similar determinant on HFF and A-431 cells.

The membrane receptor of EGF is a 170,000-dalton glycoprotein, which, upon the binding of EGF and in the presence of ATP, becomes phosphorylated at tyrosine residues (19). The EGF-sensitive kinase appears to be an integral part of the receptor molecule (13, 24).

EGF receptor from GH_3 cells is very similar to EGF receptor from A-431 cells. The difference in the phosphopeptide map could be due to the differential specificity of the EGF-receptor–kinase system in A-431 and GH_3 cells. Interestingly, EGF receptor of HFF is also phosphorylated at both serine and tyrosine residues (unpublished data). Nevertheless, EGF receptor of GH_3 cells is similar to the receptor identified in other cells from other species.

The capacity of 2G2-IgM to induce the expression of prolactin genes in GH_3 cells provides an important insight into the mechanism of action of EGF in this system. Several models were proposed for the action of EGF in this system with obvious implications on the mode of action of EGF in general: model 1, the internalized intact EGF molecule binds to nuclear EGF receptors, which induce changes in chromatin structure leading to the expression of the prolactin gene; model 2, a fragment of the internalized EGF acts as a "second messenger" to activate a putative nuclear site; model 3, EGF receptor either on the cell surface or inside the cell contains all of the biological attributes necessary for the induction of biological response. The appropriate change in the receptor induced by EGF (i.e., conformational change, phosphorylation, or proteolytic cleavage) renders activation of the receptor (11–13, 18).

The data presented in this report support model 3. The structural similarity between 2G2-IgM and EGF is very small, thus reducing the feasibility of a fragment of EGF as an internal stimulus of EGF (model 2). Schreiber *et al.* reported (12) that 2G2-IgM clustered on the cell surface and became rapidly internalized into HFF with a similar kinetics observed for the internalization of EGF into these cells. Hence, it is possible that like EGF, 2G2-IgM has intracellular sites that are required for the stimulation of various delayed responses. However, we could not detect nuclear accumulation of fluorescently labeled EGF analogues or fluorescently labeled 2G2-IgM in HFF, even in the presence of chloroquine, an inhibitor of degradation by lysosomal enzymes (unpublished data). Nevertheless, this could be due to insufficient sensitivity of the image-intensification microscopy system (2, 4) to detect a minute accumulation of labeled probe on the nucleus.

In spite of the different response mediated by EGF on GH_3 cells compared to its action on fibroblasts or epidermal cells (differentiation versus proliferation), the membrane receptors of the two systems are very similar. Hence, we propose that the different response of EGF in GH_3 is not mediated via a different class of EGF receptor; rather, it is a manifestation of another pathway that is stimulated by the same EGF–receptor–kinase system. Moreover, the EGF receptor of GH_3 cells, when properly triggered, contains all of the biological information necessary for the initiation of prolactin gene expression in these cells. The initiation could occur as a consequence of direct interactions between the activated receptor and intracellular sites or via a putative intermediate molecule that acts as an internal stimulus of the activated EGF receptor.

We acknowledge support by grants from the National Institutes of Health (CA-25820), Stiftung Volkswagenwerk, and United States–Israel Binational Foundation. A.B.S. is a recipient of a long-term European Molecular Biology Organization Fellowship, J.H. is a recipient of a travel grant of the South-African Society for the Weizmann Institute, and Z.N. is the incumbent of the Charles H. Revson Career Development Chair.

1. Carpenter, G. & Cohen, S. (1979) *Annu. Rev. Biochem.* **48**, 193–216.
2. Schlessinger, J., Shechter, Y., Willingham, M. C. & Pastan, I. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 2659–2663.
3. Haigler, H., McKanna, T. J. A. & Cohen, S. (1979) *J. Cell Biol.* **81**, 382–395.
4. Hillmann, G. & Schlessinger, J. (1982) *Biochemistry* **21**, 1667–1672.
5. Carpenter, G. & Cohen, S. (1976) *J. Cell Biol.* **71**, 1959–1971.
6. Rheinwald, J. C. & Green, H. (1977) *Nature (London)* **265**, 421–424.
7. Sun, T. T. & Green, H. (1977) *Nature (London)* **269**, 489–492.
8. Johnson, L. K., Baxter, J. D., Vlodavsky, I. & Gospodarowicz, D. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 394–398.
9. Johnson, L. K., Vlodavsky, I., Baxter, J. D. & Gospodarowicz, D. (1980) *Nature (London)* **287**, 340–343.

10. Johnson, L. K. & Eberhardt, N. L. (1982) *J. Cell. Biochem. Suppl.* 6, 139 (abstr.).
11. Schreiber, A. B., Lax, I., Yarden, Y., Eshhar, Z. & Schlessinger, J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 7535–7539.
12. Schreiber, A. B., Libermann, T. A., Lax, I., Yarden, Y. & Schlessinger, J. (1983) *J. Biol. Chem.* **258**, 846–853.
13. Schlessinger, J., Lax, I., Yarden, Y., Kanety, H. & Libermann, T. A. (1983) in *Receptors and Recognition: Antibodies to Receptors*, eds. Greaves, M. F. (Chapman & Hall, Ltd., London), in press.
14. Savage, C. R., Jr., & Cohen, S. (1972) *J. Biol. Chem.* **247**, 7609–7611.
15. Elder, J. H., Pickett, R. A., Hampton, J. & Lerner, R. A. (1977) *J. Biol. Chem.* **252**, 6511–6515.
16. Hunter, T. & Sefton, B. M. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 1311–1315.
17. Lowery, O. H., Rosenbrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275.
18. Schlessinger, J., Schreiber, A. B., Libermann, T. A., Lax, I., Avivi, A. & Yarden, Y. (1982) in *Cell Membranes: Methods and Reviews*, eds. Elson, E. L., Frazier, W. & Glaser, L. (Plenum, New York), Vol. 1, pp. 117–144.
19. Ushiro, H. & Cohen, S. (1980) *J. Biol. Chem.* **255**, 8363–8365.
20. Tashjian, A. H., Jr., Bancroft, F. C. & Levine, L. (1970) *J. Cell Biol.* **47**, 67–70.
21. Murdoch, G. H., Potter, E., Nicolaisen, A. K., Evans, R. M. & Rosenfeld, M. G. (1982) *Nature (London)* **300**, 192–194.
22. Carpenter, G., King, L. J. R. & Cohen, J. (1979) *J. Biol. Chem.* **254**, 4884–4891.
23. Cohen, S., Ushiro, H., Stoscheck, C. & Chinkers, M. (1982) *J. Biol. Chem.* **257**, 1523–1531.
24. Buhrow, S. A., Cohen, S. & Staros, J. V. (1982) *J. Biol. Chem.* **257**, 4019–4022.
25. Chinkers, M., McKanna, T. J. A. & Cohen, S. (1979) *J. Cell Biol.* **83**, 260–265.
26. Schlessinger, J. & Geiger, B. (1981) *Exp. Cell. Res.* **134**, 273–279.
27. Goldfine, I. D., Smith, G. J., Wong, K. Y. & Jones, A. L. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 1368–1372.
28. Andres, R. Y., Jeng, I. & Bradshow, R. A. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2785–2789.
29. Yankner, B. A. & Shooter, E. M. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1268–1273.