F. Gorman and M. V. Thomas, *ibid.* **275**, 357 (1977); Z. Ahmed and J. A. Connor, *ibid.* **286**, 61 (1979)].
7. S. J. Smith and R. S. Zucker, *J. Physiol. (London)* **300**, 167 (1980).
8. J. A. Connor and G. Nikolakopoulou, *Lect. Math. Life Sci.* **15**, 79 (1982).
9. R. Demoll, *Zool. Jahrb. Abt. Anat. Ontog. Tiere* **38**, 443 (1914); A. W. Clark, R. Millecchia, A. Mauro, *J. Gen. Physiol.* **54**, 289 (1969).
10. J. E. Brown *et al.* (*6*). The light-induced increase in absorption at 660 nm of arsenazo III inside *Limulus* ventral photoreceptors is largely if not entirely due to an increase in $[Ca^{2+}]_i$.
11. The absorption of intracellular dye at an isosbestic wavelength (585 nm), A_{585}, was computed from the optical transmission, T_{585}, measured both before and after injection of dye: $A_{585} = \log T_{585}$ (before) $- \log T_{585}$ (after). Changes in the absorption of intracellular dye induced by stimulation were calculated as follows. After the onset of a prolonged bright stimulus, the optical transmission at 660 nm, T_{660}, changes. The change of T_{660} is delayed with respect to the onset of the light (*10*). Therefore, we have calculated the change in absorption of the dye at 660 nm at time t_2, $\Delta A_{660}(t_2)$, by $\Delta A_{660}(t_2) = \log T_{660}(t_1) - \log T_{660}(t_2)$, where time t_1 was chosen to be as early as practical after the onset of the stimulus. Each complete scan of the diode array required 18 msec. The first scan was unusable because the stimulus light was turned on during this period. After the first scan, all the diodes in the array were read out and reset every 18 msec. Usually we took the second scan (which started at $t = 18$ msec) to be the reference scan for the calculation of ΔA_{660}.
12. J. E. Brown and J. R. Blinks, *J. Gen. Physiol.* **64**, 643 (1974).
13. The profiles of the change in absorbance at 660 nm across the cell do not change as a function of time as predicted by a simple diffusion model; the magnitude of the change declines faster at the maximum of the spatial profile than would be predicted by the magnitude of the broadening of the profile.
14. In previous studies, light-induced transients of intracellular arsenazo III were measured across large portions of *Limulus* ventral photoreceptors (*10*). Those recordings probably resulted from the fortuitous positioning of the measurement aperture over a region of the cell that contained a rhabdom. Also, light-induced transient increases of aequorin luminescence have been recorded from whole photoreceptors (*12*). Such spatially averaged luminescence records (in contrast to absorbance records) would be dominated by portions of the cell in which $[Ca^{2+}]_i$ increased most and influenced little by the much lower levels of luminescence arising from portions of the cell that had small changes in $[Ca^{2+}]_i$.
15. B. G. Calman and S. C. Chamberlain, *J. Gen. Physiol.* **80**, 839 (1982).
16. J. Stern, K. Chinn, J. Bacigalupo, J. Lisman, *ibid.*, p. 825.
17. A. Fein and J. S. Charlton, *ibid.* **66**, 823 (1975).
18. Supported by NIH grants EY 01914 and EY 01915. We thank S. R. Bolsover, J. A. Connor, U. B. Kaupp, J. E. Lisman, L. J. Rubin, and B. M. Salzberg for helpful comments on the manuscript.

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Production of an Epidermal Growth Factor Receptor-Related Protein

Abstract. *Human epidermoid carcinoma A431 cells in culture produce a soluble 105-kilodalton protein which, by the criteria of epidermal growth factor (EGF) binding, recognition by monoclonal and polyclonal antibodies to the EGF receptor, amino-terminal sequence analysis and carbohydrate content, is related to the cell surface domain of the EGF receptor. The high rate of production and the finding that with biosynthetic labeling the specific activity of this 105-kilodalton protein exceeds that of the intact receptor indicate that it is not derived from membrane-bound mature receptor but is separately produced by the cell. These cells thus separately synthesize an EGF receptor that is inserted into the membrane and an EGF receptor-related protein that is secreted.*

The epidermal growth factor (EGF) receptor is a 170-kD membrane glycoprotein that contains intrinsic tyrosine kinase activity (*1, 2*). The EGF receptor has three functional domains: an EGF binding domain located on the external cell surface (*3, 4*), a transmembrane domain, and a cytoplasmic tyrosine kinase domain where adenosine triphosphate (ATP) is a donor for phosphorylation of intracellular substrates (*5, 6*). Binding of EGF to the receptor activates its tyrosine kinase activity which phosphorylates intracellular substrates (*6, 7*), increases ion fluxes (*8, 9*) and phosphatidyl inositol turnover (*10*), enhances receptor clustering and internalization of EGF receptor complexes (*3, 4*), and enhances receptor decay (*11*). The fate of internalized EGF receptors is not known, but there is evidence for sequestration in vesicles distinct from lysozymes (*12, 13*).

Using a monoclonal antibody to the EGF receptor, we have identified a 105-kD glycoprotein in medium from cultured human epidermoid carcinoma A431 cells. To characterize this extracellular EGF receptor-related protein (ERRP) we have studied EGF binding, recognition by several monoclonal and polyclonal antibodies to the EGF receptor, amino-terminal sequence homology with the EGF receptor, and carbohydrate content. Comparison of the rate of production and of the biosynthetic specific activity of this protein with those of the mature membrane bound EGF receptor indicates that ERRP is not a degradation product of the mature EGF receptor but is synthesized separately.

A431 cells produce a 105-kD protein that is specifically adsorbed to an immobilized monoclonal antibody to EGF receptor (termed 528). With time this 105-kD protein progressively accumulates in the medium of cultured $A431_8$ cells (Fig. 1A); the Coomassie blue stained bands correspond to the amount of protein produced on less than one 10-cm culture plate. The daily secretion rate was 0.4 μg of protein per 10^6 cells (Fig. 1A). Over this time period, cellular EGF receptor content remained constant. Like the EGF receptor, the ERRP was specifically bound to the monoclonal antibody and was not adsorbed to control columns containing immobilized mouse immunoglobulin G (IgG). Because the 528 monoclonal IgG used as an immunoabsorbent is a competitive inhibitor of EGF binding (*14, 15*), competitive elution with EGF was used to purify active EGF receptor–kinase protein (Fig. 1B). Likewise, the ERRP could be specifically eluted from the 528 IgG affinity column by EGF (Fig. 1B), an indication that it was also adsorbed via an EGF binding domain. Self-phosphorylation of the EGF receptor–kinase (*5*) was confirmed by incubating the washed column with [γ-^{32}P]ATP prior to elution of the protein. Under the same conditions, ERRP exhibited no detectable protein kinase activity (Fig. 1B). The absence of tyrosine kinase activity in the eluted ERRP was confirmed by means of a soluble assay with a synthetic peptide substrate (*16*). Also, ERRP was not phosphorylated by EGF receptor–kinase, suggesting that it also lacks the sites of self-phosphorylation present in the EGF receptor (data not shown).

Both the EGF receptor and ERRP stained well with the Schiff reagent (*17*), an indication that both are glycoproteins (Fig. 1C). Treatment of the EGF receptor protein with endoglycosidase F, which cleaves both high mannose glycans and complex glycans linked through asparagine (*18*), removes about 30 kD of oligosaccharide, leaving a 140-kD protein core (Fig. 1C). Treatment of ERRP with endoglycosidase F also removes a similar amount of oligosaccharide to produce an 80-kD protein core (Fig. 1C), suggesting that ERRP contains not only an EGF binding domain but that its glycosylation is similar to the EGF receptor protein. The protein doublet detected in samples treated with endoglycosidase F presumably arose during the prolonged incubation from a small proteolytic contamination present in the enzyme preparation (*18*).

To define further the relation of ERRP produced into the culture medium and the cell surface EGF receptor protein, we examined its interaction with several antibodies to the EGF receptor. ERRP competes with EGF receptor protein for binding to monoclonal antibody 29.1 (*19*) and to polyclonal antibodies to the EGF receptor. Monoclonal antibody 29.1 does

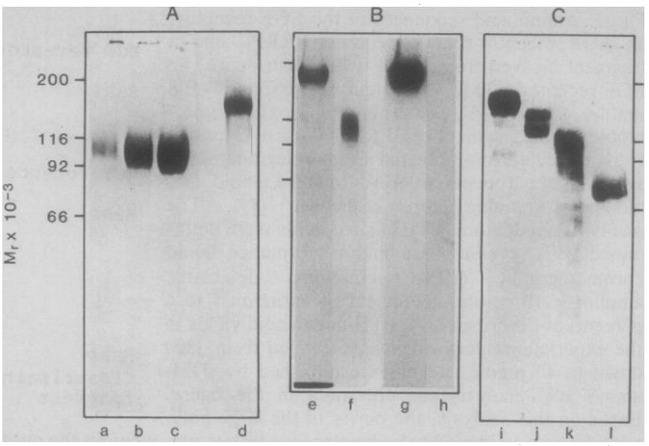

Fig. 1. Identification of an EGF receptor-related protein (ERRP) in culture medium from A431 cells. (A) Time course of accumulation of ERRP in culture medium. After incubation of the confluent A431 cultures for 15, 32, and 55 hours, the medium (Dulbecco-modified Eagle's medium, 0.5 percent calf serum) was passed through columns containing monoclonal antibody to EGF receptor (528) coupled to Sepharose. Cells from the same plates were extracted with a buffer containing 1 percent Triton X-100 and protease inhibitors, and extracts were chromatographed on parallel 528-IgG Sepharose columns. After extensive washing with buffers containing Triton X-100, NaCl, and 1*M* urea, bound proteins were desorbed with 6*M* urea, concentrated by dialysis and lyophilization, and analyzed on SDS–polyacrylamide gels. (lanes a to c) ERRP from medium harvested after 15, 32, and 55 hours incubation. (lane d) EGF receptor isolated from those cells responsible for the accumulation of ERRP. The amount of EGF receptor did not change during the experiment, and the amount shown corresponds to the cells which produced the amount of ERRP shown. (B) Competitive elution from immobilized monoclonal antibody to EGF receptor by EGF. Culture medium and cell extract were adsorbed to 528-IgG Sepharose which was extensively washed and incubated for 30 minutes at 5°C with 10 μ*M* [γ-^{32}P]ATP, 5 m*M* $MgCl_2$, 1.5 m*M* $MnCl_2$, 50 μ*M* $NaVO_3$ to permit self-phosphorylation of the EGF receptor. Bound proteins were eluted with 100 μ*M* EGF and analyzed by SDS–polyacrylamide gel electrophoresis. (lanes e and g) EGF receptor from solubilized cells; (lanes f and h) ERRP from medium. (lanes e and f) Coomassie blue stain; (lanes g and h) autoradiograms of lanes e and f, respectively. (C) Enzymatic deglycosylation of EGF receptor and ERRP. Each protein (8 μg) was incubated with 0.6 unit of endo-β-*N*-acetylglucosaminidase F (New England Nuclear) for 1 hour at 37°C and then for 12 hours at 25°C (*18*). Mock-incubated control protein and proteins treated with endoglycosidase F were analyzed on 10 percent SDS–polyacrylamide gels and stained first for glycoproteins by the Schiff procedure (*17*) and then for proteins with Coomassie blue. Schiff staining was positive only in controls (lane i, EGF receptor and lane k, ERRP) and did not stain the treated proteins, which were detected only with Coomassie blue (lanes j and l).

not affect EGF binding and thus recognizes a receptor protein domain distinct from that recognized by competitor 528 IgG. The polyclonal antibodies were affinity-purified by adsorption to Sepharose-immobilized EGF receptor protein or to immobilized ERRP before use. Under all conditions the affinity of EGF receptor antibodies for ERRP was 4 to 20 times less than for the EGF receptor itself. For example, the equilibrium binding constant (K_D) for EGF receptor binding to monoclonal antibody 29.1 and polyclonal antibodies (Fig. 2, A and B) were 3 and 2 n*M*, respectively, whereas the K_D's for ERRP binding to these two antibodies were 14 and 20 n*M*, respectively. Thus ERRP contains EGF binding, glycosylation, and antibody recognition domains similar to the EGF receptor.

To obtain a more direct comparison of ERRP and EGF receptor proteins, each was purified by affinity chromatography with monoclonal 528 IgG immobilized to Sepharose. Dansyl end group determination (*20*) revealed leucine at the amino terminus. Sequence comparison by means of automated Edman degradation revealed complete identity at the amino-terminal sequences of the EGF receptor and ERRP (Fig. 3). After digestion with clostripain and separation by high-performance liquid chromatography, one peptide from ERRP lacking arginine was also selected for sequence analysis (Fig. 3). On the basis of carboxyl-terminal analysis of ERRP with carboxypeptidase Y at different *pH* values (*21*), it appears likely that the 16-residue polypeptide is an internal fragment and does not represent the carboxyl-terminal sequence of ERRP. Carboxyl-terminal analysis of ERRP suggests that serine is the carboxyl-terminal amino acid. None of the analyzed sequences corresponded to the amino-terminal sequence of chicken erb B (*22, 23*) which is the predicted homolog of the carboxyl terminus of the intact EGF receptor protein (*19*).

ERRP was produced in serum-free as well as in serum-replete culture medium, indicating that serum proteases did not generate ERRP. The rate of production of ERRP into medium was proportional to the amount of EGF receptor protein in clonal variant A431 cells. Clone 29R2, which contains six to ten times more EGF receptor than clone 1 (*24, 28*), produced correspondingly more ERRP than clone 1 (data not shown). The large amount of EGF receptor protein present in $A431_8$ cells correlates with transloca-

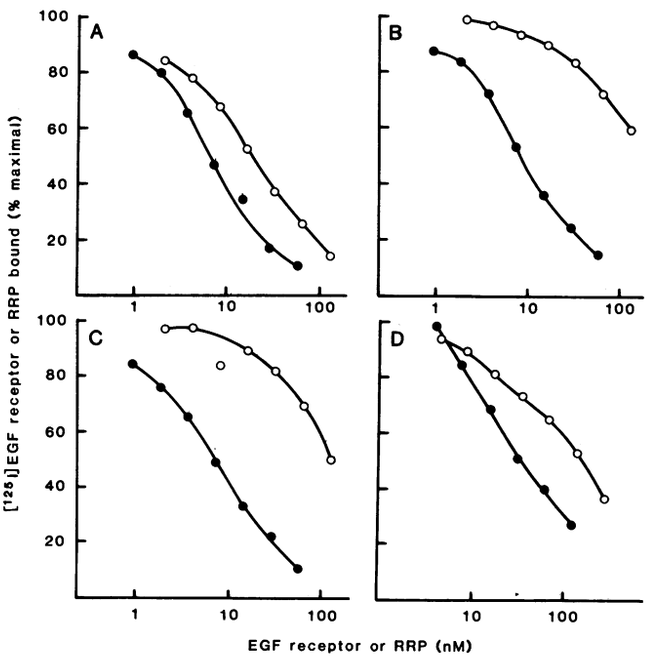

Fig. 2. Binding of EGF receptor-related protein (ERRP) to monoclonal and polyclonal antibodies to EGF receptor. Increasing amounts of purified EGF receptor protein (●) or ERRP (○) were used as competitors with either ^{125}I-labeled EGF receptors (A to C) or ^{125}I-labeled ERRP (D) for binding to (A) monoclonal antibody to EGF receptor IgG 29.1 (*19*) or to polyclonal antibody to EGF receptor generated in rabbits inoculated with EGF receptor protein purified from A431 cells. Polyclonal antibodies were affinity-purified on columns containing either EGF receptor protein (B) or ERRP (C and D) coupled to Sepharose. Immune complexes were isolated by adsorption to Formalin-fixed protein A containing *Staphylococcus aureus*, and radioactivity was quantified. The fluorescamine method with bovine serum albumin as standard (*37*) was used to quantify proteins used as competitors in this experiment. Molar concentrations were calculated from the protein molecular weights of deglycosylated EGF receptor and ERRP.

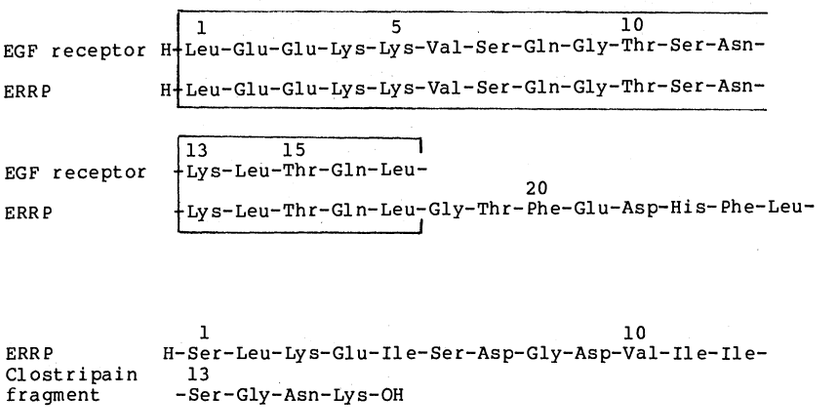

Fig. 3. Amino acid sequences of the EGF receptor, an EGF receptor-related protein (ERRP), and a fragment cleaved from ERRP with clostripain. The EGF receptor (120 μg of protein; approximately 0.8 nmole), ERRP (135 μg of protein; approximately 1.7 nmole), and a fragment of ERRP (0.7 μg; 0.43 nmole) were subjected to automated Edman degradation in separate experiments in a Wittmann-Liebold (*38*) modified spinning cup sequencer (*39*). The phenylthiohydantoin (PTH) amino acids were determined with reverse-phase high-performance liquid chromatography (HPLC) (minimal detectable amount < 10 pmole, coefficient of variation 1 to 6 percent) as described (*39*). PTH-amino acid yields in the experiments performed have ranged from 1300 down to 40 pmole. Residues established by PTH-amino acid analysis are presented in the figure. Based on this analysis, the purity of the large polypeptides was estimated to be greater than 90 percent, whereas the purity of the 16-residue polypeptide was determined to be 70 to 80 percent. The 16-residue polypeptide was cleaved from ERRP with clostripain (*21*), purified with reverse-phase HPLC on a Vydac C_{18} column, hydrolyzed with constant boiling HCl and 3 μl of thioglycol per milliliter (140°C, 15 hours) and analyzed with a Beckman 121 MB amino acid analyzer (*40*). The composition was determined to be: Asx, 3.1; Ser, 2.9 (corrected for losses during hydrolysis); Glx, 1.4; Gly, 2.3; Val, 1.1; Ile, 2.5; Leu, 1.4; Lys, 2.0. These data were in agreement with the sequence data (Fig. 3). All protein concentrations presented were determined by amino acid analysis.

tion of the portion of chromosome 7 carrying the EGF receptor gene (*26*), whereas clone 1 which has ~ 10 percent of the EGF receptor protein content of parental cells lacks this translocation (*27*). Production of the 105-kD protein does not appear to depend on translocation of chromosome 7 because it is produced by clone 1 cells, but its rate of production does correlate with the amount of EGF receptor and with the chromosome 7 translocation.

ERRP could arise from proteolysis at the cell surface of the mature EGF receptor protein, from intracellular processing from a common precursor or from a distinct biosynthetic pathway. Two approaches were used to study the origin of ERRP.

1) The rate of ERRP production was compared to the rate of degradation of the EGF receptor. The half-life of the EGF receptor was 18 hours as determined by pulse-chase experiments (data not shown). Given the measured steady state level of 6 pmole of EGF receptor per 10^6 cells, a degradation rate of 4 pmole per 10^6 cells per 24 hours can be calculated. This value is in agreement with the synthesis rate of 72,000 EGF binding sites per cell per hour (*11*) equivalent to 3 pmole of EGF receptor produced per 10^6 cells per 24 hours. The production rate of ERRP (Fig. 1) was determined to be 5 pmole per 10^6 cells per 24 hours, thus exceeding the degradation rate of the cellular EGF receptor. Because not all ERRP may be recovered from dilute medium, this represents a minimal figure. These results suggest that EGF receptor degradation cannot quantitatively account for formation of ERRP.

2) Biosynthetic labeling of ERRP was compared to that of the cellular EGF receptor. If ERRP is derived from the EGF receptor protein its specific activity should reflect that of the EGF receptor. To determine this relation, cells were incubated with [^{35}S]methionine for 7 hours to label EGF receptor and ERRP (*28*). The specific radioactivity of the EGF receptor at 2, 4, and 7 hours and the specific radioactivity of ERRP collected over the 7 hours were measured after samples were purified by affinity chromatography and sodium dodecyl sulfate–(SDS)–polyacrylamide gel electrophoresis. Table 1 shows that the specific radioactivity of the EGF receptor increased as expected over the 7 hours labeling period as unlabeled EGF receptor was replaced by newly synthesized protein. The specific radioactivity of ERRP, however, was greater than the highest specific radioactivity of the EGF receptor achieved. On a molar basis the specific radioactivity of ERRP was seven times greater than that of the average specific radioactivity of the EGF receptor. This result, which was confirmed in separate experiments with different labeling times, implies that ERRP is synthesized via a distinct pathway and cannot be derived from membrane bound EGF receptor protein.

Table 1. Comparison of specific radioactivities of EGF receptor and EGF receptor-related protein (ERRP). Dulbecco-modified Eagle's medium containing 10 μ*M* [^{35}S]methionine [10^7 cpm/ml) and dialyzed fetal calf serum (1 percent) was added to confluent A431 cells. Cells were harvested at the indicated times for purification of the cellular EGF receptor by immune-affinity chromatography. ERRP was isolated from medium collected at the end of the 7-hour pulse. Purified fractions (about 20 μg each) were subjected to SDS–polyacrylamide gel electrophoresis, and bands at 170 and 105 K_D were excised. Protein was quantified by photometry of the Coomassie blue stain extracted from the gel material, which then was used for the determination of incorporated radioactivity. The Coomassie blue extraction procedure was standardized by radioimmunoassay as described in the legend of Fig. 2. As a control for nonspecifically adsorbed radioactivity, medium from unlabeled cells was incubated for 7 hours with [^{35}S]methionine; no detectable radioactivity was associated with subsequently isolated ERRP.

Labeling time (hours)	Specific radioactivity (cpm/μg protein)
Cellular EGF receptor	
2	91
4	172
7	278
ERRP in medium	
0–7	593

Studies by Downward *et al.* (*19*) indicate that the cytoplasmic carboxyl-terminal portion of the EGF receptor is strongly homologous to the erb B transforming protein of avian erythroblastosis virus; both proteins are related to retroviral oncogenes which express tyrosine kinase activity (*22, 23*). The viral erb B protein, which is presumably homologous to chicken erb B is a 74-kD glycoprotein with antigenic determinants expressed on the cell surface (*29, 30*). The 105-kD glycoprotein identified in our studies lacks detectable tyrosine kinase activity and corresponds to the amino-terminal cell surface domain of the EGF receptor protein. Sequence analysis indicates that the two proteins are identical at their amino termini, but additional structural data are required to determine the extent of their identity. The decreased affinity of ERRP for antibodies to the EGF receptor could be due to conformational changes or to sequence differences distal to the common amino-terminal sequences. Our data suggest that the intact EGF receptor–kinase con-

sists of an 80-kD cell surface EGF binding portion with 30 kD of oligosaccharide (present in ERRP) and a 65-kD cytoplasmic tyrosine kinase and transmembrane containing portion (missing in ERRP). The amino-terminal sequence homologies indicate that the orientation of the EGF receptor is NH_2–EGF receptor–transmembrane–tyrosine kinase (erb B)–COOH.

ERRP does not appear to be derived from degradation of intact cell membrane inserted 170-kD EGF receptor. Only a fraction of the EGF receptor–kinase may mature to the membrane bound protein, and ERRP may be generated from proteolytic processing within the endoplasmic reticulum and Golgi. If this is correct, a domain containing tyrosine kinase (erb B) would be produced and either degraded or transported as a separate protein. By analogy with the insulin receptor whose tyrosine kinase activity is increased by partial proteolysis (*31*), separation of an erb B homolog from the EGF receptor portion might result in activation of the former. Alternatively, the EGF receptor–kinase and ERRP could be produced by way of separate genes or alternative splicing pathways. Using short 15-minute pulses of [^{35}S]methionine and a monoclonal anti-EGF receptor antibody, Cooper *et al.* (*32*) detected a slightly smaller precursor that matured to the 170-kD EGF receptor protein. Analysis of figure 7 from that study reveals a separate protein of ~ 100 kD which was synthesized in about the same amounts as EGF receptor protein but was not chased into mature EGF receptor by excess unlabeled methionine, rather it disappeared from the cell, consistent with it being secreted. Recent unpublished data indicates that A431 cells contain at least two EGF receptor–erb B messenger RNA (mRNA) species, a finding in agreement with that reported for chick cells which contain two mRNA's with erb B sequences (*33*). Taken together, these results suggest that ERRP is made separately from the EGF receptor. Synthesis may be from a distinct mRNA generated via alternative splicing.

It remains possible that EGF receptor degradation (*34*), as well as separate synthesis, contributes to total ERRP detected in medium, and additional studies are required to determine whether production of ERRP occurs in normal cells or is restricted to tumor cells. In A431 cells, increased synthesis of both EGF receptors and ERRP may result from translocation of the region of chromosome 7 containing the EGF receptor (*35*) and erb B (*36*) genes and their amplification. The secreted ERRP may provide a readily measurable marker of excessive production of EGF receptor and erb B proteins in human epidermoid carcinomas.

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References and Notes

1. H. Ushiro and S. Cohen, *J. Biol. Chem.* **255**, 8363 (1980).
2. G. Carpenter, *Mol. Cell Endocrinol.* **31**, 1 (1983).
3. H. Haigler, J. F. Ash, S. J. Singer, S. Cohen, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 3317 (1978).
4. J. Schlessinger, Y. Schechter, M. C. Willingham, I. Pastan, *ibid.*, p. 2659.
5. S. Cohen, H. Ushiro, C. Stoscheck, M. Chinkers, *J. Biol. Chem.* **257**, 1523 (1982).
6. T. Hunter and J. A. Cooper, *Cell* **24**, 741 (1981).
7. J. E. Buss, C. Chouvet, G. N. Gill, *J. Cell. Physiol.*, in press.
8. P. Rothenberg, L. Glaser, P. Schlessinger, D. Cassel, *J. Biol. Chem.* **258**, 4883 (1983).
9. W. H. Mollenaar, R. Y. Tsien, P. T. Van der Saag, S. W. de Laat, *Nature (London)* **304**, 645 (1983).
10. S. T. Sawyer and S. Cohen, *Biochemistry* **20**, 6280 (1981).
11. M. K. Krupp, D. T. Connolly, M. D. Lane, *J. Biol. Chem.* **257**, 11489 (1982).
12. M. C. Willingham and I. Pastan, *Cell* **21**, 67 (1980).
13. W. K. Miskimins, N. Shimizu, *J. Cell. Physiol.* **112**, 327 (1982).
14. T. Kawamoto, J. D. Sato, A. Le, J. Polikoff, G. H. Sato, J. Mendelsohn, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1337 (1983).
15. G. N. Gill *et al.*, *J. Biol. Chem.*, in press.
16. L. J. Pike, G. Gallis, J. E. Casnellie, P. Bornstein, E. G. Krebs, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 1443 (1982).
17. H. Glossmann and D. M. Neville, Jr., *J. Biol. Chem.* **246**, 6339 (1971).
18. J. H. Elder and S. Alexander, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4540 (1982).
19. J. Downward *et al.*, *Nature (London)* **307**, 521 (1984).
20. C. J. Bruton and B. S. Hartley, *J. Mol. Biol.* **52**, 165 (1970).
21. J. Spiess, J. Rivier, C. Rivier, W. Vale, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6517 (1981).
22. T. Yamamoto, T. Nishida, N. Miyajima, S. Kawai, T. Ooi, K. Toyoshima, *Cell* **35**, 71 (1983).
23. M. L. Privalsky, R. Ralston, J. M. Bishop, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 704 (1984).
24. J. E. Buss, J. E. Kudlow, C. S. Lazar, G. N. Gill, *ibid.* **79**, 2574 (1982).
25. T. Kawamoto, J. Mendelsohn, A. Le, G. H. Sato, C. S. Lazar, G. N. Gill, *J. Biol. Chem.*, in press.
26. N. Shimizu, I. Kondo, S. Gamou, M. A. Behzadian, Y. Shimizu, *Somatic Cell Genetics*, in press.
27. N. Shimizu, S. Gamou, D. M. Thompson, W. Weber, H. Masui, G. N. Gill, unpublished data.
28. G. N. Gill, J. E. Buss, C. S. Lazar, A. Lifshitz, J. A. Cooper, *J. Cell. Biochem.* **19**, 249 (1982).
29. M. J. Hayman, G. M. Ramsay, K. Savin, G. Kitchener, T. Graf, H. Beug, *Cell* **32**, 579 (1983).
30. M. L. Privalsky, L. Sealy, J. M. Bishop, J. P. McGrath, A. D. Levinson, *ibid.*, p. 1257.
31. S. Tamura, Y. Fujita-Yamaguehi, J. Larner, *J. Biol. Chem.* **258**, 14749 (1983).
32. J. A. Cooper, E. M. Scolnick, B. Ozanne, T. Hunter, *J. Virol.* **48**, 752 (1983).
33. B. Vennstrom and J. M. Bishop, *Cell* **28**, 135 (1982).
34. J. L. Gross, M. N. Krupp, D. B. Rifkin, M. D. Lane, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 2276 (1983).
35. I. Kondo and N. Shimizu, *Cytogenet. Cell Genet.* **35**, 9 (1983).
36. N. K. Spurr *et al.*, *EMBO J. (Eur. J. Mol. Biol. Org.)* **3**, 159 (1984).
37. S. Udenfriend, S. Stein, P. Böhlen, W. Dairman, W. Leimgruber, M. Weigele, *Science* **178**, 871 (1972).
38. B. Wittman-Liebold, in *Polypeptide Hormones*, R. F. Beers, Jr., E. G. Basset, Eds. (Raven, New York, 1980), pp. 87–120.
39. J. Spiess, J. Rivier, C. Rivier, W. Vale, in *Methods in Protein Sequence Analysis*, M. Elzinga, Ed. (Humana Press, Clifton, N.J., 1982), pp. 131–138.
40. J. Spiess, J. Villarreal, W. Vale, *Biochemistry* **20**, 1982 (1981).
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Antibodies to Hepatitis B Surface Antigen Potentiate the Response of Human T Lymphocyte Clones to the Same Antigen

Abstract. *Human T-helper lymphocyte clones specific for hepatitis B virus surface antigen (HBsAg) proliferate on stimulation with HBsAg in vitro. Antibodies specific for HBsAg, but no other antibodies, augment this proliferative response. In the presence of antibodies to HBsAg, the maximum response could be achieved at HBsAg concentrations that were 1 percent of those required in the absence of the antibodies. These findings suggest that antigen-specific antibodies exert regulatory controls on T cells that recognize the same antigens.*

T lymphocytes are important in the regulation of immune responses to most antigens (*1*). One characteristic of T cells is that they recognize antigens only in association with major histocompatibility gene complex–encoded molecules (Ia or DR) present on the surface of accessory cells (*2*) known as antigen-presenting cells. After T lymphocytes are triggered by the antigen, they proliferate and secrete factors that eventually will enhance or suppress the production of immunological effector molecules and cells (for example, antibodies and cytotoxic T lymphocytes).

As with most infectious diseases, resistance to hepatitis B virus (HBV) is largely mediated by protective antibodies and immune effector cells (*3*). Individuals who recover from an acute HBV infection develop immunity to the virus by producing antibodies to the viral en-