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Down-regulation of epidermal growth factor receptor correlates with plasminogen activator activity in human A431 epidermoid carcinoma cells

(plasmin)

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ABSTRACT **Human A431 epidermoid carcinoma cells in culture exhibit epidermal growth factor (EGF)-induced "down-regulation" of cell-surface and total cellular (Triton X-100 extractable) EGF receptors caused entirely by an enhanced rate (4-fold) of receptor inactivation [Krupp, M. N., Connolly, D. T. & Lane, M. D. (1982) *J. Biol. Chem.* 257, 11489–11496]. The following observations show that this enhanced rate of EGF receptor inactivation is closely correlated with an increased cellular activity of plasminogen activator (PA), a serine protease. First, EGF-induced down-regulation of cell-surface and total cellular EGF receptors and the concomitant increase in cellular PA activity occur with identical kinetics, the $t_{1/2}$ for both processes being 3–3.5 hr. Second, the EGF dose–response curves for down-regulation of total cellular EGF receptor and increased PA activity are similar. The EGF concentrations for half-maximal responses of both processes are 10–15 nM and 20 nM, respectively. Third, the removal of EGF from previously down-regulated cells results in the recovery of total cellular EGF binding activity with a concurrent loss of cellular PA activity. Fourth, blocking PA synthesis or activity with cycloheximide or dexamethasone prevents down-regulation of the EGF receptor. Fifth, the addition of leupeptin, an inhibitor of PA and plasmin action, blocks EGF-induced receptor down-regulation as well as the increase of PA activity. That EGF receptor down-regulation is independent of plasminogen *per se* in the culture medium suggests that PA-mediated events may initiate the rapid inactivation of the EGF receptor that occurs during down-regulation.**

The interaction of epidermal growth factor (EGF), a polypeptide hormone, with specific cell-surface receptors initiates numerous biochemical events (1) in target cells, most notably mitogenesis (2). Such factors as the period of exposure to EGF, the concentration of EGF, and the level of functional receptors at the plasma membrane affect the magnitude of ligand–receptor interactions and, thus, the cellular response to EGF (3–5). It has been established that cells can modulate their level of surface EGF receptors in response to EGF and thereby alter the biological response to this potent mitogen (3–5).

Like other receptors for polypeptide hormones (6), the EGF receptor exhibits ligand-induced "down-regulation" of cell-surface EGF binding capacity (3–5). This negative modulation of EGF receptor level *in vitro* is rapid, EGF concentration dependent, and EGF specific and occurs in many cell types (1, 3, 5). Reduced EGF binding capacity is the result of a lowered EGF receptor level rather than an altered affinity of the EGF receptor for its ligand (3, 5). Because down-regulation of EGF receptor in human A431 epidermoid carcinoma cells in culture includes both reduced cell-surface and total cellular (detergent-extractable) EGF receptors (3), this phenomenon results from fewer active receptors per cell rather than from a redistribution of EGF receptors from the cell surface to intracellular compartments. Furthermore, we have demonstrated that reduced EGF binding capacity in down-regulated A431 cells is not caused by an altered rate of receptor synthesis but is entirely attributable to an enhanced rate of receptor inactivation or decay (3).

Although the mechanism by which ligand-induced down-regulation promotes EGF receptor inactivation is unknown, there is some evidence with another cell-surface receptor that proteolysis modulates receptor metabolism. When proteolysis in chicken myogenic cultures is induced by chemical or viral transformation, both the steady-state concentration and the half-life of the surface acetylcholine receptor is decreased, while the rate of receptor synthesis is unaffected (7). Transformation of chicken myoblasts results in enhanced rates of acetylcholine receptor degradation (7) as well as higher levels of plasminogen activator (PA), a serine protease (8). The plasminogen dependence of this effect suggests that plasmin-mediated proteolysis (of the receptor itself or of other membrane components), generated by PA, accounts for most of the reduction in the half-life of the acetylcholine receptor (7).

In this investigation we have addressed the question of whether enhanced proteolysis could be responsible for the increased rate of EGF receptor inactivation that occurs with EGF-induced down-regulation in cultured A431 cells. More specifically, the involvement of PA, plasmin, or both in modulating EGF receptor binding capacity was investigated.

MATERIALS AND METHODS

Cell Culture. Human A431 epidermoid carcinoma cells were obtained from G. Todaro (National Cancer Institute) and maintained in Dulbecco's modified Eagle's medium (DME medium, GIBCO) with 10% (vol/vol) fetal bovine serum (GIBCO) in a 10% CO_2/90% air atmosphere at 37°C. For some experiments, cells were grown through at least five passages (1:10 split) in DME medium with 10% fetal bovine serum depleted of plasminogen (9) or in serum-free medium (10). Single-cell suspensions of A431 cells were counted with a hemocytometer after 2-min sequential incubations at 37°C with collagenase [150–200 units/mg in phosphate-buffered saline (P_i/NaCl)] and trypsin (0.05% in P_i/NaCl). Cell number over a 24-hr period was un-

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Abbreviations: EGF, epidermal growth factor; PA, plasminogen activator; DME medium, Dulbecco's modified Eagle's medium; P_i/NaCl, phosphate-buffered saline.

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affected by any chemical addition to the medium or by incubation in any of the described media.

Analysis of Binding of ^{125}I-Labeled EGF (^{125}I-EGF). Receptor-grade murine EGF was iodinated by the solid-phase lactoperoxidase method (11) using Enzymobeads (Bio-Rad Laboratories). ^{125}I-EGF was purified on a 0.7 × 23 cm Sephadex G-25 column (Pharmacia) in 0.1 M sodium phosphate buffer, pH 7.4, with 0.1% bovine serum albumin and stored at −20°C. ^{125}I-EGF had a specific activity of 1,000–3,000 cpm/fmol and was 95% precipitable with 20% (vol/vol) trichloroacetic acid.

To down-regulate A431 cells, confluent 60-mm cultures (0.8–1.5 × 10^7 cells per dish) were incubated with (and controls without) EGF in DME medium and 5% fetal bovine serum at 37°C as described below. After the indicated periods of incubation, cell monolayers were rapidly washed twice with ice-cold P_i/NaCl and allowed to equilibrate to 4°C. Subsequent procedures were performed at 4°C. For EGF binding studies, the monolayers were acid washed (3) to remove surface-bound EGF and washed rapidly (twice) with cold P_i/NaCl. To determine total cell-surface ^{125}I-EGF binding capacity, duplicate monolayers were incubated overnight at 4°C with ^{125}I-EGF at a final concentration of 10 nM EGF in Krebs–Ringer phosphate buffer and cell-associated radioactivity was determined (3). Nonspecific binding was measured similarly, but in the presence of unlabeled EGF at 1 μg/ml. Specific EGF binding activity was obtained by subtracting nonspecific binding from total binding activity.

For experiments with solubilized EGF receptor, total cellular membranes were prepared from duplicate cultures as described (3). The membranes were extracted in 2.0 ml of 20 mM Hepes, pH 7.5, with Trasylol (400 kallikrein inhibitor units/ml) containing 1% (vol/vol) Triton X-100. After a 30-min incubation at room temperature, the suspension was centrifuged in a Beckman 70.1 Ti rotor at 230,000 × *g* (r_{max}) for 45 min at 4°C. The supernate was stored at −70°C until EGF binding assays were performed.

The binding of ^{125}I-EGF to total cellular receptor—i.e., receptor solubilized from total cellular membranes with 1% Triton X-100, was carried out as detailed (3). Briefly, solubilized receptor (100 μl) was incubated at 25°C for 45 min with 100 μg of concanavalin A, ^{125}I-EGF (1 nM final concentration), 0.1% bovine serum albumin, and 0.2% Triton X-100 in 0.5 ml of 20 mM Hepes buffer, pH 7.5. Nonspecific binding was determined in the presence of unlabeled EGF at 1 μg/ml. The ^{125}I-EGF–receptor complexes were precipitated with 1 ml of 0.1 M potassium phosphate buffer, pH 7.4, containing 0.1% bovine gamma globulin and 1 ml of 21.3% (wt/vol) polyethylene glycol 8000 (Fisher) in the same buffer. The suspensions were centrifuged and the radioactivity in the pellets was determined (3).

For PA studies, cell monolayers were washed three times with cold P_i/NaCl, scraped into P_i/NaCl, and pelleted by centrifugation (400 × *g*, 5 min). The cell pellets were solubilized in 250 μl of 0.1 M Tris·HCl at pH 8.1/0.5% Triton X-100 and frozen at −20°C until total cell protein and PA were measured. Protein determinations were by the method of Lowry *et al.* (12), with bovine serum albumin as a standard.

Plasminogen Activator Assay. Aliquots of Triton X-100-extracted cells (1.0 μg of cell protein) were added to 500 μl of 0.1 M Tris·HCl (pH 8.1) containing 125 μg of bovine serum albumin and 4 μg of calf plasminogen (9). This mixture was added to dishes coated with ^{125}I-fibrin and incubated at 37°C for 1–2 hr (13). Aliquots (100 μl) were removed and their radioactivities were measured. The amount of radioactivity released by cellular PA was related to the amount released by 0.001 Plough unit of human urokinase standard (Leo Pharmaceutical, Ballerup, Denmark) included with all assays. The reaction was stopped when less than 20% of the total trypsin-removable radioactivity was released from the plates. A431 protease activity was entirely dependent upon plasminogen in the assay mixture. The fluctuations in basal levels of cellular PA represent variations in the ^{125}I-labeled fibrin and in the state of the standard urokinase.

Reagents. Sodium [^{125}I]iodide was purchased from Amersham. Murine EGF (receptor grade) was from Collaborative Research (Waltham, MA) or was prepared from mouse submaxillary glands (14) obtained from Pel-Freez. Trasylol was purchased from FBA Pharmaceuticals (New York). Concanavalin A, β-estradiol, dexamethasone, cycloheximide, chloroquine, and leupeptin were supplied by Sigma. Collagenase and trypsin were from Worthington. All other chemicals were as previously described (3) or were reagent grade.

RESULTS

Kinetics of EGF Receptor Down-Regulation and PA Induction. If inactivation of the EGF receptor that occurs during EGF-induced down-regulation (3) is related to or caused by PA- or plasmin-mediated proteolysis, there should be a correlation between the extent of EGF receptor down-regulation and the level of cell-associated PA activity. To investigate this association, the kinetics of EGF-induced down-regulation and PA activity in A431 cells were examined. Cultures were treated with 50 nM EGF for increasing periods of time, after which cell-surface and total cellular EGF binding activity and cell-associated PA levels were measured. As shown in Fig. 1 and as we have previously demonstrated (3), EGF-induced down-regulation of both cell-surface and total cellular EGF receptors occurs with similar kinetics ($t_{1/2}$ = 3 hr) and results in reduced (by 70–80%) EGF binding capacity within 20 hr after the initial exposure to EGF. A431 cells initially exhibited low basal levels of PA, which

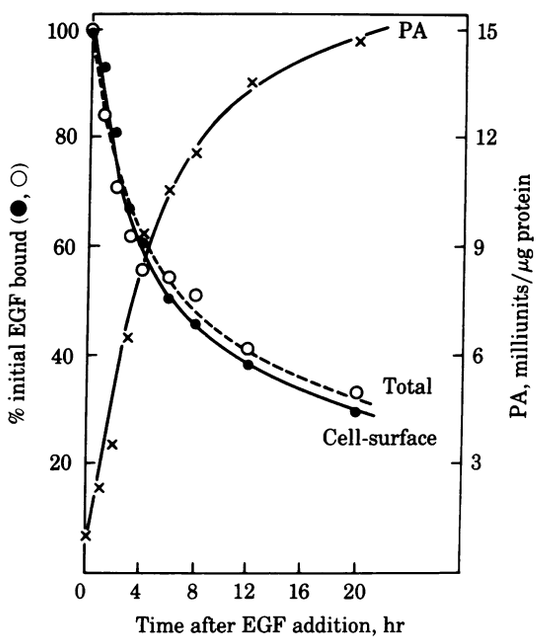

FIG. 1. Kinetics of down-regulation of the EGF receptor and the increase of cellular PA in A431 epidermoid carcinoma cells. Cell monolayers were incubated with DME medium containing 5% fetal bovine serum and 50 nM EGF for the indicated times. Specific ^{125}I-EGF binding to monolayers (cell-surface) or to total receptor in Triton X-100-solubilized extracts of total cellular membranes was measured. Parallel cultures were processed for determination of cell-associated PA activity. Each data point represents the average of two measurements from duplicate cultures.

were increased (up to 15-fold) with continuous incubation of the cells with EGF (Fig. 1). The kinetic course of the EGF-dependent increase in PA was similar to that of EGF-induced down-regulation; the $t_{1/2}$ for a half-maximal increase in PA activity was approximately 3.5 hr (Fig. 1). Thus, exposure of A431 cells to EGF resulted in enhanced PA activity with concomitant down-regulation of EGF receptor. The large number of surface EGF receptors in A431 cells [$>10^6$ per cell (3, 15)] and their high affinity for EGF precludes accurate measurement of cell-surface EGF binding at low EGF concentrations by using A431 cell monolayers. For this reason and because cell-surface EGF receptors constitute the major fraction (>80%) of the total receptor population and down-regulate concomitantly with the total receptor population (3), only total cellular EGF receptor binding was measured in subsequent experiments.

EGF Concentration Dependence and Reversibility of EGF Receptor Down-Regulation and PA Induction. Because the magnitude of EGF receptor down-regulation in A431 cells is dependent upon the EGF concentration (3), it was of interest to compare the dependence of cellular PA activity and EGF receptor level on EGF concentration. Cell monolayers were incubated with various concentrations of EGF for approximately 20 hr, after which both total cellular EGF receptor binding capacity and cellular PA activity were measured. As illustrated in Fig. 2, the EGF concentration required for half-maximal down-regulation of EGF receptor was approximately 10–15 nM EGF, with maximal down-regulation (to ≈25% of initial binding) achieved at about 50 nM EGF. As EGF receptor binding capacity decreased in response to EGF, cellular PA levels increased coordinately (Fig. 2). Half-maximal induction of PA activity occurred at approximately 15–20 nM EGF. Thus, increased cellular PA activities were closely correlated with decreased EGF binding capacity.

The reversibility of EGF receptor down-regulation upon removal of the ligand (3, 5) made it possible to further examine the association between EGF receptor level and cellular PA activity. Removal of EGF from previously down-regulated A431

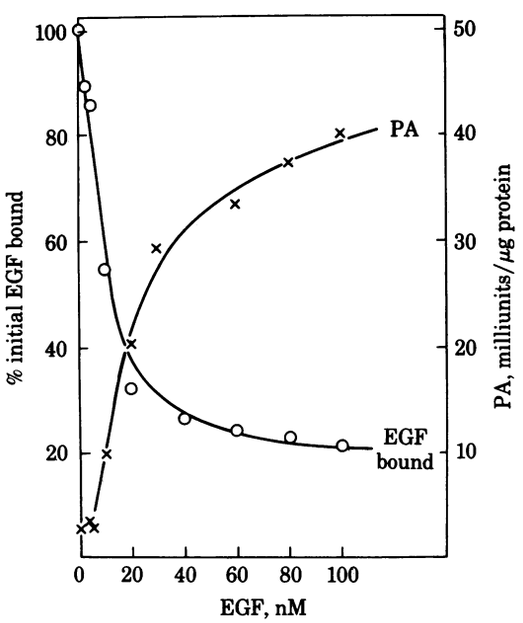

FIG. 2. EGF-concentration dependence of EGF receptor down-regulation and cellular PA activity in A431 epidermoid carcinoma cells. Cell monolayers were incubated for 18 hr with the indicated concentrations of EGF, after which ^{125}I-EGF binding to total cellular receptor and cell-associated PA activity were measured.

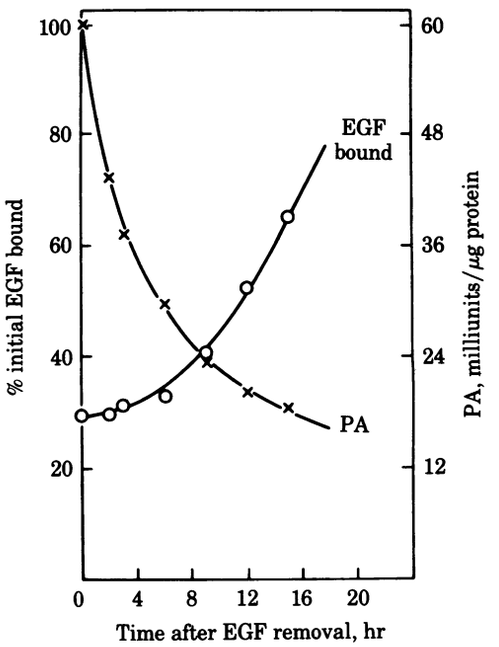

FIG. 3. Reversibility of EGF receptor down-regulation and associated cellular PA changes in A431 epidermoid carcinoma cells. A431 cultures were down-regulated for 24 hr with 50 nM EGF in DME medium and 5% fetal bovine serum. At 0 hr, cultures were washed twice with P_i/NaCl and incubated in medium without EGF. At various times ^{125}I-EGF binding to total cellular solubilized receptors and cell-associated PA activity were measured.

cells resulted both in the recovery of EGF binding and in the loss of cellular PA activity, as shown in Fig. 3. The time required for half-maximal loss of PA activity was 5–6 hr. Whereas PA levels began to fall immediately after EGF was removed, there was a 4- to 6-hr lag before EGF binding capacity began to recover. This suggests that the high cellular level of PA induced by EGF must fall substantially before the rate of EGF receptor inactivation decreases to a level sufficient for a net rise in receptor binding to occur. Taken together, the above results show that EGF binding capacity in A431 cells is closely correlated with decreased cellular PA activity.

Effect of PA Modulation on Cellular EGF Binding Capacity. Agents known to indirectly affect PA production or activity were used to modulate A431 PA levels and to determine the effect on total cellular EGF binding capacity. Cycloheximide, an inhibitor of PA synthesis (16), blocked both the EGF-induced increase in PA activity and EGF receptor down-regulation when added to A431 cell monolayers (Table 1). Similar effects were observed when A431 cells were cultured with dexamethasone, one of the most potent corticosteroid inhibitors of PA activity (17) (Table 1). In contrast, the EGF-induced down-regulation of soluble A431 EGF receptors proceeded in cells treated with β-estradiol, a steroid that has little effect on PA activity in some cell types (17), including A431 cells (Table 1). Thus, when the rise in PA activity in A431 cells caused by EGF (Figs. 1 and 2) was inhibited, normal EGF receptor levels were maintained.

To further evaluate the possible involvement of proteases in the down-regulation of the EGF receptor, the effects of the proteinase inhibitor leupeptin were investigated. Leupeptin inhibits serine proteases, including PA and plasmin (18, 19), as well as certain lysosomal proteases (18). As shown in Table 2, down-regulation of cellular EGF receptors was inhibited in A431 cells pretreated and maintained in leupeptin-containing medium. The increase in EGF binding capacity in EGF-treated

Table 1. Effect of cycloheximide and steroid hormones on PA and total cellular EGF binding activities

Addition	Cellular PA, milliunits/μg protein	EGF binding capacity, % of control
None (control)	0.54	100
EGF	26	32
Cycloheximide + EGF	0.05	94
None (control)	1.1	100
EGF	24	47
Dexamethasone + EGF	1.3	112
β-Estradiol + EGF	15	38

A431 cultures were incubated for 1 hr with 10 μM cycloheximide or for 7 hr with 1 μM steroids in DME medium with 5% fetal bovine serum. Cells were then incubated for an additional 20 hr or 18 hr, respectively, with cycloheximide or steroids, with or without 50 nM EGF. EGF specifically bound to receptor in Triton X-100-solubilized extracts of total cellular membranes was measured in duplicate cultures. Cell-associated PA activity was also measured in duplicate cultures.

cells was proportional to the amount of leupeptin in the medium (Table 2). Furthermore, leupeptin interfered with the relative increase in PA activity associated with EGF receptor down-regulation. Chloroquine, which blocks lysosomal proteolysis, was added to A431 cell monolayers to assess the role of lysosomal proteases in EGF receptor inactivation. Chloroquine had no significant effect either on the stimulation of PA activity by EGF or on the associated loss of EGF binding capacity caused by EGF (Table 2). These results suggest that a nonlysosomal serine protease(s) is involved in the initial events associated with EGF receptor down-regulation.

Effect of Plasminogen on EGF Receptor Down-Regulation. The results heretofore suggest that either PA or the resultant generated plasmin is involved in EGF receptor inactivation. To define the role of plasmin in this process, plasmin formation was prevented by growing A431 cells in medium lacking plasminogen. Plasminogen-free conditions consisted of either (*i*) chemically defined serum-free media (10) or (*ii*) DME medium with serum depleted of plasminogen (9). A431 cells grown in plasminogen-free medium maintained the ability to down-regulate total cellular EGF receptors when challenged with EGF

Table 2. Effect of leupeptin and chloroquine on cellular PA and total cellular EGF binding activities

Inhibitor	EGF, nM	Cellular PA, fold increase over control	EGF binding capacity, % of control
Leupeptin			
0 μM	0	1.0	100
0 μM	50	10.5	29
10 μM	50	3.6	63
50 μM	50	2.6	71
100 μM	50	2.1	88
Chloroquine			
0 μM	0	1.0	100
0 μM	60	20.2	59
25 μM	60	16.9	38
50 μM	60	18.3	56

A431 cell monolayers were incubated with leupeptin for 2 hr followed by an additional 18-hr incubation with or without 50 nM EGF in DME medium and 5% fetal bovine serum. Other monolayers were treated with chloroquine in the presence or absence of EGF for 18 hr. EGF specifically bound to receptor in Triton X-100-solubilized extracts of total cellular membranes was determined on duplicate cultures. Cell-associated PA activity was measured in duplicate cultures.

Table 3. Effect of plasminogen on down-regulation of the EGF receptor

Medium	EGF, nM	Cellular PA, milliunits/μg protein	EGF binding capacity, % of control
DME medium,	0	16	100
fetal bovine serum	20	22	48
with plasminogen	60	47	28
DME medium,	0	18	100
fetal bovine serum	20	40	60
without plasminogen	60	88	42
Serum-free	0	25	100
	20	40	61
	60	66	43

A431 cell monolayers were maintained through five passages in the respective media. At confluence cultures were treated with EGF for 18 hr. EGF specifically bound to receptor in Triton X-100-solubilized extracts of total cellular membranes from duplicate cultures was determined and cell-associated PA activity was measured in duplicate cultures.

at appropriate concentrations (Table 3). Importantly, the increase in PA activity attendant to down-regulation occurred both in the presence and in the absence of plasminogen (Table 3). Furthermore, there was little effect on the EGF binding capacity of A431 cell monolayers or total cellular membrane extracts treated with exogenously added plasmin (results not shown).[§] These results suggest that neither plasminogen nor plasmin *per se* is directly involved in EGF receptor inactivation during down-regulation.

DISCUSSION

A previous report from this laboratory showed that EGF-induced down-regulation of the EGF receptor in A431 cells results entirely from an enhanced rate of receptor inactivation (3). Results presented in this paper indicate that EGF receptor inactivation may be a direct or indirect result of a proteolytic event(s). The associations between decreased EGF binding capacity and increased cellular PA activity, and the converse, suggested that PA or plasmin may be involved in EGF receptor down-regulation. Plasmin, however, is most likely not responsible for EGF receptor inactivation because: (*i*) down-regulation occurs in A431 cells grown in the absence of plasminogen

[§] The effects of plasmin on the EGF receptor were assessed as follows. First, confluent A431 monolayers (1.4×10^6 cells per dish) were treated with exogenously added plasmin (10–150 units/dish; Sigma, 6.7 units/mg) for 18 hr at 4°C. After this time, (*i*) total cellular membrane extracts were prepared to measure EGF binding capacity and (*ii*) surface EGF receptor was labeled with ^{125}I-EGF by crosslinking with disuccinimidyl suberate (Pierce) to determine changes in the apparent molecular weight of the EGF-receptor complex. Extracts of crosslinked ^{125}I-EGF-EGF receptor were applied to sodium dodecyl sulfate/polyacrylamide gels and the gels were processed for autoradiography. Plasmin treatment of A431 monolayers resulted in a change in the apparent molecular weight of the EGF receptor (from approximately 160,000 to 145,000) with little loss—i.e., <10%—of the initial EGF binding activity. Second, Triton X-100-solubilized EGF receptor (equivalent to 0.025 10-cm culture dish), prepared from crude A431 membranes, was incubated with plasmin (25 units) for 28 hr at 37°C. Plasmin treatment, which caused a transitional change in apparent molecular weight of similarly prepared ^{125}I-EGF-EGF receptor (from 160,000 to 145,000 to 115,000) within minutes, resulted in no loss of soluble EGF binding capacity after 18 hr. Thus, proteolytic processing of the EGF receptor caused by plasmin had little or no effect on the EGF binding capacity of the receptor.

and serum (Table 3), and (*ii*) plasmin-treated EGF receptor retains full EGF binding activity.

Although plasmin has been implicated in receptor metabolism (7), a role for PA itself or a plasminogen-independent protease in receptor inactivation has not been suggested previously. That PA may be involved enzymatically at the cell membrane is supported by the following. First, PA is associated with and is firmly bound to cell membranes, including the plasma membrane (20, 21), and is also secreted into the extracellular environment. Also, PA retains activity at the cell surface despite the approximation of proteinase inhibitors in the serum (22). Second, there is evidence for a direct catalytic involvement of PA in mediating the morphological changes observed in phorbol ester-treated chicken cells, although the substrate affected by PA has not yet been identified (19). Therefore, because PA has been demonstrated to be active on protein(s) other than its natural substrate, plasminogen, PA could conceivably act enzymatically on the EGF receptor or on an EGF receptor-related protein. Third, macromolecules have been identified that modulate the plasma membrane levels of both urokinase and EGF. Protease nexin is a component released from fibroblasts that binds covalently to and inactivates serine proteases, including urokinase (23). Urokinase–protease nexin complexes bind to specific receptors for protease nexin and are then internalized and degraded. Similarly, EGF carrier protein nexin is a molecule secreted by cells that binds to and inactivates EGF carrier protein, an arginine endopeptidease that converts pro-EGF to EGF (24). Therefore, both protease nexin and carrier protein nexin are secreted by cells, form covalent complexes with serine proteases (urokinase and EGF carrier protein, respectively) in the extracellular milieu, and mediate the binding of these inactive protease complexes to cells via specific receptors for the nexins (23, 24). Thus, cellular mechanisms exist to modulate plasma membrane levels of EGF (via EGF carrier protein) and PA (urokinase), and hence EGF receptor binding capacity. PA may not, of course, affect the EGF receptor *per se*, but as a serine protease, may activate another protease that does. Proof for a direct or indirect involvement of PA in EGF receptor inactivation will, of course, require further experimentation.

Receptor down-regulation is a composite of several events, including the internalization, degradation, and recycling of the receptor (25). Although the site of PA involvement is not established, PA could be involved in several ways. For example, increased PA activity induced by EGF may result in the loss of EGF receptor binding capacity by direct receptor degradation. There is, however, no evidence yet to support this. Another possibility is that increased PA activity could cause an enhanced rate of EGF–EGF receptor internalization by slightly modifying either the EGF receptor (while retaining EGF binding capacity) or another membrane component so as to result in preferential internalization. Once internalized, the modified receptor could undergo degradation by other cellular proteases (26) or by lysosomal proteases in the normal degradative pathway. Alternatively, a slightly modified receptor–EGF complex could be internalized at a normal rate, but could not recycle to the cell surface. There is some evidence to suggest that limited proteolysis of the insulin receptor prevents its recycling (25). This may also be true for the EGF receptor. PA could conceivably be involved in altering any one (or more) of the parts of the receptor degradative/recycling pathways.

Nonetheless, it is evident that there is a definite correlation between PA activity and EGF receptor down-regulation. Although the role of PA in EGF receptor inactivation has yet to be defined, this enzymatic activity could represent an important modulator of receptor metabolism.

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1. Carpenter, G. (1981) in *Tissue Growth Factors*, ed. Baserga, R. (Springer, New York), pp. 89–132.
2. Carpenter, G. & Cohen, S. (1976) *J. Cell. Physiol.* **88,** 227–237.
3. Krupp, M. N., Connolly, D. T. & Lane, M. D. (1982) *J. Biol. Chem.* **257,** 11489–11496.
4. Carpenter, G. & Cohen, S. (1976) *J. Cell Biol.* **71,** 159–171.
5. Aharonov, A., Pruss, R. M. & Herschman, H. R. (1978) *J. Biol. Chem.* **253,** 3970–3977.
6. Cuatrecasas, P. & Hollenberg, M. D. (1976) *Adv. Prot. Chem.* **30,** 251–451.
7. Hatzfield, J., Miskin, R. & Reich, E. (1982) *J. Cell Biol.* **92,** 176–182.
8. Miskin, R., Easton, T. G., Maelicke, A. & Reich, E. (1978) *Cell* **15,** 1287–1300.
9. Deutsch, D. G. & Mertz, E. T. (1970) *Science* **170,** 1095–1097.
10. Barnes, D. W. (1982) *J. Cell Biol.* **93,** 1–4.
11. Marchalonis, J. J. (1969) *Biochem. J.* **113,** 299–305.
12. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193,** 265–275.
13. Crowe, R., Ozer, H. & Rifkin, D. (1978) *Experiments with Normal and Transformed Cells. A Laboratory Manual for Working with Cells in Culture* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), p. 79.
14. Savage, C. R., Jr., & Cohen, S. (1972) *J. Biol. Chem.* **247,** 7609–7611.
15. Haigler, H. T., Ash, J. F., Singer, S. J. & Cohen, S. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 3317–3321.
16. Rifkin, D. B., Beal, L. P. & Reich, E. (1975) in *Proteases and Biological Control*, eds. Reich, E., Rifkin, D. B. & Shaw, E. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 841–847.
17. Rifkin, D. B. & Crowe, R. M. (1980) *J. Cell. Physiol.* **105,** 417–422.
18. Aoyagi, T. & Umezawa, H. (1975) in *Proteases and Biological Control*, eds. Reich, E., Rifkin, D. B. & Shaw, E. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 429–464.
19. Quigley, J. P. (1979) *Cell* **17,** 131–141.
20. Quigley, J. P. (1976) *J. Cell Biol.* **71,** 472–486.
21. Solomon, J. A., Chou, I.-N., Schroder, E. W. & Black, P. H. (1980) *Biochem. Biophys. Res. Commun.* **94,** 480–486.
22. Chapman, H. A., Jr., Vavrin, Z. & Hibbs, J. B., Jr. (1982) *Cell* **28,** 653–662.
23. Low, D. A., Baker, J. B., Koonce, W. C. & Cunningham, D. D. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2340–2344.
24. Knauer, D. J. & Cunningham, D. D. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2310–2314.
25. Fehlmann, M., Carpenter, J.-L., Van Obberghen, E., Freychet, P., Thamm, P., Saunders, D., Brandenburg, D. & Orci, L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5921–5925.
26. Cassell, D. & Glaser, L. (1982) *J. Biol. Chem.* **257,** 9845–9848.