Mini-Review

Allosteric Regulation of the Epidermal Growth Factor Receptor Kinase

Joseph Schlessinger

Biotechnology Research Center, Meloy Laboratories, Rockville, Maryland 20850

SINCE the pioneering discoveries of nerve growth factor (24) and epidermal growth factor (EGF)[1] (8), it has become clear that polypeptide growth factors play a crucial role in the proliferation, survival, and differentiation of eukaryotic cells. Since then, many new growth factors have been discovered; some of them exert their biological effects on specific cell lineages, while others have broad specificity for many cell types from many tissues (17). EGF, because it is the best characterized growth factor, may serve as a model system for exploring the molecular mechanisms underlying mitogenic stimulation. Moreover, it has become clear that specific lesions in the EGF receptor, or abnormal expression of growth factors that mediate their effects by activating the EGF receptors, may be associated with certain cancers. Therefore, the elucidation of the mechanism of action of EGF will certainly provide important clues to fundamental questions concerning the action of growth factors in general and their role in oncogenesis.

The Structure of the EGF Receptor

EGF binds to a transmembrane glycoprotein and activates a protein tyrosine kinase activity (5). After purification of the human EGF receptor by immunoaffinity chromatography (45) and its partial sequencing (12), the complete primary structure of the EGF receptor was deduced from nucleotide sequence of cDNA clones (38). The following picture of the structure of the EGF receptor emerges. The mature receptor is composed of 1,186 amino acid residues that are preceded at the NH_2-terminal end by a signal peptide of 24 hydrophobic amino acids. The signal peptide is cleaved after insertion of the nascent receptor into the membrane of the endoplasmic reticulum. Cotranslationally, the receptor is glycosylated and transported through the Golgi apparatus to the plasma membrane. The mature receptor is composed of three major structural elements (Fig. 1). The first is an extracellular EGF-binding domain composed of 621 amino acid residues, anchored to the plasma membrane by a single transmembrane region of 23 hydrophobic amino acids. The transmembrane region is followed by a sequence of mostly basic residues; a feature common to many membrane proteins. The cytoplasmic domain of EGF receptor is composed of 542 amino acids. It contains a region of ~300 amino acid residues that is homologous to the catalytic domain of the protein tyrosine kinase encoded by the src gene family of oncogenes (14). Like the other protein tyrosine kinases, the catalytic domain of EGF receptor kinase contains a lysine (Lys 721) residue (Fig. 1) that is located 15 residues to the carboxy-terminal side of a consensus sequence, Gly-X-Gly-X-phe-Gly-X-Val. The lysine residue, together with the consensus sequence, probably functions as part of the ATP binding site (14).

The binding of EGF to the receptor induces the activation of the protein tyrosine kinase, which phosphorylates various cellular proteins as well as the EGF receptor itself. In intact cells, autophosphorylation occurs mainly on Tyr1173; however, at least two additional tyrosine residues located at the carboxy-terminal end of EGF receptor are phosphorylated when EGF is added to solubilized membranes or to the pure receptor (14). It was suggested that the autophosphorylation of EGF receptor regulates its capacity to phosphorylate exogenous substrates (1).

A hallmark of the extracellular domain of EGF receptor is a high proportion of cysteine residues clustered in two regions, each 160 residues long. The two cysteine-rich clusters can be aligned to form internal repeats with similar spacing between individual residues. Similar cysteine-rich domains were also found in the insulin receptor (13, 37) and in the HER2/*neu* protein, which probably functions as a membrane receptor for an as yet unknown growth factor (9, 33). Hence, the cysteine-rich domain of growth factor receptors probably evolved from a common ancestral gene (28).

The EGF Receptor and Cell Transformation

We have shown that the v-erbB oncogene of avian erythroblastosis virus encodes a truncated EGF receptor and proposed that the v-erbB protein induces transformation by functioning as an activated growth factor receptor. (12). The v-erbB protein (44) is devoid of most of the extracellular domain of EGF receptor and also of 32 amino acid residues at the carboxy-terminal end of the receptor, thus losing the major autophosphorylation site of the native receptor (Fig. 1). Nevertheless, the v-erbB protein, $gp74^{v\text{-}erbB}$, possesses intrinsic protein tyrosine kinase activity towards exogenous substrates and also undergoes autophosphorylation (19). Chicken erythroblastosis is also caused by avian leukosis virus, which transforms these cells by activating the c-erbB/EGF receptor gene through a promoter insertion mechanism. Proviral integration leads to the expression of a truncated EGF receptor with intrinsic protein tyrosine kinase activity (20). Interestingly, the v-erbB-like proteins expressed in these leukemias contain the coding information for 34 amino acids at the COOH terminus that are homologous to the respective sequence of the human EGF receptor, but

1. *Abbreviations used in this paper*: EGF, epidermal growth factor; LDL, low density lipoprotein.

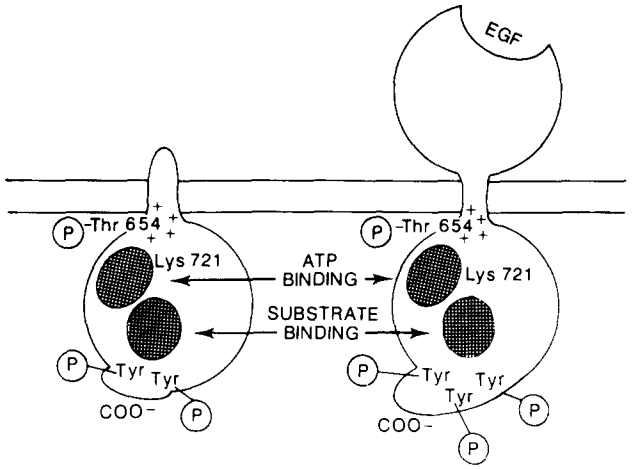


Figure 1. A model for the structure of the EGF receptor and the v-erbB protein. The EGF-receptor (*right*) is depicted as membrane protein composed of an extracellular EGF-binding domain connected to the cytoplasmic domain by a single transmembrane region. The cytoplasmic domain contains a protein tyrosine kinase with an ATP binding site (consensus Lys 721) and substrate binding region. Thr654, which is the major kinase C phosphorylation site, is located 10 amino acid residues into the cytoplasmic domain and is surrounded by basic residues. The carboxy-terminal tail of EGF receptor contains three autophosphorylation sites. The v-erbB protein (*left*) is a truncated EGF receptor devoid of most of the extracellular EGF binding domain and also lacks 32 amino acid residues from the carboxy-terminal tail, including the major autophosphorylation site (Tyr1173).

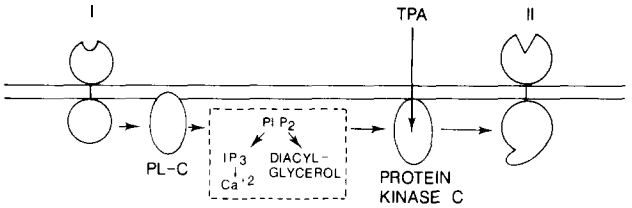


Figure 2. A model for heterologous regulation of EGF receptor functions (receptor transmodulation). According to this model the binding of growth factor to receptor I (i.e., PDGF to PDGF receptor or Bombesin to Bombesin receptor) will activate the phosphoinositol pathway by activating membrane phospholipase C (PL-C); probably through a specific G protein. This in turn will lead to the generation of diacyl glycerol and immobilization of Ca^{+2} ions necessary for the activation of kinase C. Kinase C phosphorylation of EGF receptor will modulate ligand binding affinity and protein tyrosine kinase activity.

The tumor promoter TPA will short circuit this process by binding to and activating kinase C directly.

which are deleted from v-erbB protein (30), thus suggesting that the COOH-terminal deletion is not required for erythroblastosis. Numerous studies suggest that the EGF receptor may play a role in oncogenesis through an autocrine mechanism. Various animal and human tumor cells produce a growth factor called transforming growth factor-alpha (alpha-TGF) (36). This growth factor is a member of the EGF gene family. It binds to cells bearing EGF receptor with an affinity similar to that of EGF and stimulates their proliferation. Todaro et al. (36) suggested that alpha-TGF plays a role in oncogenesis by stimulating the growth of cells bearing EGF receptors through an autocrine mechanism (36). Finally, it was shown that the EGF receptor gene is amplified and rearranged in many human brain tumors of glial origin (25). The resultant overexpression of the EGF receptor may play a role in the development or progression of these tumors.

Heterologous Regulation of EGF Receptor Functions

Binding experiments of ^{125}I-EGF to EGF receptor on living cells reveal two distinct affinity states of EGF receptor (18). High affinity sites usually represent 5–10% of the total receptor population, depending on cell type. This correlates well with the optimal receptor occupancy required for the initiation of DNA synthesis, and, therefore, it was suggested that the high affinity receptor plays a role in the mitogenic signalling process. The treatment of fibroblasts and A-431 cells with the tumor promoter 12-*O*-tetradecanoyl phorbol-13-acetate (TPA) abolished the high affinity state of the EGF receptor (4, 35) and reduced its protein tyrosine kinase activity (7, 16). Moreover, the addition of TPA to cells also blocks the stimulation of Na+/H+ exchange induced by EGF and by other growth factors (40, 41). TPA activates the Ca^{+2}-sensitive protein kinase C, which in turn phosphorylates the EGF receptor on several sites (6). One of these sites is Thr654 of the EGF receptor (10, 15); a residue located 10 amino acid residues from the membrane in the cytoplasmic domain of EGF receptor. Based on these results, it was suggested that the phosphorylation of EGF receptor on Thr654 regulates the affinity of the extracellular domain towards the ligand and the enzymatic activity of the protein tyrosine kinase domain.

Similar inhibition of the high affinity state of the EGF receptor was induced by platelet-derived growth factor and bombesin (3, 43). Both platelet-derived growth factor and bombesin elicit their mitogenic response by binding to their specific cell surface receptors. Moreover, both growth factors stimulate the phosphoinositol pathway, which in turn may lead to the phosphorylation of EGF receptor by kinase C (Fig. 2). Hence, it seems that the binding affinity of EGF receptor towards EGF is controlled by heterologous interactions with other occupied growth factor receptors possibly mediated by kinase C (31) that may act as a messenger for such receptor transmodulation or receptor cross-talk (Fig. 2). It was recently shown that in A-431 cells EGF induces the phosphorylation of EGF receptor on Thr654 through the activation of kinase C, thus raising the possibility that in these cells kinase C phosphorylation may provide a negative feedback mechanism to control receptor activity (42).

Models for Activation of the EGF Receptor

An interesting conclusion drawn from the primary structure of EGF receptor (38) is that the extracellular ligand binding domain is connected to the cytoplasmic kinase region by a single transmembrane region. This is in contrast to other transmembrane proteins that are also involved in signal transduction across the plasma membrane and that usually traverse the lipid bilayer more than once. Two distinct mechanisms can be reasoned for the transmembrane signalling of

membrane proteins with a single transmembrane region such as the EGF receptor (46–48). In an intramolecular model a vertical dislocation of the membrane hydrophobic stretch of EGF receptor is required for the transfer of a conformational change from the ligand binding domain to the cytoplasmic kinase region. However, the juxtamembranal sequence of EGF receptor contains charged amino acids at both faces of the plasma membrane, which may impose a high energy barrier for a putative vertical conformational change from the extracellular domain to the cytoplasmic region (28, 38).

An alternative mechanism involves an intermolecular allosteric process in which ligand-induced receptor oligomerization leads to the activation of the protein tyrosine kinase domain by subunit interaction between neighboring cytoplasmic domains, thus bypassing the requirement for a conformational change to propagate through the transmembrane region (Fig. 3). Recent results indicate that EGF-induced activation of its receptor kinase may involve an intermolecular step (46–48). However, once the receptor is activated, autophosphorylation seems to proceed by an intramolecular process (39, 47). It was shown that EGF-induced receptor self-phosphorylation has a parabolic dependence on the concentration of EGF receptor and that cross-linking of EGF receptor by antibodies or lectins stimulates receptor self-phosphorylation. Moreover, immobilization of EGF receptor on various solid matrices prevents EGF from activating the kinase function (46–48). It was also shown that in native gels, EGF receptor migrates in two forms: a fast-migrating form and an EGF-induced slow-migrating form. Based on various control and calibration experiments (48) it was concluded that the low form represents the monomeric 170-kD EGF receptor and the high form represents an EGF receptor dimer. The binding of EGF causes a rapid, temperature-sensitive dimerization of EGF receptors. Receptor dimerization is fully reversible and involves saturable, noncovalent interactins that are stable at neutral pH and in nonionic detergents.

Internalization of EGF Receptor

Like many other serum proteins, EGF is internalized by receptor-mediated endocytosis. Unlike the low density lipoprotein (LDL) receptor, which is preclustered in coated pits (2), EGF receptors are randomly dispersed on the cell surface (reviewed in 34). The occupied EGF receptors cluster in coated pits, and, after internalization, both EGF and its receptor are degraded by lysosomal enzymes. Hence, in contrast to the recycling of LDL receptor that is ligand-independent, EGF appears to facilitate the entry of its receptor into coated pits. The selectivity of this process may imply recognition signals that are associated with receptors that will facilitate their entry to and association with coated pits. Since clathrin and other proteins that are associated with coated pits are located in the cytoplasmic side of the plasma membrane, it is possible that the putative recognition sites of receptors reside in their cytoplasmic domains. Indeed, mutations in the cytoplasmic domain of LDL receptor impair its ability to cluster in coated pits and abolish receptor endocytosis (2, 22, 23). However, EGF receptor does not share sequence similarity with the LDL receptor or with other receptors that enter the cells via the coated pits pathway.

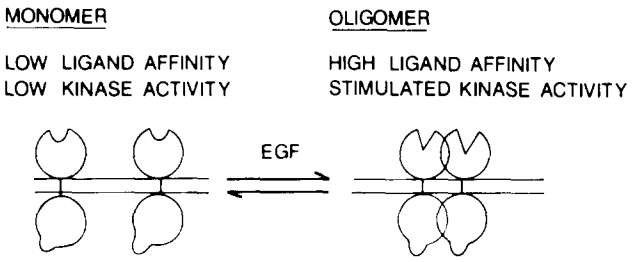


Figure 3. An allosteric oligomerization model for the activation of the EGF receptor kinase by EGF. EGF receptor is depicted as a biglobular transmembrane molecule as shown in Fig. 1. It is proposed that monomeric receptors exist in equilibrium with receptor oligomers. It is postulated that monomeric receptors possess low ligand affinity and reduced kinase activity and oligomeric receptors have high binding affinity and stimulated kinase activity. Hence, EGF binding will drive the aggregation process and thus stimulate the protein tyrosine kinase activity.

Hence, it is possible that the clustering in coated pits is mediated by chemical modifications added posttranslationally to the receptor protein by some other as yet uncharacterized processes.

It is clear from the results presented so far that EGF receptor is a multifunctional allosteric protein with several regulatory sites. These regulatory sites modulate various receptor functions such as: ligand affinity, receptor clustering and endocytosis, protein tyrosine kinase activity, and the state of receptor phosphorylation. An approach that affords the analysis of the various structural domains of the EGF receptor is the use of site-directed in vitro mutagenesis of EGF receptor cDNA combined with transfection experiments into heterologous animal cells. This approach was used to generate various EGF receptor mutants that were used for addressing questions of mechanism concerning the action and regulation of EGF receptor. Three types of EGF receptor mutants were already described; deletion mutants (27, 29, Reidel, H., J. Lee, T. J. Dull, R. M. Kris, M. D. Waterfield, J. Schlessinger, and A. Ullrich, manuscript submitted for publication), insertional mutants (32), and point mutants (26).[2]

Properties of Deletion Mutants of EGF Receptor

DNA sequences encoding the human EGF receptor and various EGF receptor deletion mutants were transfected into Chinese hamster ovary cells (CHO) that were devoid of EGF receptor. A functional EGF receptor was expressed in these cells. Like the native receptor, the EGF receptor expressed in the CHO cells has an apparent molecular mass of 170 kD. Moreover, it possesses an intrinsic protein tyrosine kinase activity that is stimulated by EGF leading to increased receptor self-phosphorylation and to the phosphorylation of exogenous substrates. The reconstituted EGF receptor exhibits typical two-affinity states toward EGF. Approximately 10% of the receptors exhibit a K_d of $\cong 3 \times 10^{-10}$ M (high affinity receptors) while the rest of the receptors have a K_d of $\cong 2 \times 10^{-9}$ M (low affinity receptors). It appears that both affinity

2. Livneh, E., R. Prywes, T. Dull, A. Ullrich, and J. Schlessinger, manuscript submitted for publication.

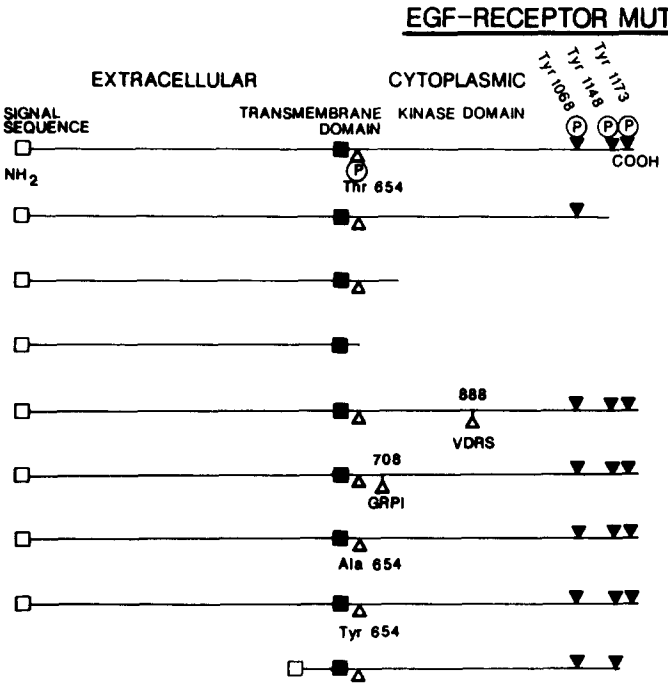
states of the receptor are encoded by a single gene and that the 170-kD EGF receptor expressed on the cell surface (Fig. 3) has the capacity to acquire two affinity states toward EGF (Fig. 4). This may be mediated by different oligomerization states of EGF receptor on the cell surface or by interactions with other unknown membrane or cytoskeletal molecules.

The reconstituted EGF receptor undergoes efficient EGF-induced endocytosis. Moreover, EGF stimulates the CHO cells expressing the EGF receptor to undergo DNA synthesis. Deletion of 63 amino acids from the COOH-terminal tail of EGF receptor, which removes two autophosphorylation sites, abolishes the high affinity state of the EGF receptor (Fig. 4). Nevertheless, this receptor mutant is able to undergo endocytosis and to respond mitogenically to the growth factor. Hence, the high affinity state of EGF receptor is not required for efficient endocytosis and DNA synthesis. Further deletions from the cytoplasmic domain give rise to low affinity endocytosis–defective receptor mutants. One of these mutants possesses 98 cytoplasmic amino acids and is devoid of most of the protein tyrosine kinase domain (Fig. 4). A larger deletion from the carboxy-terminal tail yields a receptor mutant with a short cytoplasmic tail of nine amino acids without Thr654, which is the major kinase C phosphorylation site (Fig. 4). Finally, deletion of the transmembrane region of the human EGF receptor yields an EGF receptor ligand binding domain that is secreted from the CHO cells (29).

Reidel and co-workers (Reidel, H., J. Lee, T. J. Dull, R. M. Kris, M. D. Waterfield, J. Schlessinger, and A. Ullrich, manuscript submitted for publication) generated a human counterpart of the v-erbB oncogene; namely, a cDNA construct encoding a double-truncated EGF receptor (Fig. 4). It was shown that this construct is able to transform Ratl cells, thus indicating that truncation of most of the extracellular domain and deletion of 32 residues from the carboxy-terminal end converts the EGF receptor into a transforming protein.

Insertional Mutants of the EGF Receptor

A murine retrovirus vector was used to express the human EGF receptor cDNA and two insertional mutants of the EGF receptor in mouse 3T3 cells (32). Synthetic SalI linkers encoding four amino acid residues were inserted in the kinase domain of EGF receptor to explore the role of this region on other known biological properties of the EGF receptor (32). A four amino acid insertion after residue 888 of the EGF receptor abolished protein tyrosine kinase activity, high affinity binding, internalization, and mitogenic responsiveness (Fig. 4). Another four amino mutation at residue 708 abolished the protein tyrosine kinase activity of the immunoprecipitated receptor (Fig. 4). However, this receptor mutant exhibited both the high and low affinity binding affinities towards EGF and was able to internalize ^{125}I-EGF efficiently into these cells. Surprisingly, EGF was able to stimulate DNA synthesis in these cells as efficiently as in NIH-3T3 cells expressing the wild type receptor construct (32). The properties of this mutant may suggest that the kinase activity is not required for internalization and mitogenesis. However, an alternative and more plausible interpreta-



CELLS	HIGH AFFINITY	LOW AFFINITY	KINASE ACTIVITY	ENDOCYTOSIS	DNA SYNTHESIS	TRANSFORMATION
C,N	+	+	+	+	+	−
C,N	−	+	+	+	+	−
C,N	−	+	−	−	−	−
C,N	−	+	−	−	−	−
N	−	+	−	−	−	−
N	+	+	−	+	+	−
L	+	−	+	+	+	−
R	+	+				
N	+	+	+	+	+	−
C	−	+				
R	−	−	+	ND		+

Figure 4. A summary of the properties of EGF receptor mutants. Details concerning the properties of these EGF receptor mutants are described in the text. The symbols used for the various established cell lines are as follows: mouse NIH-3T3, N; chinese hamster ovary cells (CHO), C; mouse L cells, L; and Ratl cells, R. EGF induced two to threefold thymidine incorporation into the CHO cells expressing the human EGF receptor and 5–10-fold stimulation of thymidine incorporation into the NIH-3T3 cells expressing the EGF receptor. It is noteworthy that the EGF receptor insertion mutant at residue 708 does not possess protein tyrosine kinase activity in our in vitro assays (Prywes et al., 1986). However, phosphorylation experiments in living cells indicate that under these conditions EGF is able to stimulate receptor autophosphorylation (Livneh, E., and J. Schlessinger, unpublished data). For further details see text.

tion is that the insertional mutation at the kinase domain renders the immunoprecipitated kinase unstable and therefore defective in the in vitro assays for autophosphorylation and exogenous substrate phosphorylation, while in intact cells, this receptor mutant may possess kinase activity capable of phosphorylating the physiologically important substrates. Indeed, we have recently shown (Livneh, E., and J. Schlessinger, unpublished data) that EGF is able to stimulate the autophosphorylation of this receptor mutant in living cells. Hence, more studies with new receptor mutants are required for establishing the role of the protein tyrosine kinase function in mediating the biological responses of EGF.

Point Mutations of the EGF Receptor

As shown earlier, Thr654 is the major kinase C phosphorylation site of EGF receptor. To probe the function of this amino acid residue, Thr654 was replaced either by an alanine residue (26) or by a tyrosine[2] residue utilizing in vitro site-directed mutagenesis. The Ala654 mutant of EGF receptor was expressed in mouse L cells (devoid of endogenous EGF receptor) and in RAT1 cells that possess endogenous EGF receptor. The properties of these mutants are summarized in Fig. 4. It appears that the Ala654 mutant of EGF receptor expressed in the heterologous cells did not internalize in response to TPA, although EGF was still able to induce endocytosis and degradation of the mutated receptor. On the basis of these results it was proposed that two independent mechanisms, one using Thr654 phosphorylation and a second mechanism that is EGF-dependent, play a role in the internalization of EGF receptor (26). The second mutant of EGF receptor contained a tyrosine residue instead of Thr654 (Fig. 4). In this receptor mutant Thr654 is replaced by an amino acid that is a potential phosphate acceptor site but cannot be phosphorylated by kinase C. The Tyr654 mutant of EGF receptor was expressed in NIH-3T3 (<1,000 endogenous EGF receptors/cell) and in CHO cells that are devoid of EGF receptor. Like the wild type receptor expressed on the surface of the NIH-3T3 cells, the Tyr654 receptor mutant exhibited both the high and the low affinity states toward EGF. The addition of TPA abolished the high affinity state of both the wild type and the Tyr654 receptor mutant in a similar manner.[2] Interestingly, the CHO cells expressing the Tyr654 receptor mutant exhibited a single binding affinity toward EGF (Fig. 4).

The mitogenic capacity of EGF can either be enhanced (11) or inhibited (21) by TPA, depending on cell type. It appears that in the NIH-3T3 cells expressing the wild type EGF receptor, TPA blocks the mitogenic capacity of EGF.[2] Interestingly, the inhibition mediated by TPA in cells expressing the wild type receptor does not occur in cells expressing the Tyr654 EGF receptor mutant. Namely, EGF is able to stimulate DNA synthesis in these cells even in the presence of TPA, which inhibits the mitogenic capacity of EGF in the 3T3 cells expressing the wild type receptor.[2] On the basis of these results, we propose that the phosphorylation of Thr654 by kinase C may provide a negative control mechanism for EGF-induced mitogenesis.[2]

In summary, the EGF receptor represents an allosteric protein composed of several regulatory domains with different functions. Detailed structure/function analysis using in vitro site-directed mutagenesis combined with expression of receptor mutants in heterologous cells will resolve the mechanisms underlying the regulation of the affinity state of the EGF receptor, activation of the protein tyrosine kinase, endocytosis, and, eventually, the structural elements that control DNA synthesis and oncogenesis. Since EGF and its receptor are also a good model system for other growth promoting factors, these studies will provide important clues for understanding the mechanisms underlying normal growth control and oncogenesis in general.

I would like to acknowledge fruitful discussions with E. Livneh, A. M. Honegger, R. Kris, I. Lax, T. Libermann, and A. Ullrich.

References

1. Betrics, P. J., and G. N. Gill. 1985. Self-phosphorylation enhances the protein-kinase activity of the epidermal growth factor receptor. *J. Biol. Chem.* 260:14642–14647.

2. Brown, M. S., R. G. W. Anderson, and J. R. Goldstein. 1983. Recycling receptors: the round trip itinerary of migrant membrane proteins. *Cell.* 32:663–667.

3. Brown, K. D., J. Blay, R. F. Irvine, J. P. Heslop, and M. J. Berridge. 1984. Reduction of epidermal growth factor affinity by heterologous ligands: evidence for a mechanism involving the breakdown of phosphoinsitide and action of protein kinase-C. *Biochem. Biophys. Res. Commun.* 123:377–384.

4. Brown, K. D., P. Dicker, and E. Rozengurt. 1979. Inhibition of epidermal growth factor binding to surface receptors by tumor promoters. *Biochem. Biophys. Res. Commun.* 86:1037–1043.

5. Carpenter, G., and S. Cohen. 1979. Epidermal growth factor. *Annu. Rev. Biochem.* 48:193–216.

6. Castagna, M., Y. Takai, K. Kaibuchi, K. Sano, U. Uikkawa, and Y. Nishizuka. 1982. Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. *J. Biol. Chem.* 257: 7847–7851.

7. Cochet, C., G. N. Gill, J. Meisenheider, J. A. Cooper, and T. Hunter. 1984. C-kinase phosphorylates the epidermal growth factor-receptor and reduces its epidermal growth factor stimulated tyrosine protein kinase activity. *J. Biol. Chem.* 259:2553–2558.

8. Cohen, S. 1962. Isolation of a mouse submaxillary gland protein accelerating incisor eruption eyelid opening in the newborn animal. *J. Biol. Chem.* 237:1555–1562.

9. Coussens, L., T. L. Yang-Feng, Y. C. Liao, E. Chen, A. Gray, J. McGrath, P. H. Seeburg, T. A. Libermann, J. Schlessinger, U. Francke, A. Levinson, and A. Ullrich. 1985. Tyrosine kinase receptor with extensive homology to EGF-receptor shares chromosomal location with neu oncogene. *Science (Wash. DC).* 230:1132–1139.

10. Davis, R. J., and M. P. Czech. 1985. Tumor-promoting phorbol diesters cause the phosphorylation of epidermal growth factor receptors in normal human fibroblasts at threonine-654. *Proc. Natl. Acad. Sci. USA.* 82:1974–1978.

11. Dicker, P., and E. Rozengurt. 1978. Stimulation of DNA synthesis by tumor promoters and pure mitogenic factors. *Nature (Lond.).* 279:723–726.

12. Downward, J., Y. Yarden, E. Mayes, G. Scrace, N. Totty, P. Stockwell, A. Ullrich, J. Schlessinger, and M. D. Waterfield. 1984. Close similarity of epidermal growth factor receptor and V-erb-B oncogene protein sequences. *Nature (Lond.).* 307:521–527.

13. Ebina, Y., L. Ellis, K. Jarnagin, M. Edery, L. Graf, E. Clausner, J.-H. Ou, F. Masiarz, X. W. Kan, I. D. Goldfine, R. A. Roth, and N. J. Rutter. 1985. The human insulin receptor cDNA: the structural basis for hormone activated transmembrane signalling. *Cell.* 40:747–758.

14. Hunter, T., and J. A. Cooper. 1985. Protein-tyrosine kinases. *Annu. Rev. Biochem.* 54:897–930.

15. Hunter, T., N. Ling, and N. A. Cooper. 1984. Protein Kinase-C phosphorylation of the EGF-receptor at a threonine residue close to the cytoplasmic face of the plasma membrane. *Nature (Lond.).* 314:480–483.

16. Iwashita, S., and C. F. Fox. 1984. Epidermal growth factor and potent tumor promoters induce epidermal growth factor receptor phosphorylation in a similar but distinctively different manner in human epidermal carcinoma A-431 cells. *J. Biol. Chem.* 259:2559–2567.

17. James, R., and R. A. Bradshaw. 1984. Polypeptide growth factors. *Annu. Rev. Biochem.* 53:259–292.

18. King, A. C., and P. Cuatrecasas. 1982. Resolution of high and low affinity epidermal growth factor receptors: inhibition of high affinity component by low temperature, cycloheximide and phorbol esters. *J. Biol. Chem.* 257:3053–3060.

19. Kris, R., I. Lax, M. Gullick, M. Waterfield, A. Ullrich, M. Fridkin, and J. Schlessinger. 1985. Antibodies against a synthetic peptide as a probe for the kinase activity of the avian EGF-receptor and V-erb-B proteins. *Cell.* 40: 619–625.

20. Lax, I., R. Kris, I. Sasson, A. Ullrich, M. J. Hayman, H. Beug, and J. Schlessinger. 1985. Activation of C-erb-B in avian leukosis virus-induced erythroblastosis leads to the expression of a truncated EGF-receptor kinase. *EMBO (Eur. Mol. Biol. Organ.) J.* 4:3179–3182.

21. Lechner, J. F., and M. E. Kaighn. 1980. EGF-growth promoting activity is neutralized by phorbol esters. *Cell Biol. Int. Rep.* 4:23–28.

22. Lehrman, M. A., J. L. Goldstein, M. S. Brown, D. W. Russell, and W. J. Schneider. 1985. Internalization defective LDL-receptors produced by genes with nonsense and frameshift mutations that truncate the cytoplasmic domain. *Cell.* 41:735–743.

23. Lehrman, M. A., W. J. Schneider, T. C. Sudhof, M. S. Brown, J. L. Goldstein, and D. W. Russell. 1985. Mutation in LDL-receptor: Alu-Alu-recombination deletes exons encoding transmembrane and cytoplasmic domains. *Science (Wash. DC).* 227:140–146.

24. Levi-Montalcini, R. 1966. The nerve growth factor: its mode of action on sensory and sympathetic nerve cells. *Harvey Lect.* 60:217–259.

25. Libermann, T. A., H. R. Nussbaum, N. Razon, R. Kris, I. Lax, M. Soreq, N. Whittle, M. D. Waterfield, A. Ullrich, and J. Schlessinger. 1985. Amplification, enhanced expression and possible rearrangement of the EGF-receptor gene in primary human brain tumors of glial origin. *Nature (Lond.).* 313:144–147.

26. Lin, C.-R., S.-W. Chen, C. S. Lazar, D. C. Carpenter, G. N. Gill, R. N. Evans, and M. G. Rosenfeld. 1986. Protein kinase-C phosphorylation at Thr654 of the unoccupied EGF-receptor and EGF binding regulate functional receptor loss by independent mechanisms. *Cell.* 44:839–848.

27. Livneh, E., M. Benveniste, R. Prywes, S. Felder, Z. Kam, and J. Schlessinger. 1986. Large deletions in the cytoplasmic kinase domain of epidermal growth factor receptor do not affect its lateral mobility. *J. Cell Biol.* 103:327–331.

28. Livneh, E., L. Glaser, D. Segal, J. Schlessinger, and B. Z. Shilo. 1985. The drosphila EGF-receptor gene homolog conservation of both hormone binding and kinase domains. *Cell.* 40:599–607.

29. Livneh, E., R. Prywes, O. Kashles, N. Reiss, I. Sasson, Y. Mory, A. Ullrich, and J. Schlessinger. 1986. Reconstitution of human EGF-receptors and its deletion mutants in cultured hamster cells. *J. Biol. Chem.* 261:12490–12497.

30. Nilsen, T. W., P. A. Maroney, R. G. Goodwin, F. M. Rottman, L. B. Crittenden, M. A. Raines, and H.-J. Kung. 1985. C-erb-B activation in ALV-induced erythroblastosis: novel RNA processing and promoter insertion result in expression of an amino-truncated EGF-receptor. *Cell.* 41:719–726.

31. Nishizuka, Y. 1984. The role of protein kinase-C in cell surface signal transduction and tumor promotion. *Nature (Lond.).* 308:693–698.

32. Prywes, R., E. Livneh, A. Ullrich, and J. Schlessinger. 1986. Mutations in the cytoplasmic domain of EGF-receptors affect EGF-binding and receptor internalization. *EMBO (Eur. Mol. Biol. Organ.) J.* 5:2179–2190.

33. Schechter, A. L., D. G. Stern, L. Vaidyanathan, S. J. Decker, J. A. Drebin, M. I. Greene, and R. A. Weinberg. 1984. The *neu* oncogene: an *erb*-B-related gene encoding a 185,000-M_r tumor antigen. *Nature (Lond.).* 312: 513–516.

34. Schlessinger, J., A. B. Schreiber, A. Levi. I. Lax, T. Libermann, and Y. Yarden. 1983. Regulation of cell proliferation by epidermal growth factor. *CRC Crit. Rev. Biochem.* 14:93–111.

35. Shoyab, M., J. E. DeLarco, and G. J. Todaro. 1979. Biologically active phorbol esters specifically alter affinity of epidermal growth factor receptors. *Nature (Lond.).* 279:387–391.

36. Todaro, G. J., C. Fryling, and J. E. DeLarco. 1980. Transforming growth factors produced by certain human tumor cells: polypeptides that interact with human EGF-receptors. *Proc. Natl. Acad. Sci. USA.* 77:5258–5262.

37. Ullrich, R., J. R. Bell, E. Y. Chen, R. Herrera, L. M. Petruzzelli, T. J. Dull, A. Gray, L. Coussens, X.-C. Liao, M. Tsubokawa, A. Mason, P. H. Seeburg, C. Grunfeld, D. M. Rosenfeld, and J. Ramachandran. 1985. *Nature (Lond.).* 313:756–761.

38. Ullrich, A.,L. Coussens, J. S. Hayflick, T. J. Dull, A. Gray, A. W. Tam, J. Lee, Y. Yarden, T. A. Libermann, J. Schlessinger, J. Downward, E. L. V. Mayes, N. Whittle, M. D. Waterfield, and P. H. Seeburg. 1984. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A-431 epidermoid carcinoma cells. *Nature (Lond.).* 309:418–425.

39. Weber, W., P. J. Bertics, and G. N. Gill. 1984. Immunoaffinity purification of the EGF-receptor. *J. Biol. Chem.* 259:14631–14636.

40. Whiteley, B., D. Cassel, Y. Zhuang, and L. Glaser. 1984. Tumor promotor phorbol 12-myristate 13-acetate inhibits mitogen stimulated Na+/H+ exchange in human epidermoid carcinoma A-431 cells. *J. Cell Biol.* 99:1162–1166.

41. Whiteley, B., T. Duel, and L. Glaser. 1985. Modulation of the activity of the platelet-derived growth factor receptor by phorbol myristate acetate. *Biochem. Biophys. Res. Commun.* 129:854–861.

42. Whiteley, B., and L. Glaser. 1986. EGF promotes phosphorylation at Threonine-654 of the EGF-receptor: possible role of protein kinase-C in homologous regulation of the EGF-receptor. *J. Cell Biol.* 103:1355–1362.

43. Wrann, M., C. F. Fox, and R. Ross. 1980. Modulation of epidermal growth factor receptors on 3T3 cells by platelet-derived growth factor. *Science (Wash. DC).* 210:1363–1364.

44. Yamamoto, T., T. Nishida, M. Miyajima, S. Kawai, T. Ooi, and K. Toyoshima. 1983b. The *erb*-B gene of avian erythroblastosis virus is a member of the *src* gene family. *Cell.* 35:71–78.

45. Yarden, Y., I. Harari, and J. Schlessinger. 1985. Purification of an active EGF-receptor kinase with monoclonal anti-receptor antibodies. *J. Biol. Chem.* 260:315–319.

46. Yarden, Y., and J. Schlessinger. 1985. The EGF-receptor: evidence for allosteric activation and intramolecular self-phosphorylation. *In* Growth Factors in Biology and Medicine. Pitman, London. 23–45.

47. Yarden, Y., and J. Schlessinger. 1986. Self-phosphorylation of EGF-receptor: evidence for a model of intermolecular allosteric activation. *Biochemistry.* In press.

48. Yarden, Y., and J. Schlessinger. 1986. EGF induces rapid, reversible aggregation of the purified EGF-receptor. *Biochemistry.* In press.