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Regulatory Control of the Epidermal Growth Factor Receptor Tyrosine Kinase

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A. Regulation of the EGF Receptor Tyrosine Protein Kinase Activity by EGF

The epidermal growth factor (EGF) receptor has been shown to posses an intrinsic tyrosine protein kinase activity that is stimulated by the binding of EGF [1]. In addition, two other ligands for the EGF receptor can stimulate the tyrosine protein kinase activity in intact cells: TGF-α (transforming growth factor α) and a soluble biosynthetic precursor of TGF-α [2, 3]. Analysis of the kinetics of autophosphorylation and of the activity of the receptor to phosphorylate exogenous substrates indicates that the autophosphorylation of the receptor causes an increase in its tyrosine protein kinase activity [4]. This increase in the tyrosine protein kinase activity of the receptor caused by phosphorylation represents a mechanism by which amplification of the signaling mechanism of the EGF receptor can occur subsequent to the binding of EGF to the receptor.

B. Phosphorylation of the EGF Receptor by Protein Kinase C

Protein kinase C is regulated by diacylglycerol and the cytosolic concentration of Ca^{++} [5]. It has recently been demonstrated that protein kinase C is a major cellular receptor for tumor-promoting phorbol diesters (e.g., PMA [1]) which bind to protein kinase C at the diacylglycerol binding site [5]. Treatment of cells with either PMA or diacylglycerol results in activation of the phosphotransferase activity of protein kinase C [5], the association of cytosolic protein kinase C with the inner surface of the plasma membrane [6, 7], and an increase in the phosphorylation state of the EGF receptor on serine and threonine residues [8–13]. The increased phosphorylation of the EGF receptor occurs at a site that is a substrate for protein kinase C (Fig. 1) and at additional sites [9, 10].

C. Substrate Specificity of Protein Kinase C

The protein kinase C phosphorylation site on the EGF receptor has been identified as threonine^{654} [9, 12]. It is located in a highly basic region of the EGF receptor that is close to the cytoplasmic surface of the plasma membrane. Similar locations of protein kinase C phosphorylation in the primary structure of pp60$^{c\text{-}src}$ [14] and in the interleukin 2 (IL-2) receptor [15] have been reported (Fig. 2). The marked similarity between the protein kinase C phosphorylation sites on the EGF receptor, pp60$^{c\text{-}src}$, and the IL-2 receptor suggests that the proximity of a potential phosphorylation site to the plasma membrane surface may be an important factor in determining the substrate specificity of protein kinase C. Recently, we

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[1] *PMA*, 4-phorbol 12β-myristate 13α-acetate

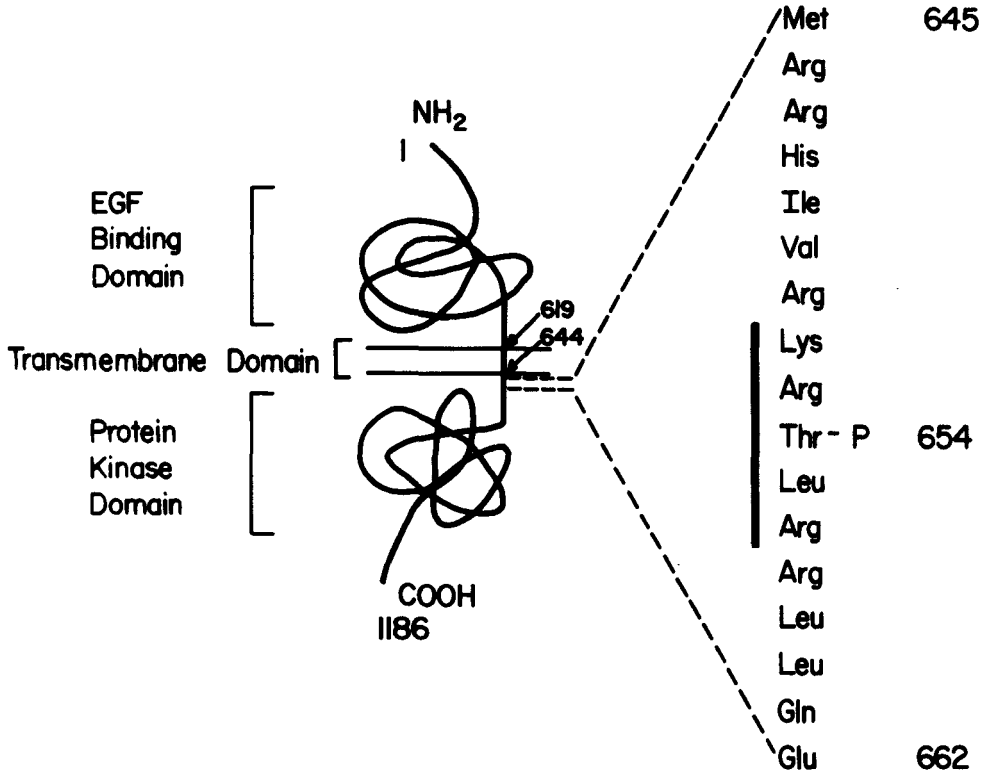

Fig. 1. Structure of the protein kinase C phosphorylation site on the EGF receptor (threonine^{654}), presented schematically. The deduced structure of the tryptic phosphopeptide containing threonine^{654} is indicated by a *bar*. (Reprinted, with permission, from Davis and Czech [9])

identified the protein kinase C phosphorylation site on the transferrin receptor as serine^{24} [16]. Inspection of the primary structure of the transferrin receptor indicates that serine^{24} is not located close to the transmembrane domain of the receptor in the primary sequence (Fig. 2). However, it is possible that the tertiary structure of the transferrin receptor is arranged so that serine^{24} is located close to the cytoplasmic surface of the plasma membrane. We conclude that the protein kinase C phosphorylation site on many integral membrane proteins may not have a primary structure that is homologous to the protein kinase C phosphorylation site on the EGF receptor (threonine^{654}). However, the hypothesis that similarity may exist in the tertiary structure of integral membrane proteins around the protein kinase C phosphorylation site remains to be tested.

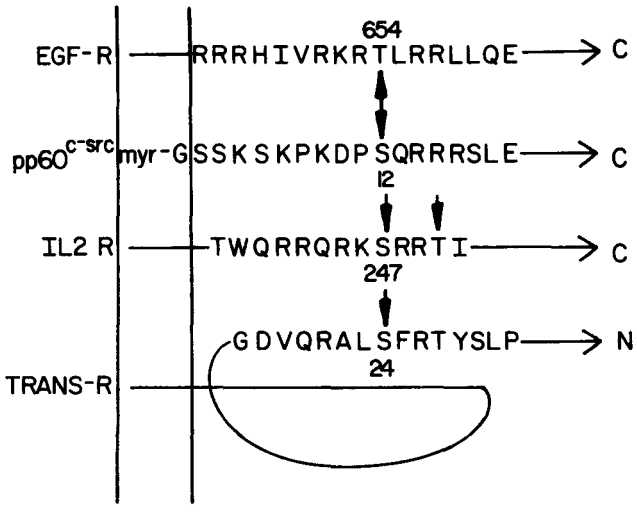

Fig. 2. Substrate specificity of protein kinase C. Protein kinase C phosphorylation sites on the EGF receptor (threonine^{654} [9, 12]), pp60$^{c\text{-}src}$ (serine^{12} [14]), IL-2 receptor (serine^{247} and threonine^{250} [15]), and transferrin receptor (serine^{24} [16])

D. Regulation of the EGF Receptor by Phorbol Diesters

Addition of the tumor-promoting phorbol diester PMA to A431 cells causes an inhibition of the high-affinity (Kd = 30–50 p*M*) binding of EGF [8] and TGF-α [2]. This high-affinity component of binding to A431 cells regulated by phorbol diesters represents

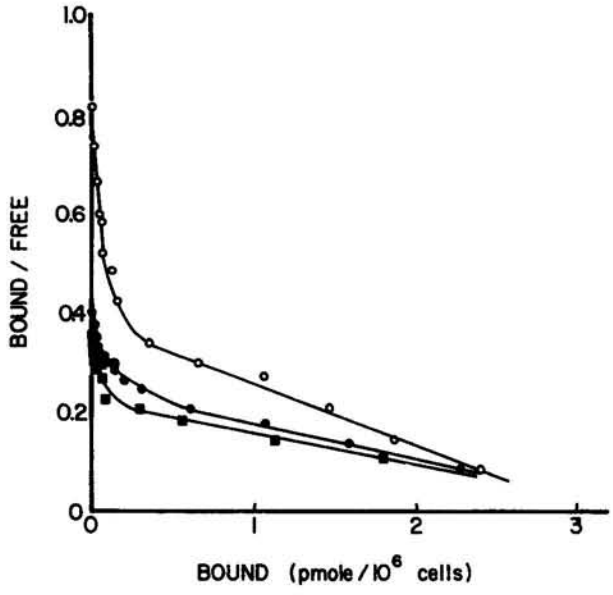

Fig. 3. Inhibition of ^{125}I-EGF binding by PMA and diC_8. A431 cell monolayers were incubated with no treatment (○), 10 n*M* PMA (■), or 3 μ*M* diC_8 (●) for 30 min at 37 °C. The cells were then cooled to 0 °C, and different concentrations of ^{125}I-EGF were added for 4 h. The monolayers were then washed with cold medium and solubilized with 0.4 *M* NaOH, and radioactivity associated with the cells was measured with a gamma counter. Nonspecific binding was measured in the presence of a 500-fold excess of EGF. The data are plotted according to the method of Scatchard. (Reprinted, with permission, from Davis et al. [20])

only 10% of the total binding of EGF or TGF-α that is observed. Most of the binding observed is to a component of low affinity (Kd = 0.3 n*M*) that is not regulated by PMA. A Scatchard plot of EGF binding to A431 cells treated with and without PMA is presented in Fig. 3. Two lines of evidence indicate that the regulation of the high-affinity binding of EGF and TGF-α is linked to the phosphorylation of the EGF receptor at threonine^{654}. First, the phosphorylation of the EGF receptor at this site has been shown to correlate closely with the regulation of the binding of ^{125}I-EGF to A431 cells by PMA [17]. Second, mutagenesis of the EGF receptor at threonine^{654} through replacement of this residue by alanine has been reported to prevent the action of PMA to regulate the binding of ^{125}I-EGF to cells [18].

A second reported action of PMA on the EGF receptor is inhibition of the tyrosine kinase activity of the receptor [11, 19, 20]. This is illustrated by the experiment presented in Fig. 4. It has been shown that this decrease in the tyrosine protein kinase activity of the EGF receptor is a result of the phosphorylation of the EGF receptor at threonine^{654} by protein kinase C [11].

The molecular basis of the perturbation of the EGF receptor (ligand binding and

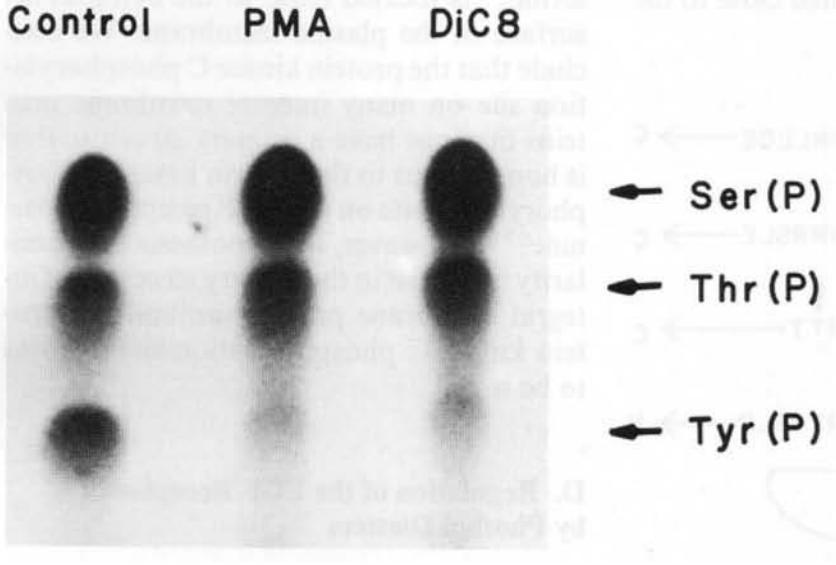

Fig. 4. Inhibition of tyrosine kinase activity of the EGF receptor by diC_8 and PMA. A431 cells labeled with [^{32}P]phosphate were treated with 10 n*M* PMA or 3 μ*M* diC_8 for 30 min. All the cells were then treated with 10 n*M* EGF for 10 min, and the EGF receptors were isolated by immunoprecipitation and polyacrylamide gel electrophoresis. Phosphoamino acid analysis was performed by partial acid hydrolysis and thin-layer electrophoresis (pH 3.5) of the [^{32}P] phosphoamino acids. Shown is an autoradiograph of the resolved [^{32}P]phosphoamino acids. (Reprinted, with permission, from Davis et al. [20])

tyrosine protein kinase activity) by phosphorylation at threonine^{654} by protein kinase C is not understood. However, this phosphorylation site is in an interesting region of the EGF receptor (Fig. 1). The site is nine amino acids from the cytoplasmic side of the predicted transmembrane domain of the EGF receptor, in a region that links the EGF receptor ligand-binding domain to the receptor tyrosine protein kinase domain. If a conformational change occurs subsequent to the binding of EGF to the receptor, the sequence surrounding the transmembrane domain will be of great importance for the transmission of this signal to the tyrosine kinase domain. The very basic sequence around threonine^{654} may be involved in the interaction of the receptor with other proteins or phospholipids by an electrostatic mechanism. The introduction of a phosphate group into this sequence could be expected to alter these interactions and may be sufficient to perturb the function of the EGF receptor.

E. Regulation of the EGF Receptor by Diacylglycerol

Diacylglycerol is able to stimulate the activity of protein kinase C [5]. It has been shown that the addition of exogenous diacylglycerol to A431 cells mimics the actions of PMA on the EGF receptor [20–22]. Thus, treatment of A431 cells with diacylglycerol causes an inhibition of the high-affinity binding of ^{125}I-EGF (Fig. 3) and decreases the tyrosine protein kinase activity of the EGF receptor (Fig. 4). These effects of diacylglycerol are associated with the phosphorylation of the EGF receptor at the same sites observed after treatment of the cells with PMA [20]. The structural requirements for diacylglycerols to regulate the EGF receptor have been investigated in detail. It was found that for symmetric sn-1,2-diacylglycerols with saturated acyl chains the optimal responses were observed with sn-1,2-dioctanoylglycerol (diC_8), and that the 3′ hydroxyl group was essential for the biological activity of the diacylglycerol [21].

We have recently investigated the hypothesis that the EGF receptor is regulated physiologically by changes in the activity of protein kinase C caused by alterations in the level of endogenous diacylglycerol. In these experiments the regulation of the EGF receptor by platelet-derived growth factor (PDGF) was examined in WI-38 human fetal lung fibroblasts [23, 23]. It has been reported that PDGF rapidly stimulates the hydrolysis of phosphatidyl inositol 4,5 bisphosphate which results in an increase in the level of diacylglycerol [25] and inositol-1,4,5-trisphosphate. The inositol-1,4,5-trisphosphate causes the release of Ca^{++} from intracellular stores and results in an increased cytosolic free Ca^{++} concentration [26, 27]. The dual action of PDGF to increase the level of diacylglycerol and free Ca^{++} would be expected to stimulate the activity of protein kinase C. We confirmed this by demonstrating that PDGF caused the phosphorylation of the EGF receptor at threonine^{654} [23]. The functional consequences of this action of PDGF were the inhibition of the tyrosine protein kinase activity of the EGF receptor and the inhibition of the high-affinity binding of ^{125}I-EGF to the fibroblasts [23].

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