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Molecular Analysis of Signal Transduction by Growth Factors

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Cell–cell interaction is an essential requirement for the integrated function of a multicellular organism during development and mature life. Secreted molecules and their specific cell surface receptors are key components of this cellular communication network. Ligand–receptor interaction on the cell surface is translated into activation of intracellular pathways, initiating a sequence of events that eventually results in a specific cellular response. Currently, two general mechanisms of cellular signal transduction are relatively well understood: coupling of receptors to various effectors by means of G proteins (Gilman, 1987) or coupling through the activation of a tyrosine-specific protein kinase activity that is intrinsic to the receptor molecule. Signaling by tyrosine kinase (TK) activation is shared by at least five known hormones and growth factors and their corresponding receptors. These receptor tyrosine kinases (RTKs) constitute a family of structurally related receptor polypeptides with a rapidly increasing number of members (Yarden & Ullrich, 1988).

In recent years it has become clear that the study of receptor function and receptor-activated signaling pathways will provide a better understanding of basic biological problems in the areas of cell biology, endocrinology, and development. As a first step toward the detailed analysis of growth factor activated cellular signaling mechanisms, structural characterization of the RTK family resulted in complete primary structures of all known RTKs. One surprising result of these efforts further reinforced the pivotal role of RTK molecules in signal transduction: structurally modified RTK genes were found to be part of the genomes of acutely oncogenic retroviruses (Heldin & Westermark, 1984; Sherr, 1987). Moreover, secretion of various ligands for RTKs was found to be widely associated with the transformed phenotype (Sporn & Roberts, 1985; Ross et al., 1986) and may therefore play a crucial role in oncogenesis. More recently, the availability of complementary DNA (cDNA) clones encoding RTKs enabled construction of structurally altered receptors, which provided insights into the molecular mechanism of signal transduction. To date, the results of these investigations, which are the subject of this review, indicate that RTK subclasses, despite their structural differences and possibly evolutionary origins, use similar mechanisms of transmembrane signaling with the TK activity being the enzymatic basis for all receptor-generated cellular signals. Further expansion of detailed understanding of signal transduction mechanisms is expected to pave the way for clinical studies of endocrine disorders and cancer.

Structural Aspects of RTKs

Partial amino acid sequence analyses of peptides derived from purified receptors enabled determination of the full-length nucleotide sequences of mRNA molecules encoding the receptors for epidermal growth factor (EGF) (Ullrich et al., 1984), insulin (Ullrich et al., 1985; Ebina et al., 1985), platelet-derived growth factor (PDGF) (Yarden et al., 1986), and insulin-like growth factor 1 (IGF-1) (Ullrich et al., 1986). The structure of the putative receptor for the macrophage growth factor (CSF-1; Sherr et al., 1985) was determined on the basis of its homology to the viral *fms* oncogene (Coussens et al., 1986). A similar approach yielded the structure of a receptor-like molecule encoded by the cellular homologue of the viral *kit* oncogene (Yarden et al., 1987). Finally, another putative receptor encoded by the HER2/*neu* protooncogene (also called c-*erb*B-2) was independently characterized by three groups (Coussens et al., 1985; Bargmann et al., 1986a; Yamamoto et al., 1986a). Surprisingly, a common structural organization emerged from the analysis of the deduced primary structures. The presence of an amino-terminal signal peptide and a single internal hydrophobic sequence confers the same transmembrane orientation for all RTKs. The extracellular ligand binding domain resides in the amino-terminal half of the molecule, whereas the catalytic function, a tyrosine-specific kinase, is contained in the cytoplasmic, carboxy-terminal

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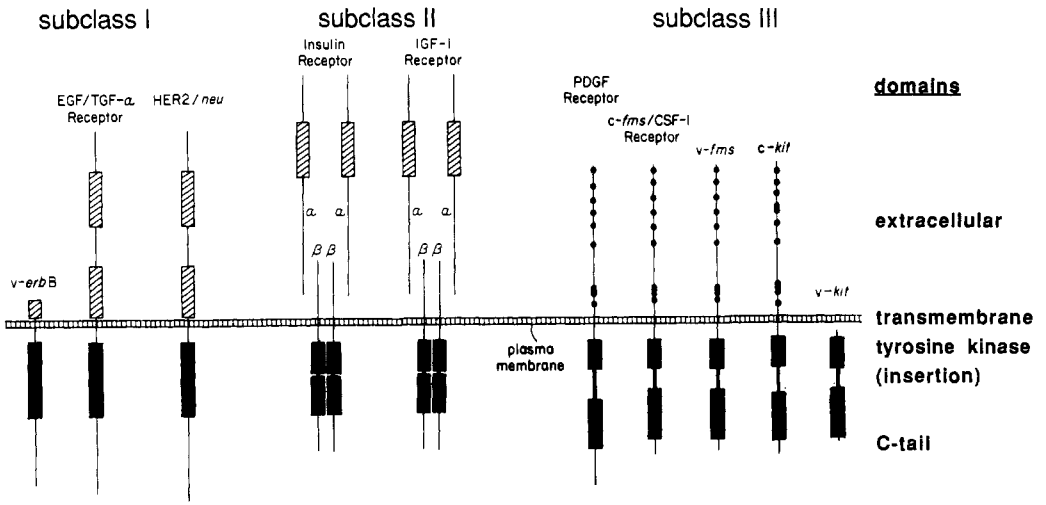

FIGURE 1: Subclasses and structural features of the family of receptor tyrosine kinases. All members and their known viral oncogenic counterparts are schematically shown. Hatched regions represent cysteine-rich repeat domains, and closed boxes demarcate the tyrosine kinase domains. Closed circles represent individual cysteine residues in the extracellular portions of subclass III RTKs.

portion of the receptor (see Figure 1). This topology provides a common overall structural basis for signal transduction. Closer primary sequence examination reveals the existence of subdomains and structural subgroups within the RTK family.

(*a*) *Extracellular Domain.* The ligand binding domain is an extensively glycosylated, protease-resistant structure. Consistent with their unique binding specificities, primary structure heterogeneity is found when RTK sequences are compared. Nevertheless, limited homology (40–50%) exists between the ectodomains of EGF receptor and HER2/*neu* product and also between insulin and IGF-1 receptors. The corresponding domains of PDGF receptor, CSF-1 receptor, and the c-*kit* gene product display less, but still significant, homology, but they are completely unrelated to the other RTKs. The most conserved structural feature within RTK subclasses (see below) is the distribution and spacing of cysteine residues. Whereas insulin and IGF-1 receptors contain a single cysteine-rich region, two clusters of 20–25 cysteine residues are found in the ectodomains of EGF receptor and the HER2/*neu* gene product. On the other hand, a strictly conserved pattern of 10 cysteine residues is found in the ectodomains of PDGF receptor, CSF-1 receptor, and the c-*kit* gene product.

(*b*) *Transmembrane Domain.* Membrane anchorage is achieved by a single stretch of 23–26 amino acids, which are highly hydrophobic but show no conservation of specific sequences within the RTK family.

(*c*) *Cytoplasmic Domain.* Most of the intracellular portion of RTK molecules represents sequences that specify the tyrosine-specific protein kinase function. This region displays extensive sequence homology within the protein tyrosine kinase family (Hunter & Cooper, 1985), but this is even more prominent within the following subfamilies: EGF receptor and the HER2/*neu* gene product; insulin and IGF-1 receptors; PDGF and CSF-1 receptors and the c-*kit* gene product. The TK domain sequences of the latter group are characterized by a 70–100 amino acid long insertion sequence found in the middle of the catalytic domain separating the nucleotide-binding region (Kamps et al., 1984) from the putative major tyrosine acceptor site (Smart et al., 1981). Unlike the surrounding catalytic sequences, this distinct and highly hydrophilic portion displays remarkable sequence heterogeneity among RTKs but is conserved among species. Noncatalytic sequences flank the enzymatic portion at both sides. A 50 amino acid region separates the ATP-binding domain from the membrane junction, and a variable-length hydrophilic carboxy-terminal tail (C-tail) is located distal to the TK domain. Whereas C-tail sequences display heterogeneity, limited structural conservation is found in the region between the transmembrane and TK domains within the most closely related groups of RTKs.

In conclusion, the RTK molecule is a mosaic of structural domains that are either distinct or shared by several receptors: a receptor-specific ectodomain is connected to a conserved catalytic portion, which is flanked or interrupted by yet other unique noncatalytic sequences. Conceivably, combination of receptor-specific sequences and shared catalytic function confers not only ligand specificity but also selectivity toward cytoplasmic effector molecules.

Shared structural features and homologous primary structures reveal the existence of three groups of receptors within the RTK family (see Figure 1):

(1) Subclass I includes EGF receptor and HER2/*neu* gene product and their *Drosophila* homologue (Livneh et al., 1985; Wadsworth et al., 1985) and is characterized by two or three cysteine-rich sequence repeat regions in their ectodomains.

(2) Subclass II includes insulin and IGF-I receptors and their *Drosophila* homologue (Petruzzelli et al., 1986). They are characterized by a single cysteine-rich sequence and a heterotetrameric structure of two extracellular α chains disulfide linked to two β chains that span the membrane and carry the TK function.

(3) Subclass III includes PDGF receptor, CSF-1 receptor, and c-*kit* gene product. Structural landmarks of this group are related ectodomains sharing cysteine residue distribution and insertion sequences within the tyrosine kinase domain.

Recently, new members of the family of transmembrane tyrosine kinases have been discovered. In addition to the already characterized PDGF receptor, a related human receptor was identified that may be specific for A-chain PDGF (D. Bowen-Pope, personal communication). Furthermore, the cellular homologues of the viral oncogenes *sea* (Hayman et al., 1985) and *ros* (Neckameyer et al., 1985), the fusion genes *trk* (Martin-Zanca et al., 1986) and *ret* (Takahashi & Cooper, 1987), the protooncogene *met* (Park et al., 1987), and the *sevenless* gene of *Drosophila* (Haffen et al., 1987) have been characterized as receptor-like tyrosine kinases. However, their DNA-deduced primary structures do not match the criteria of the subclasses described above. They therefore appear to constitute a new subclass with relatively large ectodomains and

longer cytoplasmic juxtamembrane regions.

Functional Aspects of RTKs

(*a*) *Ligands and the Pleiotropic Cellular Response.* The spectrum of target tissues for the known RTK ligands includes most of the body organs. Moreover, multiplicity of ligands that bind to the same receptor appears to augment the versatility of the RTK family. Thus, the receptor for epidermal growth factor also binds a related molecule, the transforming growth factor α (TGF-α; Derynck et al., 1984), and a factor encoded by vaccinia virus (Stroobant et al., 1984). Similarly, PDGF receptor binds a dimer of B chains (Kelly et al., 1985; Robbins et al., 1983; Josephs et al., 1984; Collins et al., 1985; Stroobant & Waterfield, 1984), a dimer of A chains produced by certain tumor cells (Heldin et al., 1986; Betsholtz et al., 1986a), and also a factor encoded by the viral *sis* oncogene (Waterfield et al., 1983; Doolittle et al., 1983; Wang & Williams, 1984). RTKs of subclass II also exhibit multiplicity since insulin and IGF-1 bind to each other's receptor.

Despite variation in the structures of both the ligands and their corresponding receptors, the cellular responses mediated by various RTKs display similar patterns and chronologies and in most cell types culminate in cell cycle progression. In addition, nonproliferative metabolic and cellular effects such as maintenance and differentiation, as well as cytokinetic and phagocytic responses, have been described for various ligands of RTKs. Generally, ligand-stimulated RTK activation triggers numerous cellular events that include activation of ion transport, membrane protein kinases, pinocytosis and ruffling, and changes in the cytoskeleton, followed by activation of a number of cytoplasmic pathways including polyamine synthesis, glycolysis and ribosomal protein S6 phosphorylation, and eventually increased synthesis of proteins, RNA, and DNA. Currently, the biochemical mechanism by which these pleiotropic responses are induced is unknown. However, it is believed that the tyrosine kinase function of the RTK molecule triggers multiple cytoplasmic systems and leads to elevated expression of specific genes, most notably the nuclear protooncogenes *fos* and *myc* (Kelly et al., 1983; Greenberg & Ziff, 1984; Kruijer et al., 1984; Muller et al., 1984; Bravo et al., 1985; Stumpo & Blackshear, 1986). Mechanistically, the action of PDGF, and probably CSF-1, differs from that of EGF, insulin, and IGF-1, since its mitogenic effect requires only a short interaction with the receptor (Pledger et al., 1977), and when given alone it is the most potent growth factor for mouse fibroblasts (Rozengurt, 1986). The unique potency of PDGF may reflect its ability to activate protein kinase C through an increased turnover of phosphatidylinositol (Habenicht et al., 1981).

(*b*) *Cellular Transformation by RTKs.* The pivotal role of RTKs and their ligands in cell regulation is best exemplified by the recent identification of retroviral oncogene products as structurally modified RTKs or their ligands. v-*erb*B, the transforming gene of the avian erythroblastosis virus (H strain), encodes a truncated EGF receptor lacking most of the extracellular ligand binding domain and 34 amino acids at the carboxy terminus (Downward et al., 1984a; Ullrich et al., 1984). v-*fms*, the oncogene of the McDonough strain of feline sarcoma virus, is a receptor for CSF-1 (Sherr et al., 1985), which lacks 11 carboxy-terminal amino acids (Coussens et al., 1986), and v-*kit*, the oncogene of the Hardy-Zuckerman-4 strain of feline sarcoma virus, encodes almost exclusively the tyrosine kinase domain of a putative receptor that is related to PDGF and CSF-1 receptors (Yarden et al., 1987). Another putative receptor, the product of the HER2/*neu* gene, acquires transforming potential upon replacement of a single amino acid at the transmembrane domain (Bargmann et al., 1986b). Constitutive and ligand-independent stimulation of these structurally altered RTK molecules is thought to provide a continuous mitogenic signal that leads to cellular transformation.

Constitutive production of a ligand molecule may also lead to uncontrolled cell proliferation. Thus v-*sis*, the oncogene of the simian sarcoma virus, encodes a protein that is almost identical with the B chain of PDGF (Waterfield et al., 1983; Doolittle et al., 1983) and can lead to cellular transformation upon interaction with PDGF receptor (Huang et al., 1984; Johnson et al., 1985; Betsholtz et al., 1986b). Although PDGF-like material, including the A-chain homodimer, is produced by many types of transformed cells (Bowen-Pope et al., 1984; Betsholtz et al., 1986a), it is probably not the primary characteristic responsible for transformation. In experimental systems the CSF-1 receptor is also capable of mediating transformation under conditions of autocrine ligand stimulation (Roussel et al., 1987). Similarly, the oncogenic potential of intact EGF receptor in combination with autocrine stimulation by TGF-α leads to transformation of fibroblasts in culture and appears to play an important role in squamous carcinomas and glioblastomas and in cells transformed by certain RNA and DNA viruses (Cowley et al., 1984; Hendler & Ozanne, 1984; Hunts et al., 1985; Libermann et al., 1985; Rosenthal et al., 1986; Yamamoto et al., 1986a,b; Derynck, 1986; Stern et al., 1987; Fizini et al., 1987; Riedel et al., 1988a).

Structure–Function Relationships

Biochemical analyses of the EGF receptor (Schlessinger, 1987) and the insulin receptor (Rosen, 1987) have established the notion that the RTK molecule is a multidomain allosteric enzyme that is regulated by both homologous and heterologous mechanisms. The availability of cloned cDNA sequences encoding the various RTKs adds a new dimension to these studies by enabling more refined assignments of receptor functions, including transforming potential, to specific structural features. Moreover, construction of chimeric receptor molecules has demonstrated mechanistic similarities within the RTK family.

(*a*) *Mutations within the Extracellular Domain.* Partial deletions of amino-terminal segments of EGF receptor resulted in reduced affinity to the ligand (Lax et al., 1988), and an almost complete deletion, exemplified by the v-*erb*B gene product, completely abolished EGF binding. Interestingly, neither the truncated receptor nor a secreted form of EGF receptor, which includes the entire extracellular domain (Yarden & Schlessinger, 1987a), is capable of self-dimerization, but the partial deletion mutants appear to participate in such dimers with intact receptors (Lax et al., 1988). Deletion mutants of HER2/*neu* gene product are partially (C. I. Bargmann and R. A. Weinberg, personal communication) or fully transforming (Di Fiore et al., 1987) and display dramatically elevated kinase activity (Y. Y. and R. A. Weinberg, unpublished results), suggesting a negative regulatory role for the extracellular domain.

(*b*) *Mutations within the Transmembrane Domain.* Replacement of a single valine residue by a glutamic acid residue in the rat *neu* gene product is sufficient to render this protein oncogenic (Bargmann et al., 1986b), probably through elevated tyrosine kinase activity (C. I. Bargmann, D. F. Stern, and R. A. Weinberg, personal communication). Importantly, replacement of valine residue 664 by glutamine but not by lysine, glycine, or aspartic acid has this effect. Thus, altered hydropathy of the transmembrane domain alone cannot explain

the observed biological effects. Experiments with chimeric receptors suggest, on the other hand, that the origin of the transmembrane domain did not affect transmembrane signaling functions or characteristics of the transmitted signal (H. Riedel and A. Ullrich, unpublished results).

(*c*) *Juxtamembrane Domain Mutations.* Protein kinase C mediated phosphorylation of a threonine residue (Thr_{654}) located in the juxtamembrane domain of EGF receptor is thought to lead to loss of high-affinity sites of EGF binding and to a concomitant attenuation of EGF receptor tyrosine phosphorylation (Friedmann et al., 1984; Hunter et al., 1984; Cochet et al., 1984; Iwashita & Fox, 1984; Davis & Czech, 1985; Downward et al., 1985). Consistent with this, an Ala_{654} EGF receptor mutant is resistant to phorbol ester induced reduction of high-affinity binding (Lin et al., 1986). Thus, the EGF receptor juxtamembrane domain appears to be the prime mediator of heterologous modulation of receptor activities. Similarly, an anti-peptide antibody directed to the analogous region of insulin receptor inhibited insulin-induced receptor self-phosphorylation (Herrera et al., 1985).

(*d*) *Tyrosine Kinase Domain Mutations.* Replacement of the lysine residue at the ATP binding site of the EGF receptor (Honegger et al., 1987a) and the insulin receptor (Ebina et al., 1987; McClain et al., 1987; Chou et al., 1987) and insertional mutations of EGF receptor (Prywes et al., 1986; Livneh et al., 1987) or the HER2/*neu* gene (C. I. Bargmann and R. A. Weinberg, personal communication) completely abolish receptor kinase activity. In comparison, an insulin receptor mutated at putative self-phosphorylation sites within the catalytic region displayed only partial kinase activity in vivo (Ellis et al., 1986). Analyses of these mutant receptors resulted in a uniform conclusion: the tyrosine kinase region, and thus by inference the catalytic activity, is indispensable for all the tested metabolic effects of EGF and insulin receptors, including ion and nutrient fluxes, activation of cytoplasmic enzymes, and induction of nuclear oncogenes. The kinase region is implicated in ligand-induced but not antibody-induced endocytosis followed by receptor degradation (Russell et al., 1987; McClain et al., 1987; Morgan et al., 1987; Honegger et al., 1987b). Various mutants of the transforming *neu* oncogene revealed a third kinase-dependent function: transformation potential was found to correlate with the endogenous tyrosine kinase activity (C. I. Bargmann and R. A. Weinberg, personal communication).

(*e*) *Carboxy-Terminal Domain Mutants.* Partial truncation of the C-tails of the EGF receptor (Livneh et al., 1986) and the insulin receptor (Ellis et al., 1986; McClain et al., 1988; Maegawa et al., 1988) did not impair the mitogenic function of the corresponding receptor, nor did this affect kinase function or down regulation. However, insulin-stimulated glucose uptake and glycogen synthesis were found to be impaired in cells bearing truncated receptors. Lack of effect on catalytic functions is consistent with experimental results obtained with antibodies directed against these regions of EGF and insulin receptors (Gullick et al., 1985; Herrera et al., 1985). Since self-phosphorylation sites are included in the distal parts of EGF and insulin receptors, the absence of an effect on the mitogenic function is surprising. Indeed, replacement of tyrosine residue 1173 of the EGF receptor, the major site of self-phosphorylation (Downward et al., 1984b), resulted in reduction of both kinase activity and maximal mitogenic response to EGF (Gill et al., 1987). The C-tail of CSF-1 receptor, when grafted into the analogous part of the v-*fms* product, reduces the transforming potential of the viral protein (Browning et al., 1986), and furthermore mutation of tyrosine residue 969, a putative self-phosphorylation site of CSF-1 receptor, restores full transforming potential upon a chimeric v-*fms*/c-*fms* gene (Roussel et al., 1987).

(*f*) *Chimeric RTKs.* Hybrid receptor molecules consisting of extracellular and cytoplasmic domains derived from heterologous RTKs demonstrate the power of a genetic approach to the study of signal transduction. Ligand binding and kinase activity are preserved in these hybrid molecules, indicative of the functional autonomy of the corresponding major structural domains. However, productive coupling of these functions is reconstitutable only in certain chimerae: the EGF receptor coupled to the insulin receptor (Riedel et al., 1986) or to the v-*erb*B gene product (Riedel et al., 1987) or the insulin receptor coupled to the v-*ros* gene product (Ellis et al., 1987). Combinations between RTKs and non-RTK molecules such as the receptor for interleukin 2 (Bernard et al., 1987) or v-*abl* gene product (Prywes et al., 1986) failed to translate ligand binding into kinase activation. Specificity of the biological signals evoked by the successfully reconstituted receptors depends on the presence of noncatalytic sequences, as suggested by a biologically active chimera of EGF receptor and insulin receptor (Riedel et al., 1986) and an inactive hybrid of insulin receptor and v-*ros* gene product (Ellis et al., 1987).

CONCLUSIONS

Assignments of Biological Functions to Structural Features. The remarkable similarity of the structural organization of RTK family members should reflect functional homologies at molecular and cellular levels. Although presently confined to a few RTKs, lessons learned from genetic approaches appear to support common, yet distinct, modes of signal transduction.

(*a*) *Ligand Binding.* Both high- and low-affinity ligand binding sites are due to single gene products. However, whereas the low-affinity component is an inherent property of the ectodomain, the high-affinity component, at least in the case of the EGF receptor, is determined by multiple cytoplasmic features including the juxtamembrane domain (Lin et al., 1986) and the carboxy-terminal tail (Livneh et al., 1986). The catalytic function appears not to affect ligand binding (Prywes et al., 1986; Livneh et al., 1986, 1987; Honegger et al., 1987a).

(*b*) *Signal Transduction.* The observed compatibility of heterologous receptor domains suggests similar transduction mechanisms for EGF and insulin receptors (Riedel et al., 1986), possibly due to receptor oligomerization (Biswas et al., 1985; Yarden & Schlessinger, 1987a,b; Boni-Schnetzler et al., 1987).

(*c*) *Biological Effects.* A functional tyrosine kinase and, by inference, the intact receptor catalytic domain are crucial for all the metabolic effects of EGF and insulin. Although the downstream elements are not known, they do not appear to be receptor specific since molecularily transfected RTK molecules are biologically active even in cells that do not normally express the receptors. The catalytic function, however, is not sufficient for signal generation, since cytoplasmic noncatalytic elements, mostly in the carboxy-terminal region, confer signal specificity (Riedel et al., 1988b).

(*d*) *Transforming Potential.* As for metabolic effects, functional tyrosine kinase is essential for the transforming potential of RTKs of subclasses I and III (Roussel et al., 1987). However, unlike metabolic effects, truncations or modifications at both the ectodomain and the carboxy-terminal tail synergistically augment the oncogenic capacity of various RTKs.

(*e*) *Endocytosis.* The same receptor may utilize different pathways of internalization. Lysosomal destination and eventually receptor degradation require tyrosine kinase activity

(Prywes et al., 1986; Livneh et al., 1986; Chou et al., 1987; Morgan et al., 1987; McClain et al., 1987; Chen et al., 1987) but are independent of the noncatalytic C-tail (Livneh et al., 1986; McClain et al., 1988). In contrast, endocytosis followed by ligand degradation and recycling of the EGF receptor to the cell surface appears to be independent of the tyrosine kinase activity (Honegger et al., 1987b).

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The Epidermal Growth Factor Receptor as a Multifunctional Allosteric Protein†

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Epidermal growth factor (EGF) is a polypeptide growth factor of 53 amino acid residues (Carpenter & Cohen, 1979), which mediates its biological responses by binding to and activating a specific cell surface receptor termed the EGF receptor. As a growth factor receptor, the EGF receptor is unique because it is specifically recognized and activated by three distinct growth factors encoded by separate genes (EGF, TGF-α, and VVGF). These growth factors have similar disulfide backbone structures, but their overall sequence is only 24% identical. Nevertheless, all three growth factors inhibit the binding of each other to the EGF receptor with similar affinities, suggesting that they bind to a similar region of the EGF receptor.

The EGF receptor is a glycoprotein of M_r 170 000, and it possesses intrinsic protein tyrosine kinase activity (Ushiro & Cohen, 1980). Growth factor receptors with intrinsic protein kinase activity can be divided into at least three distinct receptor classes, all of which have probably evolved from common ancestral genes. Generation of various transfected cell lines expressing native, mutant, and chimeric receptors has allowed the dissection of common and distinct aspects of receptor structure, as well as elucidating the mode of receptor action and regulation (Yarden & Ullrich, 1988). In this paper, we will describe recent studies that provide new insights into the structure of the EGF receptor, the role of various domains of the EGF receptor, the mechanism of receptor activation, and the potential role of the EGF receptor in oncogenesis.

Following the purification of EGF receptor (Yarden et al., 1985) and its partial sequencing (Downward et al., 1984a,b), the complete primary structure of the EGF receptor was deduced from the sequence of cDNA clones (Ullrich et al., 1984). The mature EGF receptor is composed of three major regions: a large glycosylated extracellular EGF binding region, which is anchored to the plasma membrane by a single membrane spanning region of 23 hydrophobic amino acids; a cytoplasmic portion containing the kinase domain, which contains consensus residues typical of the tyrosine kinase gene family [reviewed in Hunter and Cooper (1985) and Yarden and Ullrich (1988)]. The Lys-721 residue and consensus sequence Gly-X-Gly-X-Phe-Gly-X-Val, located 15 residues upstream to the lysine residue, probably function as part of the ATP binding site (Russo et al., 1985) in the kinase domain. The binding of EGF to the receptor induces activation of the protein tyrosine kinase (Ushiro & Cohen, 1980) leading to self-phosphorylation and phosphorylation of various cellular substrates. In intact cells, autophosphorylation occurs mainly on Tyr-1173 while at least two additional tyrosine residues located at the C-terminal end of the EGF receptor are also phosphorylated when EGF is added to solubilized membranes or to pure EGF receptor (Downward et al., 1984a). It has been suggested that autophosphorylation of EGF receptor releases a negative constraint leading to enhanced phosphorylation of exogenous substrates by the EGF receptor (Betrics & Gill, 1985).

Quantitative binding experiments with radiolabeled EGF indicate that the stoichiometry of ligand binding to the EGF receptor is 1:1 (Weber et al., 1984). Nevertheless, analyses of binding experiments of ^{125}I-EGF to intact cells according to the method of Scatchard reveal nonlinear plots, which are interpreted as an indication of the presence of different receptor classes with distinct affinities toward EGF. Hence, high-affinity EGF receptors with an apparent K_d of (1–3) $\times 10^{-10}$ M comprise 5–10% of the total receptors while the remaining low-affinity receptors have an apparent K_d of (2–15) $\times 10^{-9}$ M (King & Cuatrecasas, 1982). The treatment of cells expressing EGF receptor with the tumor promoter phorbol myristate acetate (PMA) or with growth factors such as PDGF or bombesin, each binding to its own distinct receptor, abolishes the high-affinity state of the EGF receptor and also reduces the tyrosine kinase activity of the EGF receptor (Shoyab et al., 1979; Cochet et al., 1984; Iwashita & Fox, 1984; Wrann et al., 1980). This process termed "receptor transmodulation" is probably mediated by the Ca^{2+}-sensitive kinase C, which has been shown to phosphorylate the EGF receptor on several sites [reviewed in Schlessinger (1986) and Hunter and Cooper (1985)]. One of these sites is Thr-654 (Hunter et al., 1984; Davis & Czech, 1985), which is located 10 amino acid from

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