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Topical Review

Epidermal Growth Factor Receptor: Elements of Intracellular Communication

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Introduction

Since the pioneering studies in the 1950's (Krebs & Fisher, 1956; Rall et al., 1956; Krebs et al., 1959), it has become increasingly established that, by modulating protein function, reversible protein phosphorylation has a dominant role in cellular regulation. Receptor recognition of hormones, neurotransmitters, autacoids, and growth factors is transduced from the plasma membrane to intracellular sites of response generation through the activation of protein kinases. Less, however, is known about the potential role of protein phosphatases in these signaling events.

Members of certain receptor families utilize guanine nucleotide binding proteins to mediate their actions through signal transduction pathways. Binding of agonists to these receptors results in the activation of specific G proteins leading to stimulation or inhibition of specific effector systems. The adenylate cyclase and the phosphoinositide cascades are the most well-defined effector systems, and both led to increased serine and threonine phosphorylation through the activation of protein kinase A, protein kinase C and Ca^{2+}/calmodulin protein kinases (Kikkawa et al., 1989; Berridge, 1990; Taylor et al., 1990). To date, receptors (Dohlman et al., 1991) coupled to G proteins have as a common feature a topology hallmarked by seven transmembrane sequences. This suggests that a complex receptor configuration may be essential for the mechanisms involved in receptor-G protein interaction prior to G protein activation of effector systems.

In contrast, peptide growth factor receptors have a single transmembrane sequence separating an extracellular ligand-binding domain and a cytoplasmic tyrosine kinase catalytic domain (Ullrich & Schlessinger, 1990). For these receptors, signal transduction is directly initiated as a result of growth factor binding to the extracellular domain and the subsequent rapid activation of cytoplasmic tyrosine kinase activity. While G protein-mediated effector systems that enhance ser/thr protein kinase activities, growth factors initiate tyrosine phosphorylation of their specific receptors and other cellular proteins (Ullrich & Schlessinger, 1990). Distal to tyrosine phosphorylation, growth factors do activate, by mechanisms that are not as yet clear, several serine/threonine kinases, such as MAP kinase and *raf* kinase (Carpenter & Cohen, 1990). Interestingly, a single membrane-spanning receptor topology is also exhibited by the ANF receptor, which has one transmembrane sequence separating a guanylated cyclase in the cytoplasmic domain and a ligand-binding external domain (Garbers, 1991). In this receptor, ligand binding results in downstream activation of the cyclic GMP-dependent ser/thr protein kinase. For neither the ANF receptor nor the growth factor receptors is there data indicating how a single transmembrane domain mediates activation of an enzyme activity in the cytoplasmic domain following ligand binding to the extracellular domain.

Phosphotyrosine is a rare phosphoamino acid in contrast to phosphoserine and phosphothreonine. The functional consequence of tyrosine phosphorylation of proteins is not as well understood as serine and threonine phosphorylations, but the number of distinct vertebrate protein tyrosine kinases stands at more than 40 (Hunter, 1989). Importantly, several facts demonstrate that tyrosine phosphorylation has a key role in cell growth regulation and in oncogenic transformation (Ullrich & Schles-

Key Words growth factors · receptors · phosphorylation · phospholipases

Table 1. Physiological responses to EGF

Rapid biochemical effects related to signal transduction	References
Receptor tyrosine autophosphorylation	Carpenter et al., 1979
Calcium uptake from extracellular medium	Sawyer & Cohen, 1981; Moolenaar et al., 1986
Calcium release from intracellular stores	Moolenaar et al., 1984; Morris et al., 1984; Johnson et al., 1986
Na^+/H^+ antiport activity	Rothenberg et al., 1983
Phosphoinositide hydrolysis	Wahl & Carpenter, 1988a
Formation of diacylglycerol	Smith et al., 1983 Serrero, 1987
Long term biological responses	**References**
Stimulation of cell proliferation	Raymond et al., 1986; Centrella, 1987; Reilly et al., 1987
Inhibition of acid secretion	Dembinski et al., 1986; Shaw et al., 1987
Inhibition of differentiation	Kim et al., 1987
Stimulation of oocyte maturation	Downs et al., 1988
Stimulation of vasoconstriction	Berk et al., 1985; Muramatsu et al., 1985, 1986

singer, 1990; Hunter, 1989). For example, nearly all growth factor receptors are directly or indirectly coupled to tyrosine kinase activation. Many known oncogenes are tyrosine kinases and, in a few instances, it has been demonstrated that tyrosine kinase receptors are protooncogenes. Also, the structures and expanding numbers of recently described phosphotyrosine phosphatases (Fischer et al., 1991) suggest that active regulation of tyrosine phosphorylation may occur at this point. The control of phosphatase activity and substrate specificity remain important areas of investigation.

One of the most studied and best characterized peptide growth factors is epidermal growth factor (EGF), initially described 30 years ago by Stanley Cohen (Cohen, 1962). To date there has been only one protein identified on the cell surface with which EGF interacts: the EGF receptor. The remainder of this review will focus on the EGF receptor, particularly its activation and signal transduction mechanisms, as the best understood example of a single transmembrane receptor.

Receptor Structure is Related to Biological Responses

The EGF receptor is a glycoprotein ($M_r \simeq 170{,}000$) of which approximately 40,000 daltons is N-linked carbohydrate. Ligand binding to the glycosylated external domain activates tyrosine kinase activity in the cytoplasmic domain (Carpenter et al., 1979; Ushiro & Cohen, 1980; Ullrich et al., 1984). The ligand-binding domain of the EGF receptor is specifically recognized by a family of growth factors encoded by different genes. These EGF-like ligands are transforming growth factor-α, pox virus growth factors, amphiregulin, and the heparin-binding EGF-like growth factor (Stroobant et al., 1980; Marguardt et al., 1984; Shoyab et al., 1988; Higashiyama et al., 1991). One of the most dramatic biological effects of EGF is the regulation of cell growth and differentiation, especially in epithelial cells and tissues (Carpenter & Wahl, 1990). However, EGF has other biological activities in addition to the stimulation of cell proliferation and the response to exogenous EGF may depend on cell type and physiological circumstances. Importantly, the exact physiological role(s) of endogenous EGF in the intact animal remains unknown. Some of the responses to EGF are presented in Table 1, (reviewed in Carpenter & Wahl, 1990).

By taking advantage of the fact that the A-431 epidermal carcinoma cell line overexpresses EGF receptors [2.6×10^6 receptors/cell (Haigler et al., 1978)], purification (Cohen et al., 1980) and subsequent cDNA cloning (Ullrich et al., 1984) of the human EGF receptor has been possible. These data suggest a receptor structure diagrammatically illustrated in Fig. 1.

The mature EGF receptor is composed of three major regions: an extracellular hormone binding domain, a hydrophobic transmembrane region, and a cytoplasmic domain. The extracellular domain contains the amino terminus, two high-cysteine content regions, and an EGF binding site, which is putatively localized between the high cysteine regions (Lax et al., 1988). There is also evidence that se-

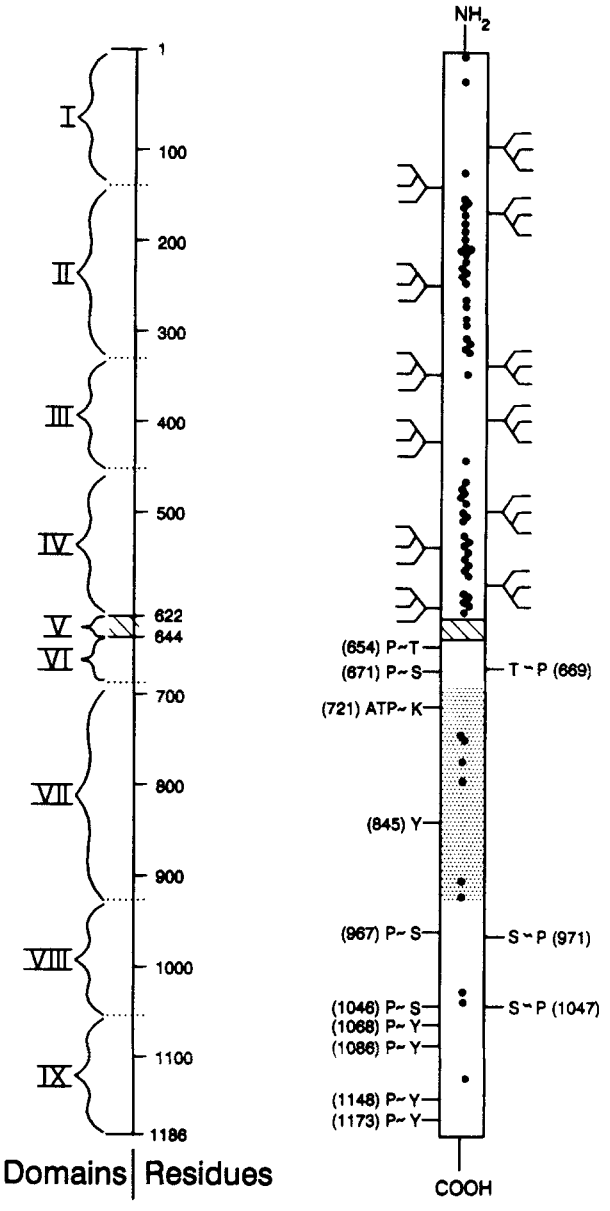

Fig. 1. Schematic Representation of the EGF Receptor. The crosshatched area indicates the location of the transmembrane sequence, while the stippled area within the cytoplasmic domain shows the location of the *src*-like tyrosine kinase sequence. Phosphorylation tyrosine ($P \sim Y$), serine ($P \sim S$), and threonine ($P \sim T$) residues are indicated. In the extracellular domain the location of cysteine residues (·) as well as *N*-type glycosylation sites (by branched structures) are indicated. (Reprinted by permission from Carpenter and Cohen, 1990.)

quences near the amino terminus may contribute to the ligand binding site (Lax et al., 1988, 1989). The external domain of the receptor is abundantly glycosylated with 10–11 N-linked oligosaccharide chains, at least three of which are high-mannose type and the others of the complex type (Mayes & Watterfield, 1984; Cummings et al., 1985). Addition of these oligosaccharide chains is required for correct receptor folding and processing to the cell surface (Soderquist & Carpenter, 1984; Slicker & Lane, 1985). In addition, the presence of mannose-6-phosphate, probably on one of the high-mannose type oligosaccharide chains, has been reported (Todderud & Carpenter, 1988). However, a function of this modified carbohydrate, which in other proteins serves as a lysosomal targeting signal, has not been established.

The cytoplasmic portion of the EGF receptor contains tyrosine kinase activity and encodes sequences shared with other proteins which have this enzymatic activity, such as *src* (Ullrich et al., 1984). The tyrosine kinase domain contains a conserved binding site for ATP that involves lysine 721 (Russo et al., 1985). The carboxy terminal region of the EGF receptor has received particular attention as it is subject to autophosphorylation, which can be intramolecular, intermolecular, or both. At least four sites of tyrosine autophosphorylation near the carboxy terminus have been identified in the intact receptor; Tyr 1173, 1148, 1086, 1068 (Downward et al., 1984; Hsuan et al., 1989; Margolis et al., 1989*a*). Tyr 992 is phosphorylated in carboxy-terminal truncation mutants of the EGF receptor and may constitute a fifth autophosphorylation site (Walton et al., 1990).

It is well established that the intracellular juxtamembrane region of the EGF receptor is subject to serine and threonine phosphorylation by other protein kinases—protein kinase C at Thr 654 (Hunter et al., 1985) and MAP kinase at Thr 669 (Northwood et al., 1991; Takishima et al., 1991). Okadaic acid, a tumor promoter and a potent inhibitor of ser/thr phosphatases 1 and 2A, produces hyperphosphorylation of the EGF receptor (Hernández-Sotomayor et al., 1991), suggesting ser/thr phosphatases may also participate in EGF receptor regulation. Other sites of ser/thr phosphorylation near the autophosphorylation domain have been identified, but not functionally characterized (Heisermann & Gill, 1988).

Tyrosine Phosphorylation Generates Second Messengers Molecules

A key question for the control of cell growth is how the binding of EGF (or other EGF-like growth factors) to its cognate receptor results in tyrosine kinase activation and the coordinate activation of subcellular process, such as gene expression, metabolism, transport, which together produce increased DNA and protein synthesis and ultimately stimulate cell division. Site-directed mutagenesis of

Table 2. Substrates of the EGF receptor

Substrate	Function	References
PLC-γ1	PIP_2 hydrolysis	Margolis et al., 1989*b*; Meisenhelder et al., 1989; Wahl et al., 1989*b*
PI-3 Kinase	PI phosphorylation at D-3	Whittman et al., 1988
GAP	Modulation of GTPase activity of *ras*	Ellis et al., 1990; Molloy et al., 1990
Lipocortin I (Annexin I)	Ca^{2+}/lipid-binding protein	Fava & Cohen, 1984
c-*erb*B-2	Receptor-like molecule with tyrosine kinase activity	Akiyama et al., 1988; Stern & Kamps, 1988

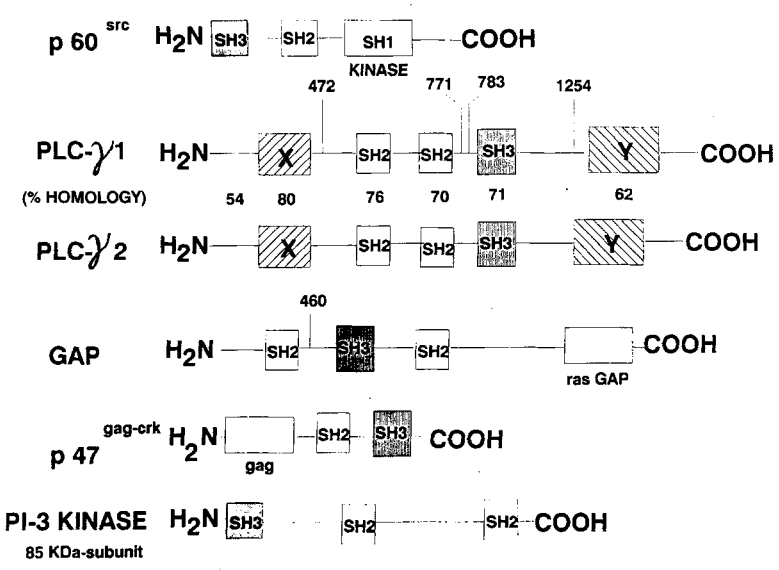

Fig. 2. The SH2 Family of Signaling Proteins. *SH*, *src* homologous regions 2 and 3; *X* and *Y*, common regions among the β, γ, δ PLC isozymes. The percentages of sequence identity between various regions of PLC-γ1 and PLC-γ2 are indicated. Tyrosine phosphorylated residues on PLC-γ1 and GAP are indicated. The oncogene product *v-src* is also diagrammed (Mayer et al., 1988).

Lys 721 in the EGF receptor abolishes tyrosine kinase activity, but not ligand binding. Analyses of cells transfected with this mutant receptor has established that tyrosine kinase activity is necessary for the generation of all biological responses measured to date (Chen, et al., 1987; Honneger et al., 1987; Glenney et al., 1988; Moolenaar et al., 1988). Therefore, a substantial effort has been made by a number of laboratories to identify EGF receptor tyrosine kinase substrates.

Following the stimulation of intact cells with EGF, a number of cellular proteins, in addition to the EGF receptor itself, become tyrosine phosphorylated. Some of these kinase substrates have been identified and their biochemical properties characterized. A list of the most well-characterized substrates for the EGF receptor is shown in Table 2. There are three proteins whose functions suggest a role in signal transduction pathways: the *ras* GTPase-activating protein or GAP (Ellis et al., 1990; Molloy et al., 1990), the 85 kDa subunit of PI-3 kinase (Whittman & Cantley, 1988; Bjorge et al., 1990; Miller & Ascoli, 1990), and PLC-γ1 (Margolis et al., 1989*b*; Meisenhelder et al., 1989; Wahl et al., 1989*b*). Despite the fact that these proteins have quite different enzymatic properties and are structurally distinct, they do share conserved, noncatalytic domains termed *src* homology (SH) regions 2 and 3 (Fig. 2). The SH2 domain is a sequence of approximately 100 amino acids originally identified in the *src* family of tyrosine kinases (Sadowski et al., 1986; Pawson, 1988; Koch et al., 1991). In terms of function, it has been shown that SH2 domains associate with tyrosine phosphorylated proteins (Moran et al., 1990). Following stimulation with PDGF or EGF, PLC-γ1, GAP, and PI-3 kinase become physically associated with the activated PDGF or EGF receptors due, at least in part, to the interaction SH2 domains (Moran et al., 1990) with autophosphorylated regions of the receptors.

GTPase-Activating Protein is Tyrosine Phosphorylated

*ras*GAP enhances the GTPase activity of *ras*, and thereby acts as a negative regulator returning *ras*

from the presumptive active GTP-bound state to the inactive GDP-bound state (Trahey & McCornick, 1987). Tyrosine phosphorylation of GAP by EGF receptor has been demonstrated *in vivo* and *in vitro* (Ellis et al., 1990; Molloy et al., 1990), and one site of tyrosine phosphorylation has been mapped at Tyr 460 (Liu & Pawson, 1991). Interestingly, although most GAP is present in the cytoplasm, tyrosine phosphorylated species of GAP are preferentially distributed to the membrane fraction, presumably complexed with *ras* (Molloy et al., 1990). However, the overall stoichiometry of GAP phosphorylation is low (<5%), and whether GAP tyrosine phosphorylation modulates GAP or *ras* function remains to be demonstrated.

PI-3 Kinase is Tyrosine Phosphorylated

PI-3 kinase phosphorylates the inositol ring of phosphatidyl inositol PI, PI-4P and PI-4,5P_2 at the D-3 position (Whitman et al., 1988). Purified PI-3 kinase is thought to be a heterodimer containing 85 and 110 kDa proteins (Carpenter et al., 1990). Since the cDNA sequence of the 85-kDa protein does not indicate the presence of an ATP binding site (Escobedo et al., 1991; Otsu et al., 1991; Skolnik et al., 1991), catalytic activity is thought to reside in the 110-kDa protein, and the 85-kDa molecule, which is phosphorylated at tyrosine residues, may be a regulatory subunit. After EGF treatment of intact cells, PI-3 kinase activity in A-431 cells is associated with EGF receptor immunoprecipitates (Bjorge et al., 1990), and in Leydig cells PI-3 kinase activity is increased (by EGF) (Miller & Ascoli, 1990). However, these results have not been reported consistently in different laboratories and association of the EGF receptor with PI-3 kinase system remains unclear. There is, as yet, no direct demonstration between tyrosine phosphorylation of the 85-kDa subunit and changes in enzymatic activity. In addition, the metabolism and cellular function(s) of the 3-phosphorylated phosphoinositides are not understood. They are not hydrolyzed by known phospholipase and may, therefore, serve a regulatory role in modulating the activity of membrane-localized proteins, perhaps as cofactors (Auger et al., 1989; Carpenter et al., 1990).

Phospholipase C-γ is Tyrosine Phosphorylated and Activated

Phospholipase C (PLC) is a family of enzymes that catalyze the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2), generating two potential intracellular second messengers: diacylglycerol and inositol 1,4,5-trisphosphate (IP_3) (Rhee et al., 1991). Diacylglycerol, an endogenous activator of protein kinase C, may have an important role in the signal transduction process for a number of external stimuli (Nishizuka, 1989). IP_3 is released into the cytoplasm and interacts with specific receptors on the endoplasmic reticulum, resulting in the release of Ca^{2+} into the cytoplasm (Berridge, 1990). As a result, intracellular free Ca^{2+} levels are increased more than 10-fold and Ca^{2+}-dependent processes are activated.

PIP_2-specific PLC activity is ubiquitous in mammalian cells, plants, and microorganisms (Shulkla, 1982; Rhee et al., 1991). Several distinct PLC isozymes have been purified and these plus additional forms, identified by homology cloning, have been molecularly cloned and sequenced (Rhee et al., 1991). According to the sequence data, mammalian PLCs can be divided into four families, some with multiple members (α, β_1, β_2, γ_1, γ_2, δ_1, δ_2, δ_3), raising the number of unique PLC isozymes to at least eight. PLC-γ1 and γ2 have a high level of sequence identity and contain domains, represented in Fig. 2 by the boxes *X* and *Y*, common to PLC β and δ. These *X* and *Y* regions are thought to represent portions of the catalytic domain. PLC-γ1 and 2, however, are the only PLC isozymes which have *src* homology (SH) regions, designated *SH2* and *SH3* in Fig. 2. Molecular biological studies have demonstrated that deletion of the entire SH region reduces PLC activity by ~20% relative to the intact enzyme, indicating that this region is not involved in catalysis *per se* (Bristol et al., 1988; Emari et al., 1989). When *X* or *Y* domains are altered, there is a complete loss of enzyme activity, suggesting that *X* and *Y* are essential for basal enzyme activity (Rhee et al., 1991).

To date, PLC-γ1 is the best-characterized EGF receptor substrate and may provide a unique example to understand how tyrosine phosphorylation mediates changes in the function of substrate proteins.

PLC-γ1 is a Specific Tyrosine Phosphorylation Substrate

Since PLC is a key component of the phosphoinositide pathway and EGF increases the formation of inositol phosphates in several cell lines (reviewed in Wahl et al., 1989*a*), a functional link between the EGF receptor and PLC is suggested. The first experimental evidence to elucidate this was the increased recovery (10-fold) of PLC activity, obtained from extracts of EGF-treated A-431 cells from a phosphotyrosine immunomatrix (Wahl et al.,

Table 3. Tyrosine phosphorylation of PLC-γ1

	Yes	No
EGF receptor	(Margolis et al., 1989*b*; Meisenhelder et al., 1989; Wahl et al., 1989*b*)	Insulin receptor (Nishibe et al., 1990*b*)
*erb*B-2 receptor	(Fazioli et al., 1991)	
PDGF receptor	(Meisenhelder et al., 1989; Wahl et al., 1989*c*; Kumjian et al., 1991)	CSF-1 receptor (Downing et al., 1989)
FGF receptor	(Burgess et al., 1990)	
NGF receptor	(Kim, U.-H., et al., 1991)	
T cell receptor	(Granja et al., 1991; Park et al., 1991; Secrist et al., 1991; Weiss et al., 1991)	
IgM receptor	(Carter et al., 1991; Padeh et al., 1991)	

1988*b*). This suggested that either PLC or a tightly associated protein was phosphorylated on tyrosine residues in the presence of EGF. The former possibility was directly tested by metabolic labeling of cultured cells with [^{32}P] orthophosphate and immunoprecipitation of PLC isozymes with specific monoclonal antibodies (Meisenhelder et al., 1989; Wahl et al., 1989*b*) or probing PLC-γ1 from EGF-treated cells with phosphotyrosine antibodies (Margolis et al., 1989*b*). EGF treatment of intact cells induced a rapid increase in the phosphorylation of PLC-γ1, and phosphoaminoacid analysis revealed the presence of increased levels of phosphotyrosine and phosphoserine. Tyrosine phosphorylation of PLC-γ1 occurs with a very high stoichiometry; up to 70% of the total cellular pool of PLC-γ1 is tyrosine phosphorylated within two minutes of EGF addition to cells incubated at 37° or 4° (Margolis et al., 1989*b*; Meisenhelder et al., 1989; Wahl et al., 1990).

Tyrosine phosphorylation of PLC-γ1 is a reasonable selective reaction. As shown in Table 3, a number of tyrosine kinase-linked receptors increase PLC-γ1 tyrosine phosphorylation in intact cells, while at least two tyrosine kinases do not produce phosphorylation of PLC-γ1. Also, in vitro studies show that the EGF receptor selectively phosphorylates PLC-γ1, but not PLC β or PLC δ (Nishibe et al., 1989) and that the insulin receptor fails to phosphorylate PLC-γ1 (Nishibe et al., 1990*b*). Therefore, PLC-γ1 would seem to be a selective substrate for the EGF and certain other tyrosine kinase receptors.

Tryptic phosphopeptide mapping of PLC-γ1 phosphorylated in vivo or in vitro indicated similar patterns of tyrosine phosphorylation sites (Meisenhelder et al., 1989; Nishibe et al., 1989; Wahl et al., 1990). Sequencing of these sites showed that PLC is tyrosine phosphorylated in vitro by the EGF receptor at residues 472, 771, 783, and 1254 (J.W. Kim et al., 1990). Sequencing studies of PLC-γ1 phosphorylated in intact cells exposed to EGF directly confirmed phosphorylation sites 771 and 1254 (Wahl et al., 1990). In that study, a third peptide, purified in insufficient quantity for sequencing, had chromatographic properties similar to the Tyr 783 peptide obtained by *in vitro* phosphorylation.

Although several groups demonstrated that PLC-γ1 was tyrosine phosphorylated in response to EGF *in vivo* or *in vitro*, initial measurements of enzyme activity did not show that this resulted in a change in catalytic activity (J.W. Kim et al., 1990). Recently, however, it has been shown that EGF-treatment of A-431 cells induced a considerable increase in PLC-γ1 catalytic activity, when the activity of this isozyme was measured in PLC-γ1 immunoprecipitates (Nishibe et al., 1990*a*). A significant change in the assay, which revealed differences in activity of the tyrosine phosphorylated and nonphosphorylated PLC-γ1 species, was the addition of Triton X-100 to produce a mixed micelle with the substrate. These studies also showed that when PLC-γ1 immunoprecipitates, obtained from untreated cells, are incubated with purified EGF receptor and ATP, the catalytic activity of the phospholipase is increased two- to fourfold. The activated enzyme could be dephosphorylated by a phosphotyrosine phosphatase with a 70% decrease in enzymatic activity. Other evidence that tyrosine phosphorylated PLC-γ1 is enzymatically different from the nonphosphorylated enzyme was obtained by adding profilin to the in vitro assay (Goldschmidt-Clermont et al., 1991). Profilin is a cytoplasmic actin-binding protein which also binds PIP_2 with a high affinity. In the presence of profilin the hydrolysis of PIP_2 by unphosphorylated PLC-γ1 was inhibited, but the activity of the tyrosine phosphorylated enzyme was not inhibited.

The role of individual PLC-γ1 tyrosine phosphorylation sites has been investigated by substitut-

ing phenylalanine for tyrosine at each of the three phosphorylation sites in PLC-γ1 and overexpressing the mutant enzymes in NIH 3T3 cells (H.K. Kim et al., 1991). The transfected cells were then assayed for the formation of IP_3 in response to PDGF. Phenylalanine substitution at Tyr 783 completely blocked the activation of PLC-γ1 by PDGF, whereas mutation at Tyr 1254 inhibited the response by 40% and mutation at Tyr 771 enhanced the response by 50%. These data indicate that phosphorylation on Tyr 783 is essential for PLC-γ1 activation.

PLC-γ1 exists mostly (80%) as a cytoplasmic enzyme, while its substrate is located at the plasma membrane. EGF treatment of intact cells does induce a redistribution of PLC-γ1 from the cytoplasm to a component in the membrane fraction (U.-H. Kim et al., 1990; Todderud et al., 1990). Since the substrate for this enzyme, PIP_2, is a membrane component, this redistribution appears necessary. The mechanism of the PLC-γ1 translocation, however, remains to be elucidated.

Role of SH Domains are Critical in PLC-γ1 Tyrosine Phosphorylation

As mentioned above, PLC-γ contains *src* homology (SH) regions 2 and 3 and SH2 domains bind tyrosine phosphorylated proteins (Matsuda et al., 1991). Based on sequence similarities, proteins with SH3 domains are thought to be associated with the cytoskeleton (Rodaway et al., 1989). As shown in Fig. 2, PLCγ, *ras*GAP, and PI-3 kinase, each have two SH2 domains and one SH3 domain, suggesting that SH domains may be a common feature in proteins that interact with activated receptor tyrosine kinases.

Several reports indicate that following stimulation of cells with EGF, PLC-γ1 not only is tyrosine phosphorylated, but is also physically associated with the activated EGF receptor. The latter conclusion is based on co-precipitation experiments in which complexes between the activated EGF receptor and PLC-γ1 have been detected following precipitation with antibody, to PLC-γ1 (Margolis et al., 1989*b*; Meisenhelder et al., 1989). However, quantitation of these complexes indicates that only small amounts (less than 5%) of each molecule are present. Hence, the complexes may be transient enzyme : substrate complexes. Interaction between PLC-γ1 and the EGF receptor is thought to be mediated by the SH2 domains of PLC-γ1 and phosphorylated autophosphorylation sites at the carboxy terminus of the EGF receptor. SH2 domains from PLC-γ1 have been isolated from bacterial expression systems and shown to associate with the activated EGF receptor (Moran et al., 1990). Additional data suggest that the site of interaction between PLC-γ1 and the EGF receptor is contained in a 200-residue fragment derived from the carboxy-terminus of the receptor, which contains four autophosphorylation sites (Margolis et al., 1990). Interaction of PLC-γ1 with this receptor fragment only occurred when the tyrosine residues were phosphorylated. Taken together this evidence suggests that SH2 domains may function to target or facilitate interaction of substrate proteins, such as PLC-γ1, with activated tyrosine kinase molecules.

PLC-γ1 is also Serine Phosphorylated

Increased tyrosine phosphorylation of PLC-γ1 is not the only covalent change detected after EGF treatment. In vivo treatment with EGF significantly increases the amount of phosphoserine (Meisenhelder et al., 1989; Wahl et al., 1989*b*) beyond that present under basal conditions. While experimental data do support a role for tyrosine phosphorylation of PLC-γ1 in the modulation of catalytic activity, the function of phosphoserine residues is not understood. Using PLC-γ1 immunoprecipitated from EGF-treated cells, it was shown that dephosphorylation of phosphoserine residues with the specific ser/thr phosphatase PP2A did not alter the enzymatic activity of the EGF-activated enzyme (Carpenter et al., 1992). Also, serine phosphorylation is not a prerequisite for PLC-γ1 to be tyrosine phosphorylated by the purified EGF receptor (Carpenter et al., 1992). Protein kinase A does phosphorylate PLC-γ1 at serine residues in vivo and in vitro (Kim et al., 1989) without affecting the enzymatic activity, but growth factors, such as EGF, do not activate A kinase. Therefore, identification of the EGF-regulated serine kinase that phosphorylates PLC-γ1 after EGF treatment remains to be determined.

Summary

While EGF has an important function in cell growth regulation, the molecular mechanisms by which intracellular signal connect the EGF : receptor complex on the plasma membrane with the initiation of DNA synthesis and mitogenesis is not well understood. The discovery that *ras*GAP, PI-3 kinase and PLC-γ1 are substrates for the EGF receptor tyrosine kinase has provided a beginning in understanding the biochemistry underlying growth factor receptor transduction.

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