Intracellular Signaling by Growth Factors

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Growth factors are involved in a variety of cellular responses such as growth, differentiation, migration, metabolism, and transformation. Binding of the growth factor to its corresponding cell surface receptor results in activation of the receptor's intrinsic tyrosine kinase activity, and subsequently in activation of complex multistep signal transduction cascades. Activation of these interconnected signaling pathways eventually leads to a biological response, which involves changes in gene expression and protein synthesis. The biological response has been shown to be receptor-specific and also cell-type (tissue)–specific, indicating that various receptors activate distinct signal transduction pathways in one tissue and that one receptor activates different pathways in various tissues. What determines receptor specificity and tissue specificity? In this context, this article will focus on certain receptors with intrinsic tyrosine kinase activity, including receptors for platelet-derived growth factor (PDGF), epidermal growth factor (EGF), insulin, and nerve growth factor (NGF).

RECEPTOR TYROSINE KINASES

GROWTH FACTOR receptors, also called receptor tyrosine kinases (RTKs), comprise a large family of receptors with more than 50 members. They all share a similar molecular topology: a large, glycosylated extracellular ligand-binding domain, a single hydrophobic transmembrane region, and an intracellular domain that can be further divided into the juxtamembrane region, a tyrosine kinase domain that contains the catalytic activity, and the carboxy-terminal tail.[1] On the basis of sequence similarity and distinct structural characteristics, RTKs have been divided into more than 12 subclasses. The EGF receptor (EGF-R) belongs to the subclass receptors. They are monomeric and contain two cysteine-rich clusters in the extracellular domain and a *src*-like tyrosine kinase domain in the cytoplasmic portion. Subclass 2 receptors include the insulin (IR) and insulin-like growth factor-I (IGF-I-R) receptors. They have a heterotetrameric structure containing two α- and two β-subunits that are linked by disulfide bonds. The α-subunit contains one cysteine-rich cluster, and the β-subunit harbors the catalytic activity. The PDGF receptors (PDGF-R), termed PDGFα-R and PDGFβ-R, are members of subclass 3 receptors. They share extensive sequence homology and have the same configuration of structural domains. The extracellular domain contains five immunoglobulin-like repeats, and the cytoplasmic region has a tyrosine kinase domain that is interrupted by a kinase insertion sequence. Two distinct classes of proteins have been identified as receptors for NGF: the $p75^{LNGF}$ protein, which binds NGF with low affinity and lacks tyrosine kinase activity, and the NGF receptor (NGF-R), encoded by the Trk gene. The NGF-R (subclass 7) contains a cytoplasmic tyrosine kinase domain; however, no obvious structural elements have been identified in the extracellular region[3] (Fig 1).

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RTK-MEDIATED SIGNAL TRANSDUCTION

Tyrosine-mediated cellular signals are generated by binding of a specific ligand to the extracellular part of a RTK, followed by receptor dimerization and tyrosine phosphorylation of the cytosolic part. This triggers the recruitment of substrates with *src* homology 2 (SH2) domain to specific phosphotyrosine residues, and leads to the assembly of these primary signal transfer factors at the inner surface of the plasma membrane in proximity to molecules involved in subsequent steps of signal transduction. The protein-protein interaction mediated by SH2 domains and phosphorylated-tyrosine residues is an essential feature of signaling by RTKs.[4] The crystal structure of the SH2 domain showed two defined pockets that complex tightly with peptides containing phosphotyrosine and flanking amino acids.[5] Of special importance is the third amino acid that follows the tyrosine residue to determine SH2 binding specificity. For example, the binding motif YMXM has been shown to be a high-affinity binding site for the p85 phosphatidylinositol-3-OH kinase (PI3-kinase) subunit, whereas the YVNI motif preferentially binds to the SH2 domain of GRB-2. It has therefore been suggested that activation of specific pathways is determined by the receptors' ability to interact with a certain set of SH2 domain–containing proteins. Several substrate interaction sites on RTKs such as PDGF-R, EGF-R, NGF-R/Trk, and IR substrate-1 (IRS-1), the main docking protein for SH2 domain–containing proteins involved in IR- and IGF-I-R–mediated signal transduction, have been identified. The PDGF-R tyrosine phosphorylation sites Y-1021 and Y-1009, located in its carboxy terminus, are binding sites for phospholipase C gamma (PLCγ) and PTP1D/Syp/SHPTP2, respectively. Y-771 in the kinase insertion domain binds the *ras*-GTPase-activating protein (GAP), and Y-740 and Y-751 represent binding sites for p85/PI3-kinase and *nck*. The closely related cytoplasmic tyrosine kinases *src, fyn,* and *yes* bind to Y-579 and Y-581 in the PDGF-R juxtamembrane region.[3,6]

The EGF-R has five tyrosine residues (Y-992, Y-1068, Y-1086, Y-1148, and Y-1173) within the carboxy-terminal tail that are phosphorylated upon ligand-mediated receptor activation. These tyrosine residues mediate binding of PLCγ, GAP, Syp/PTP1D/SHPTP2, p85/PI3-kinase, SHC, GRB-2, GRB-7, and *nck*.[7-14] In contrast to the PDGF-R, in

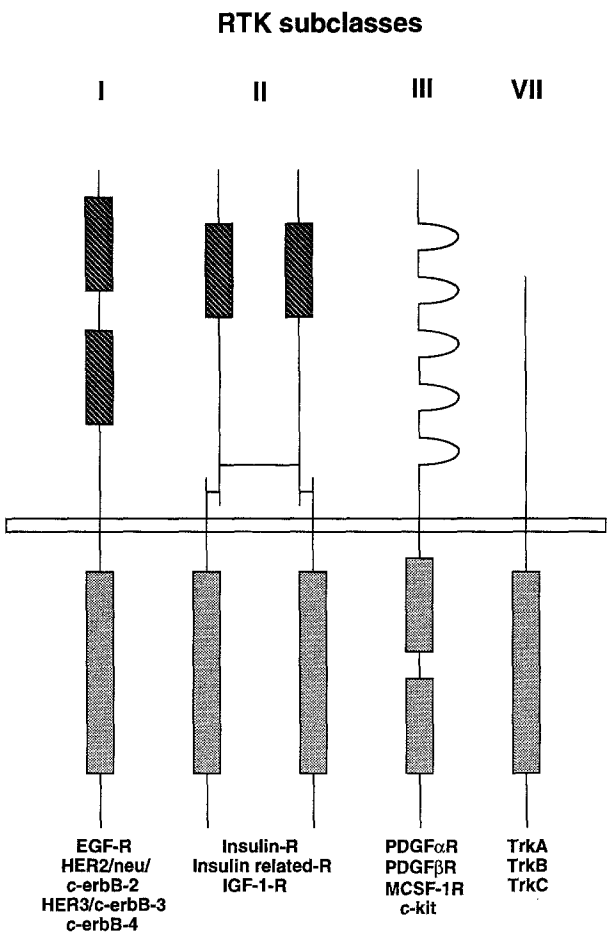

Fig 1. Schematic representation of RTK subclasses. Structural elements: cysteine-rich clusters (▨), tyrosine kinase domains (□), and immunoglobulin-like domains (semicircles).[3]

which every single phosphorylated tyrosine residue mediates specific binding of a certain SH2 domain–containing protein, the EGF-R autophosphorylation sites are highly flexible in their capacity to mediate association of SH2-containing substrates.[15]

For the NGF-R, Y-490, in the juxtamembrane region, Y-752 in the kinase core domain, and Y-785 in the short carboxy terminus of Trk are the interaction sites for SHC, p85/PI3-kinade, and PLCγ, respectively.[16-18]

The IR is phosphorylated on seven tyrosine residues (Y-953, Y-960, Y-1146, Y-1150, Y-1151, Y-1316, and Y-1322) in response to insulin.[3,19] Tyrosine 960, located in the juxtamembrane region, has been shown to be important for IRS-1 phosphorylation on tyrosine residues and for insulin-mediated signal transduction.[19,20] Phosphorylation of Y-1146, Y-1150, and Y-1151 correlates with activation of the receptors' intrinsic tyrosine kinase activity, and it has recently been shown that tyrosine 1150 is bound in the active catalytic site, leading to autoinhibition of kinase activity.[21] Interestingly, this tyrosine residue is conserved in all RTKs, suggesting a common autoinhibitory function. The role of tyrosine 1316 and 1322, located in the carboxy terminus, is not fully understood. In contrast to other RTKs, none of the IR autophosphorylation sites have been shown to be binding sites for SH2 domain–containing proteins to date. A number of cellular substrates for the IR have been identified. These include IRS-1,[22] SHC,[23] focal adhesion kinase,[24] pp62,[25] and two distinct pp60 proteins, which can be distinguished by their ability to bind either PI3-kinase or GAP.[26] IRS-1 contains 22 potential tyrosine phosphorylation sites, of which at least eight are phosphorylated by the activated IR. These phosphorylated tyrosine residues have been shown to be binding sites for SH2 domain–containing proteins like the p85/PI3-kinase subunit (Y-460, Y-608, and Y-939), Syp/PTP1D/SHPTP2 (Y-1172), GRB-2 (Y-895), *nck* and *crk*, [27] and PLCγ and GRB-7 (K. Seedorf and A. Ullrich, unpublished results, August 1993).

The PDGF-R, EGF-R, NGF-R, and IR have been shown to activate extracellular signal–regulated kinases, also known as mitogen-activated protein (MAP) kinases.[28,29] How they mediate activation of MAP kinases has been partially elucidated recently.[30-38] The EGF-R binds, in its activated form, GRB-2, thereby linking the receptor to the guanine nucleotide-exchange protein, son of sevenless (SOS),[39] which replaces *ras*-bound GDP by GTP, resulting in activation of *ras*. The GTP-binding protein *ras* has been shown to interact with *raf*,[40] a serine threonine kinase that, upon activation, activates the MAP kinase kinase (MEK), which finally activates MAP kinase.[41] The IR phosphorylates IRS-1, thereby allowing binding of the GRB-2–SOS complex to IRS-1, resulting in *ras, raf,* MEK, and MAP kinase activation. The NGF-R does not bind GRB-2; however, it binds and phosphorylates SHC on tyrosine residues, allowing the GRB-2–SOS complex to interact with SHC via the GRB-2 SH2 domain. The EGF-R binds not only GRB-2 but also SHC, suggesting the existence of two alternative pathways leading to MAP kinase activation.[42] The PDGF-R does not interact with GRB-2 or SHC. It has recently been shown that receptor-bound, tyrosine-phosphorylated Syp/PTP1DSHPTP2 binds GRB-2, suggesting that this protein complex allows interaction with SOS and thereby activates *ras*.[43] Alternatively, the PDGF-R, as well as the IR, have been shown to phosphorylate SHC on tyrosine residues, resulting in the binding of GRB-2 and most likely in the activation of MAP kinase. A second, *ras*-independent pathway has been proposed that involves PLCγ and protein kinase C (PKC). Upon activation, PLCγ catalyzes hydrolysis of phosphatidylinositol-4,5-biphosphate (PIP2) into the second messengers diacylglycerol (DAG) and inositol triphosphate (IP3). IP3 induces the release of Ca^{2+} from intracellular stores and, together with DAG, activates certain PKC isotypes.[44-46] Activated PKC is then able to activate *raf*,[47,48] allowing activation of MEK and MAP kinase.

The conservation and redundancy of MAP kinase activation implies that this signaling pathway is highly important. MAP kinase has been shown to phosphorylate transcription factors and induce transcription of immediate early genes like c-*fos* and c-*jun*. It is involved in activation of glycogen synthase by phosphorylation of RSK S6 kinase, which phosphorylates protein phosphatase-1, which dephosphorylates and thereby activates glycogen synthase.[41,49] Further-

more, it has recently become evident that MAP kinase–mediated phosphorylation of PHAS-1, which in its unphosphorylated form represses mRNA translation by complexing elongation factor 4E (eIF-4E), causes the complex to dissociate, so that eIF-4E can initiate translation.[50]

Another serine threonine kinase, ribosomal $p70^{s6k}$/$p85^{s6k}$, is activated in response to various growth factors including PDGF, EGF, and insulin. S6 kinase phosphorylates S6 protein, resulting in preferential translation of messenger RNAs containing a polypyrimidine tract at their 5′ end. The pathway that leads to $p70^{s6k}$/$p85^{s6k}$ activation is not understood. However, there is clear evidence that rapamycin inhibits S6 kinase activation in a specific manner, without inhibiting PI3-kinase activity.[51,52] These data suggest that the recently cloned mammalian target of rapamycin,[53] not PI3-kinase, is an important mediator of S6 kinase activation.

PI3-kinase interacts with a number of RTKs (eg, IR, K. Seedorf and A. Ullrich, unpublished results, August 1993; PDGF-, EGF-, and NGF-R), as well as with IRS-1, through its SH2 domain–containing p85-kd regulatory subunit, thereby activating the 110-kd catalytic subunit.[3] PI3-kinase catalyzes the phosphorylation at the D-3 position of the inositol ring of PI, PI(4)P, and PI(4.5)P_2, generating PI(3)P, PI(3,4)P_2, and PI(3,4,5)P_3. These second messengers have been shown to play an important role in cell growth and metabolism[54]; however, the downstream targets are still unidentified. It has recently been shown that PI3-kinase is also a direct downstream target of *ras*[55] and that furthermore it not only phosphorylates phosphoinositides but contains intrinsic protein serine kinase activity, which has been shown to phosphorylate its own p85 subunit[56] and, in addition, IRS-1 in an insulin-dependent fashion.[57] The mammalian catalytic domain of PI3-kinase is homologous to yeast Vps34,[58] a protein involved in protein sorting. It has therefore been speculated that PI3-kinase is involved in vesicle trafficking in mammalian cells.[59] The finding that PI3-kinase inhibitors block insulin-induced glucose transport (GLUT4 translocation)[60] supports this idea and indicates that PI3-kinase is part of the unsolved signaling pathway leading to glucose uptake. The role of *crk, nck,* and Syp/PTP1D/SHPTP2 in RTK-mediated signal transduction is not yet understood. The current knowledge of IR signaling is summarized in Fig 2.

CELL-TYPE–SPECIFIC SUBSTRATE INTERACTION

The receptors for EGF, PDGF, insulin, NGF, and many others activate mitogenic pathways when expressed in NIH3T3 fibroblasts. Under physiological conditions, the biological response of these receptors is often different and far more complex. The IR is expressed on almost every cell type, and its major physiological function is to control

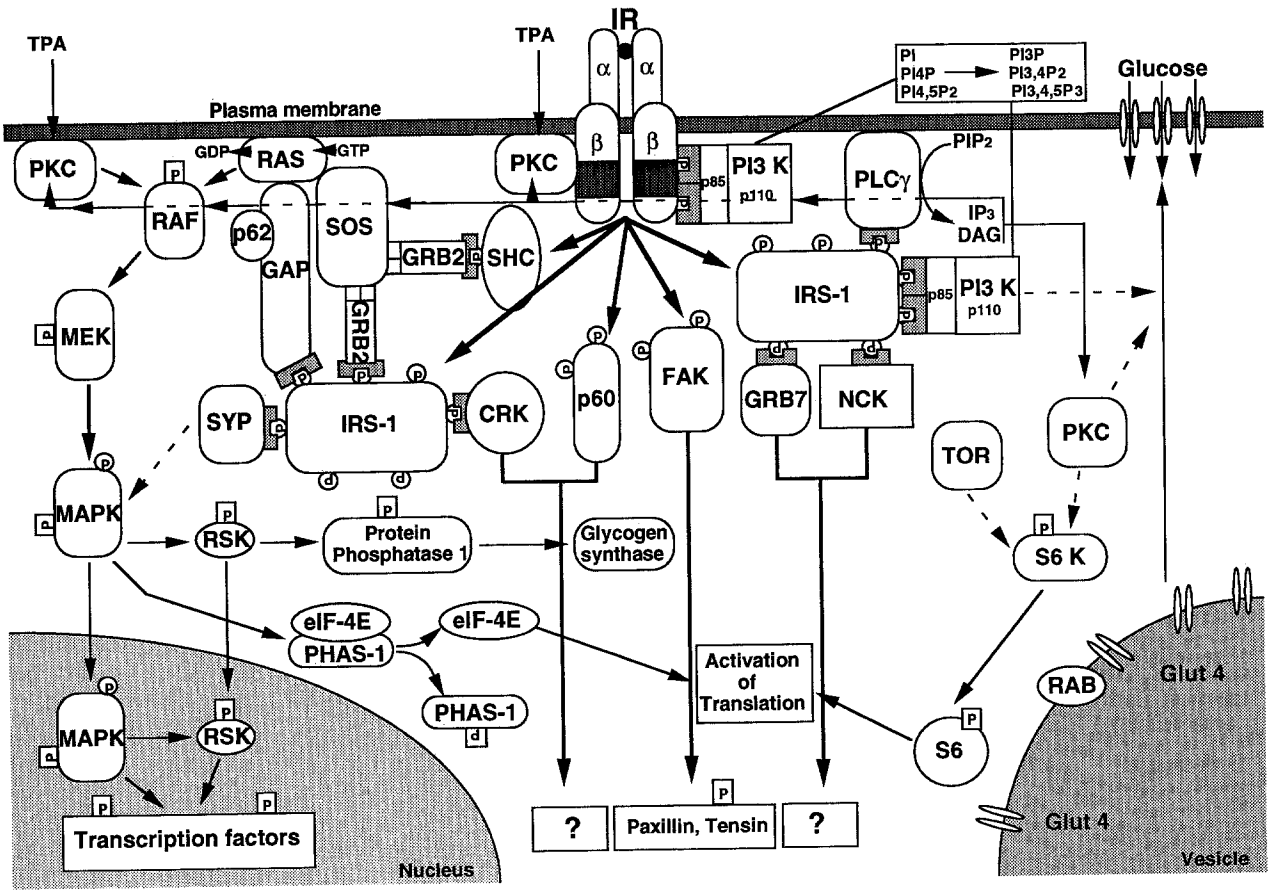

Fig 2. Summary of insulin-mediated signal transduction pathways.

metabolic processes such as glucose and lipid metabolism in muscle, fat, and liver tissue.[27] NGF is a peptide hormone that is essential for development and survival of sympathetic nerves and certain populations of nerves in the central nervous system.[61] PDGF is a potent mitogen acting on fibroblasts, smooth muscle cells, and glial cells. Receptors for PDGF also have been shown to be expressed on other cell types, including capillary endothelial and neuronal cells, and have been implicated as regulators of cell proliferation during development and in wound healing.[62-64] The EGF-R mediates a mitogenic response in various cell types; however, the *Drosophila melanogaster* EGF-R homolog DER has been shown to be an essential regulator of embryogenesis.[65] These findings, together with the observation that certain SH2 domain–containing RTK substrates like GRB-7 and VAV show a specific tissue distribution,[2] suggest that specific RTKs mediate different functions in different tissues. This naturally would require that these receptors interact with a cell-type–specific subset of proteins in order to activate tissue-specific signal transduction pathways. This hypothesis was recently investigated by so-called association experiments. This method allows identification of specific receptor-binding proteins in cell lines that originate from various tissues (K. Seedorf and A. Ullrich, unpublished results, September 1993). To make this investigation completely comparable, chimeric receptors were used that are all composed of the extracellular domain of the EGF-R fused to the cytoplasmic portion of the PDGF-R, HER2, and IR, respectively, resulting in EP-R, HER1-2, and EIR. These chimeric receptors have been shown to be biologically active in response to EGF[66-68] and, importantly for this comparative analysis, can all be immunoprecipitated by an antibody directed against the extracellular domain of the EGF-R.

Association Experiments

The protocol was as follows: (1) Transient overexpression of RTK in 293 fibroblasts, (2) activation of RTK by addition of EGF for 5 minutes, (3) cell lysis and immunoprecipitation of RTK using EGF-R–specific antibody, (4) stringent wash, (5) addition of ^{35}S-methionine– labeled Triton X-100–soluble cell extracts originating from different tissues and incubation for 2 hours, (6) stringent wash, and (7) analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

Results from this type of experimental design clearly demonstrated that different RTKs interact with the same, but also with tissue-specific, proteins in vitro, suggesting that RTKs expressed in various tissues are coupled to the same, but also to tissue-specific, pathways. The EIR chimera, for example, binds p85/p110 PI3-kinase from NIH3T3 (fibroblast), BFS1 (skin sarcoma), G8 (myoblast), 3T3L1 (preadipocyte), ASBXIV (lung carcinoma), and CaD2 (mammary carcinoma) cell extracts and, in addition, unidentified proteins of 98, 50, 41, and 29 kd. In NIH3T3, G8, and 3T3L1 cells, but not in others, the EIR binds a protein with 60 kd. Results for the EIR are summarized in Table 1.

Table 1. Results of Association Experiments for the EIR

Molecular Weight (kd)	Cell Type					
	NIH3T3	BFS1	G8	3T3L1	ASBXIV	CaD2
200	–	–	+	–	–	–
110	+++	+++	+++	+++	+++	+++
98	++	++	++	++	++	++
85	+++	+++	+++	+++	+++	+++
65	–	+	–	+	+++	+
64	+	++	+	++	+	+
60	+++	–	+	+	–	–
55	+++	+++	+++	+++	+++	+++
53	+++	+++	+++	+++	+++	+++
48	+++	–	–	–	–	–
44	–	+++	–	+	+	+
41	++	++	++	++	++	++
37	–	+	+	++	+	+
33	–	+	+	++	+	–
29	++	++	++	++	++	++
24	++	–	–	+	++	+

NOTE. Intensity of binding is indicated by the number of + and undetectable binding by –. The 110- and 85-kd proteins have been shown to be the two subunits of PI3-kinase (K. Seedorf and A. Ullrich, unpublished results).

DURATION OF SIGNAL TRANSDUCTION

A comparison of the effects of two diverse growth factors, EGF and NGF, in responsive cells presented a dilemma. Whereas NGF treatment for several days results in growth arrest of PC12 pheochromocytoma cells and induces differentiation of these chromaffin-like cells to a sympathetic neuron–like phenotype, EGF acts as a mitogen on PC12 cells. Although the biological effects of both growth factors are clearly different, no major differences with respect to substrate phosphorylation and induction of early response genes were detectable.[69] Recently, it has been shown that MAP kinase activation by NGF is sustained and leads to its nuclear translocation, whereas activation by EGF is transient and does not lead to pronounced nuclear translocation.[70] EGF may therefore be unable to initiate differentiation of PC12 cells, because activation of p42 MAP and p44 MAP kinase is not maintained long enough to ensure entry of the active kinases into the nucleus in the amounts needed to initiate the transcriptional events required for differentiation. This suggests that the transient activation of MAP kinase by EGF does not result from downregulation at a step in the pathway that is common to NGF and EGF, and that rapid inactivation of the EGF response is likely to occur at a step that is near (or at) the level of the EGF-R. If this idea is correct, the prediction can be made that if inactivation of the EGF-R is slowed or prevented, EGF should promote differentiation of PC12 cells in a manner similar to NGF. This is indeed the case: PC12 cells with a 50-fold overexpression of the wild-type EGF-R or an EGF-R desensitization–negative mutant no longer acted in response to EGF as a mitogen, but instead triggered differentiation of these cells. The activation of MAP kinase and MEK triggered by EGF was transient in both untransfected and overexpressing PC12 cells; however, the decline in overexpressing cells was slower, and after 90 minutes

MAP kinase and MEK activities remained fivefold higher in EGF-R mutant–expressing cells and threefold higher in wild-type EGF-R–expressing cells. In addition, the nuclear translocation of MAP kinase in EGF-R–overexpressing PC12 cells was just as striking as that induced by NGF. These results strongly suggest that the distinct effects of NGF and EGF on PC12 cell differentiation can be explained by differences in the extent and duration of activation of MAP kinase in response to the two factors, without involving a signal transduction pathway specific to NGF (Fig 3).[71] It is important to note that it has recently been shown that MAP kinase is not only required but also sufficient to induce differentiation of PC12 cells.[72]

FEEDBACK INHIBITION OF RTKs

Binding of a ligand to its corresponding RTK triggers activation of pathways that ultimately lead to a biological response.[2,4,73] Since constitutive activation of these pathways results in disturbance of normal cellular response mechanisms and ultimately cell transformation, internal control and negative feedback mechanisms are required. In addition, as in PC12 cells, there is increasing evidence that the duration of RTK activation is a important factor in determining the final biological response. The PKC system likely mediates such a regulatory role.

The PKC serine/threonine kinases, a family of at least 12 isoenzymes with distinct tissue-distribution characteristics, have been subdivided on the basis of different primary structures and enzymatic properties into Ca^{2+}-dependent or conventional PKCs and Ca^{2+}-independent or novel PKCs.[74] Extensive evidence is available for their involvement in control of cell proliferation, differentiation, and motility.[75] While certain PKC isotypes are activated by phorbol esters such as phorbol 12-myristate 13-acetate (TPA), their physiological ligand is the second messenger, DAG,[44,45] a product of the phosphoinositide-specific PLC, whose γ-subtype was shown to be activated by mitogens such as EGF and PDGF through receptor-mediated tyrosine phosphorylation.[2] In addition to DAG, PLCγ-catalyzed hydrolysis of PIP2 yields IP3, a regulator of intracellular Ca^{2+}, which together with DAG fully activates certain PKC isoforms.

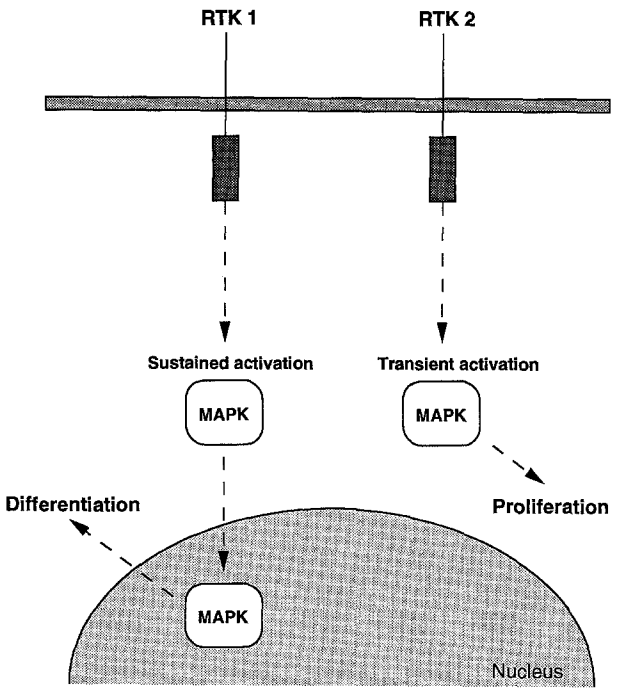

Fig 3. Schematic comparison of MAP kinase (MAPK)-mediated biological responses in PC12 cells.

Among its diverse effects on the physiology of cells,[44] PKC was shown to phosphorylate and activate the serine/threonine kinase *raf*-1,[47,48] which triggers the MAP kinase signaling pathway and ultimately leads to transcriptional activation of specific genes.[41,76] In addition to this positive influence on growth factor signals, PKC activation is known to downregulate the signaling potential of EGF-R and IR. Phosphorylation of the EGF-R at Thr 654 attenuates high-affinity EGF binding[77] and causes a decrease in EGF-stimulated tyrosine kinase activity and DNA synthesis.[78,79] Similarly, the signaling activity of the IR, which appears to be primarily phosphorylated on C-terminal serine and threonine residues,[80] is impaired after TPA treatment of some cell types due to a reduction of its tyrosine kinase activity,[81,82] whereas in other cells an activation of RTK activity was observed.[83] Moreover, as recently shown, phorbol ester treatment of CHO cells overexpressing PKCα and the IR resulted in phosphorylation of the receptor on threonine and serine residues without an effect on tyrosine phosphorylation, but caused inhibition of insulin-stimulated PI3-kinase activity.[84] Interestingly, although the EGF-R and PDGF-R share common signaling pathways, they differ with respect to PKC-dependent negative feedback mechanisms. Although TPA treatment of A431 cells blocks EGF-induced PI turnover,[85] it does not inhibit that induced by PDGF,[86] suggesting differential regulation of RTKs by the PKC system within the same cell.

In addition to its differential effects on receptor-ligand interactions and signal transduction, PKC has been implicated in receptor internalization and degradation. Chen et al[87] identified a distinct region within the EGF-R C-terminus that appears to be required for ligand-dependent downregulation and degradation. Interestingly, the high-affinity binding site for PLCγ, Tyr 992, is located within this region,[88] which suggests that PKC activation via PLCγ-generated second messengers is involved in receptor downregulation. Nevertheless, the molecular signals that are involved are still poorly understood, and their interpretation is a matter of controversy.

To elucidate the complex role of PKC in the cellular signaling network, we transiently overexpressed 293 fibroblasts with either the EGF-R or the chimeric receptors EP-R, EIR, and HER1-2 (EGF-R/human EGF-R type 2 [HER2] chimera) and investigated short- and long-term effects on RTK signaling parameters in the presence or absence of cotransfected PKCα.

In the absence of PKCα, TPA treatment results within minutes in decreased EGF-R and HER2 tyrosine phosphorylation, while PDGF-R and IR phosphorylation is upregu-

lated. These effects are not mediated by endogenous PKC-dependent RTK phosphorylation, but apparently by activation or inactivation of RTK-specific phosphatases, as indicated by neutralization of this short-term effect upon treatment of cells with sodium orthovanadate, a potent phosphatase inhibitor. In the presence of overexpressed PKCα, all investigated RTKs formed a stable protein-protein complex with PKCα upon TPA treatment, which at the same time resulted in a mobility shift of the receptors. Under these experimental conditions, the TPA effect on RTK activity was not reversible by orthovanadate, indicating that receptor phosphorylation on tyrosine residues is regulated by PKC-dependent phosphorylation on serine and threonine residues. Alternatively, binding of activated PKC to the receptor directly results in receptor inactivation. The TPA-mediated effects on receptor tyrosine phosphorylation, neutralization by orthovanadate, and TPA-induced RTK-PKC complex formation can also be demonstrated in NIH3T3 fibroblasts stably overexpressing these RTKs. RTK-PKC complex formation can also be induced by ligand addition in these cells. Ligand-induced RTK-PKC interaction must be regarded as a long-term effect. It is visible after 30 minutes and declines after 60 minutes to undetectable levels, whereas TPA induces a more rapid and more sustained complex formation. This difference between ligand- and TPA-induced complex formation might be explained by the finding that growth factors transiently activate PLCγ,[46] which then leads to transient activation of PKC, whereas TPA directly and constitutively activates PKC.

What is the biological function of this protein-protein interaction? An involvement of PKC in receptor downregulation has been suggested for many years. Exposing cells to phorbol esters results in decreased EGF binding and can be observed in most cell types. Immunofluorescence and electron-microscopic localization of these receptors showed that a significant percentage of receptors become internalized.[89] PKC-mediated internalization and degradation could clearly be demonstrated in 293 cells. Overexpression of the RTK alone and subsequent treatment with EGF or TPA had no effect on receptor degradation, whereas coexpression of PKCα resulted in a marked loss of radiolabeled receptors, but only upon TPA treatment. Determination of RTK-PKC complex formation showed that EGF was not able to induce this protein-protein interaction, whereas TPA induced receptor-PKC interaction, receptor phosphorylation, and simultaneously receptor degradation. As mentioned earlier, EGF and TPA do not mediate receptor downregulation when the receptor is overexpressed alone, suggesting that the endogenous downregulation pathways are not sufficiently stimulated to activate degradation of these elevated amounts of receptors. Coexpression of PKCα and TPA treatment can restore this pathway, whereas EGF still has no effect. If the assumption is correct that the ligand-activated receptor activates PLCγ, which hydrolyzes PIP2 into DAG and IP3 and thereby activates PKC, then PLCγ should be the limiting factor in the latter case. Simultaneous overexpression of the receptor, PLCγ, and PKC should therefore restore the entire pathway and render it EGF-inducible. This is indeed the case. Coexpression of PLCγ caused the HER1-2 receptor to be efficiently downregulated in response to EGF stimulation, an effect that was enhanced by PKCα co-overexpression. In the latter case, EGF- and TPA-induced receptor downregulation were indistinguishable, demonstrating that activation of PKC via receptor-activated PLCγ and the corresponding second messengers was equivalent to direct activation of PKC by the artificial ligand TPA.

In summary, these data show that PKC mediates short- and long-term effects on RTKs. The immediate effects,

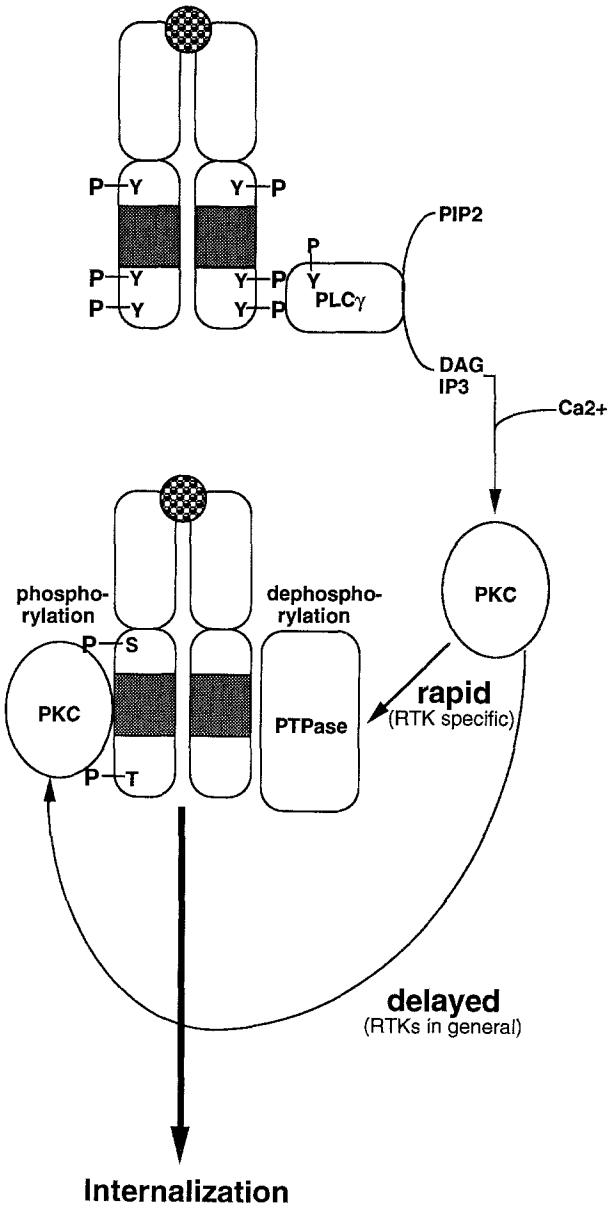

Fig 4. Model of PKC-mediated actions on RTKs. The rapid response, leading to RTK dephosphorylation, involves phosphotyrosine-specific phosphatases (PTPase) and is RTK-specific. The delayed effects are induced by translocation of PKC to the plasma membrane, resulting in binding and phosphorylation of RTKs on serine and threonine residues, and subsequently in internalization and degradation of RTKs in general.

which appear to involve TPA-responsive, tyrosine-specific phosphatases, are RTK-specific and regulate the phosphorylation state of the receptors on tyrosine residues. In contrast, the long-term effects of PKC seem to be more general and are induced by translocation from the cytosol to the plasma membrane and formation of stable complexes with RTKs, concomitant with phosphorylation of these receptors followed by their internalization and degradation (K. Seedorf, M. Sherman, and A. Ullrich, in press) (Fig 4).

CONCLUSION

The RTK-mediated biological response is receptor-specific and cell-type–specific. There is increasing evidence that specificity of signal transduction is determined by various parameters, including specific RTK-substrate interaction, affinity of RTKs for certain substrates, and tissue-specific substrate expression. In addition, the duration of signal transduction pathway activation clearly has an important impact on the response and determines, at least in PC12 cells, whether these cells proliferate or differentiate. The time factor might also determine why receptors that share the same substrates, for example, IR and IGF-I-R, mediate different responses: regulation of metabolism versus growth. There are two main candidates that have the potential to regulate the duration of signal transduction at the receptor level: phosphotyrosine-specific phosphatases that dephosphorylate and thereby inactivate the signaling capacity of RTKs, and PKC. There are numerous data published demonstrating that PKC is capable of regulating RTK tyrosine content, affinity for ligands, and receptor downregulation, all parameters that have the potential of regulating the duration of signal transduction activation in a specific manner.

Despite recent progress, many more pieces have to be added to the puzzle to fully understand these complex regulatory mechanisms.

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