

Phosphorylation of Cell Surface Receptors: A Mechanism for Regulating Signal Transduction Pathways

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Introduction

SPECIFIC receptors, located in the plasma membranes of cells, are crucially involved in mediating the acute and chronic effects of a wide variety of hormones, drugs, growth factors, antigens, and other ligands. Some membrane receptors are involved in transporting various important molecules into the cell by a process known as receptor-mediated endocytosis. An important insight which has emerged from recent research is that both the function and subcellular distribution of many such receptors are dynamically regulated.

Phosphorylation/dephosphorylation represents perhaps the most intensively studied reversible covalent modification of enzymes which is known to have regulatory significance (1, 2). Considerably more is known about the kinases than the phosphatases. Only recently has it begun to be appreciated that the function of noncatalytic proteins, such as receptors, may also be regulated in this way. We review here the important plasma membrane receptor systems thus far studied whose function and/or cellular trafficking has been demonstrated to be regulated by phosphorylation. Available information suggests a number of principles which may be generally applicable as well as several testable hypotheses.

β -Adrenergic Receptor-Coupled Adenylate Cyclase

The β -adrenergic receptor-coupled adenylate cyclase system may be considered to be prototypical of a wide variety of receptors which are coupled to their effector systems via guanine nucleotide regulatory (G) proteins. G proteins represent a family of closely related proteins which are heterotrimeric consisting of GTP binding and hydrolyzing α -subunits as well as β - and γ -subunits (3-

5). Receptors may either stimulate adenylate cyclase via the stimulatory guanine nucleotide regulatory protein (G_s) or inhibit it via the inhibitory guanine nucleotide regulatory protein (G_i). It is now readily apparent that some forms of regulation of adenylate cyclase may be achieved through phosphorylation of its protein components. One of the most extensively investigated forms of this type of regulation is that of desensitization. Desensitization is defined as a process through which prolonged exposure of target cells to hormone, drug, or neurotransmitter agonists results in a diminished cellular responsiveness to further agonist stimulation.

Desensitization of β -adrenergic receptor-coupled adenylate cyclase is generally divided into two major categories (6-8). One form is referred to as agonist specific or homologous and is distinguished by the fact that only stimulation by β -adrenergic agonists is attenuated. Conversely, agonist-nonspecific or heterologous desensitization is characterized by diminished responsiveness to additional receptor agonists and to nonreceptor activators such as guanine nucleotides and fluoride ion. Investigations of homologous desensitization have demonstrated sequestration or down-regulation of the receptors away from the cell surface in addition to an uncoupling of receptor-adenylate cyclase interaction (6-8). Homologous desensitization does not appear to be mediated by cAMP as this form of refractoriness can be demonstrated in the absence of cAMP generation (6-8). Heterologous desensitization, by contrast, does not involve receptor sequestration or down-regulation but instead is primarily associated with functional uncoupling of the receptors from adenylate cyclase. Moreover, heterologous desensitization appears to be mediated, at least partially, by cAMP (6-8). Since heterologous desensitization is associated with decrements in fluoride ion and guanine nucleotide-stimulated activities (effectors which bypass the receptors) this form of desensitization must, at a minimum, involve alterations in either the regulatory proteins and/or the catalytic moiety of adenylate cyclase.

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Recent studies have, in fact, demonstrated that the stimulatory and perhaps the inhibitory guanine nucleotide regulatory proteins (G_s and G_i , respectively) are functionally modified in heterologous desensitization. Kassis and Fishman (9) demonstrated that G_s extracted from heterologously desensitized fibroblasts and reconstituted into S49 cyc^- cell membranes (S49 cyc^- cells lack G_s but possess the other components of adenylate cyclase) was functionally impaired relative to controls. Similarly, heterologous desensitization induced by prostaglandin E_1 in liver (10) and by human CG in ovaries (11) results in an impaired functionality of G_s as determined with S49 cyc^- membrane reconstitution. In contrast, Rich *et al.* (12) have reported that glucagon-induced heterologous desensitization in MDCK cells was not associated with G_s alterations but instead involved increases in the apparent levels of the inhibitory guanine regulatory protein, G_i . This suggests that alterations in the G_s/G_i stoichiometry may be another mechanism by which heterologous desensitization is achieved.

Additional evidence for a functional alteration in G_s in heterologous desensitization has come from studies with avian erythrocytes. Briggs *et al.* (13) found that when G_s was extracted from catecholamine-desensitized turkey erythrocytes and carefully quantitated by labeling with [^{32}P]NAD $^+$ and cholera toxin, desensitization resulted in a significant reduction in the ability of G_s to reconstitute adenylate cyclase activity in S49 cyc^- membranes. Although heterologous desensitization can apparently result in impaired G_s functionality, this does not exclude other potential lesions in the adenylate cyclase system. In fact, the observation that catecholamine-stimulated adenylate cyclase activity is desensitized by about 50% whereas the fluoride ion and guanine nucleotide activities are reduced by only 10–20% after agonist-induced desensitization is indicative of other processes which occur as well (6, 13).

Indeed, using [^{32}P]orthophosphate incorporation, it has been shown that during the heterologous desensitization in both avian and amphibian erythrocytes, the β -adrenergic receptor undergoes phosphorylation (14–16). Sibley *et al.* (15) have investigated this phosphorylation process in detail using turkey erythrocytes. In these cells, the β -adrenergic receptor is stoichiometrically phosphorylated under basal conditions containing 0.7–1.0 mol phosphate/mol receptor with this stoichiometry increasing to 2–3 mol/mol upon maximal desensitization (15). This phosphorylation occurs exclusively on serine residues in the receptor (16, 17). We have also shown that the phosphate/receptor stoichiometry is tightly correlated with the degree of desensitization (15). For instance, the time courses for receptor phosphorylation and adenylate cyclase desensitization are identical as are the rates of resensitization and the return of the phos-

phate/receptor stoichiometry to control levels (15). Moreover, incubation of the cells with membrane-permeable cAMP analogs causes submaximal phosphorylation of the β -adrenergic receptor which is correlated with the partial desensitization of adenylate cyclase which these analogs evoke (15). These data thus indicate that in erythrocytes, heterologous desensitization is tightly correlated with phosphorylation of the β -adrenergic receptor.

Since cAMP analogs can reproduce the catecholamine-induced receptor phosphorylation and desensitization in avian (15) and amphibian erythrocytes (16), then presumably the cAMP-dependent protein kinase is involved in this desensitization. In fact, Benovic *et al.* (18) have recently demonstrated a cAMP-mediated phosphorylation of mammalian lung β -adrenergic receptor that is similar to the process exhibited by erythrocytes. Using pure receptor and pure cAMP-dependent protein kinase, it is shown that isoproterenol enhanced the rate of receptor phosphorylation (on serine residues) by about 2-fold. Reconstitution of the phosphorylated receptor with the G_s protein demonstrated diminished agonist-promoted receptor-mediated stimulation of the GTPase activity compared with controls. Thus, the cAMP-dependent protein kinase-promoted phosphorylation is functionally significant. Since any hormone or drug that raises intracellular cAMP levels will presumably lead to such β -adrenergic receptor phosphorylation, this type of regulation is of the heterologous type.

A fascinating question concerning heterologous desensitization is why incubation of avian erythrocytes with cAMP analogs produces only partial effects (15). One hypothesis, suggested by Benovic *et al.* (18), is that in order to obtain maximal phosphorylation of the β -adrenergic receptor, agonist occupancy must occur. Another possibility is that the receptor phosphorylation is not completely mediated by cAMP and that other protein kinase systems may phosphorylate the β -adrenergic receptor as well. In this regard, we (19) and others (20) have shown that phorbol esters, compounds which potentially activate protein kinase C, are capable of stimulating β -adrenergic receptor phosphorylation concomitantly with adenylate cyclase desensitization. Interestingly, in duck erythrocytes (19) the phorbol ester-induced receptor phosphorylation is nonadditive with that produced by isoproterenol, suggesting a common mechanism or pathway of action.

Recently, protein kinase C has been shown to directly phosphorylate the purified β -adrenergic receptor *in vitro* albeit to a lower stoichiometry than that of the cAMP-dependent protein kinase (21). The phosphorylation by protein kinase C occurs on serine residues and is not enhanced by agonist occupancy of the receptor. Importantly, the sites on the receptor that are phosphorylated

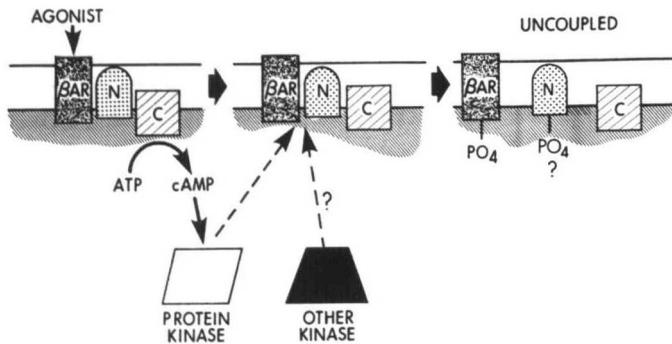


FIG. 2. Heterologous desensitization mechanisms of adenylate cyclase.

purified a novel protein kinase with phosphorylates the β -adrenergic receptor and may be involved in homologous desensitization. Referred to as the β -adrenergic receptor kinase (or β ARK), it is quite ubiquitous in mammalian tissues thus far examined and appears to be cytosolic. It has the unique property of phosphorylating only the agonist-occupied form of the receptor. The unoccupied or antagonist-occupied receptor is not a substrate for the enzyme. β ARK is distinct from other protein kinases such as the cAMP-, cGMP-, Ca^{2+} /calmodulin-, and Ca^{2+} /phospholipid-dependent protein kinases. Moreover, β ARK does not phosphorylate such general kinase substrates as casein or histones. Upon addition of β -adrenergic agonist to the cell, β ARK is translocated from the cytosol to the plasma membrane, where it phosphorylates the agonist-occupied receptor (31). The exact sites of β ARK phosphorylation on the β -adrenergic receptor are not yet known, although, in analogy with the rhodopsin/rhodopsin kinase system (see below), the serine/threonine-rich carboxy terminus (Fig. 1) is a likely region.

A clear consequence of β -adrenergic receptor phosphorylation in homologous desensitization is a functional uncoupling of the receptors from the G_s proteins. Sibley *et al.* (32) demonstrated that phosphorylated β -adrenergic receptors purified from amphibian erythrocytes are functionally impaired in reconstituting receptor- G_s interactions in phospholipid vesicles when compared to controls. Similarly, purified β -adrenergic receptor which has been phosphorylated by β ARK shows an impairment of function (J. L. Benovic *et al.*, manuscript in preparation).

The molecular events which may be involved in homologous desensitization of the β -adrenergic receptor are shown in Fig. 3. Phosphorylation and uncoupling of the receptors from adenylate cyclase activation occur within minutes after agonist occupancy of the receptors (27, 32). Shortly thereafter the receptors become sequestered or internalized within the cells into low density membrane particles or vesicles, although the exact nature of this sequestered compartment is currently unknown, as

MOLECULAR MECHANISMS OF HOMOLOGOUS DESENSITIZATION

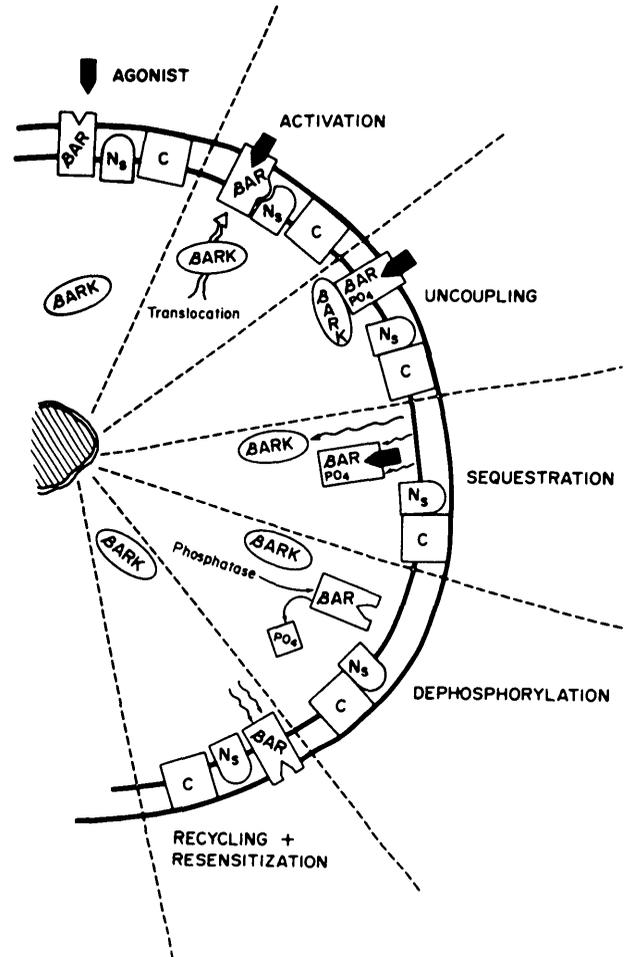


FIG. 3. Homologous desensitization of β -adrenergic receptor-coupled adenylate cyclase.

are the mechanisms of receptor translocation (6). For example, it is not known whether coated pits are involved or whether the receptor phosphorylation event triggers the redistribution of the receptors from the plasma membrane. Within the sequestered compartment the receptors become dephosphorylated and functionally regenerated (32). High levels of β -adrenergic receptor phosphatase activity appear to be associated with the sequestered membrane compartment (32). When agonist is removed or its concentration decreased sufficiently, the receptors can redistribute back to their basal levels in the plasma membranes.

As reviewed above, phosphorylation of the receptor components of the adenylate cyclase system appears to be important in dampening the hormonal signal. Recent studies, however, have suggested that phosphorylation of the catalytic components of the enzyme system may serve as an amplification mechanism. This was first suggested from experiments that involved treating cells with phorbol esters, compounds that potently activate

protein kinase C (33), and examining their effects on adenylate cyclase activity. It was observed that phorbol ester treatment resulted in enhanced basal adenylate cyclase activity as well as that stimulated by a variety of hormonal and nonhormonal effectors (34–39). This amplification effect has also been demonstrated by the direct addition of activated protein kinase C to plasma membranes (40).

Using amphibian erythrocytes, we have demonstrated that incubation of these cells with phorbol esters results in a dramatic 100–300% amplification of β -adrenergic agonist-, prostaglandin-, guanine nucleotide-, as well as Mn^{2+} -, and forskolin-stimulated enzyme activities (34). The observation that the forskolin- and Mn^{2+} -stimulated activities are increased is especially interesting since these compounds can directly stimulate the catalytic unit of adenylate cyclase. This suggests that protein kinase C may phosphorylate the catalytic unit resulting in amplified activity. In fact, using erythrocytes, we have demonstrated that activation of protein kinase C with phorbol esters results in stoichiometric phosphorylation of the catalytic unit of adenylate cyclase (41). Under basal conditions there is no observable phosphate incorporated into the catalytic unit purified from [^{32}P]-labeled cells, however, from phorbol ester-treated cells, the catalytic unit is phosphorylated to about 3 mol phosphate/mol enzyme. We have also shown that purified protein kinase C can directly phosphorylate the adenylate cyclase catalytic unit purified from bovine brain (41). This protein kinase C-catalyzed phosphorylation of the adenylate cyclase catalytic unit and amplification of enzyme activity may provide a physiological mechanism by which receptor systems that promote phosphatidylinositol turnover and protein kinase C activation can modulate receptor systems coupled to adenylate cyclase.

Other G Protein-Coupled Receptors

It has become increasingly apparent that diverse biological phenomena are characterized by adaptive processes analogous to the desensitization observed in β -adrenergic receptor-coupled adenylate cyclase systems. Not surprisingly, many of the other G protein-coupled receptors display desensitization phenomena qualitatively similar to those described above. Thus far, little information is available concerning the molecular mechanisms involved. It will be of interest to see whether these are identical to those uncovered for the β -adrenergic receptors.

A number of receptors, including α_1 -adrenergic and muscarinic cholinergic, which are coupled via G proteins to phosphatidylinositol turnover and protein kinase C activation (33, 42) demonstrate regulatory phenomena similar to adenylate cyclase-coupled receptors. Most no-

tably, the α_1 -adrenergic receptor-stimulated phosphatidylinositol turnover response in cultured smooth muscle cells has been shown to be desensitized by phorbol ester treatment (43, 44). Moreover, this desensitization is associated with phosphorylation of the α_1 -adrenergic receptor (44). More recently, it has been shown that α_1 -adrenergic agonists also promote α_1 -adrenergic receptor desensitization, sequestration, and phosphorylation in a temporally correlated fashion (45). Unlike the situation for the adenylate cyclase-coupled β -adrenergic receptors, there is as yet no evidence for specific receptor kinases involved in these phosphorylation reactions. In fact, it appears that protein kinase C itself directly phosphorylates the α_1 -adrenergic receptor and that the rate but not the extent of this reaction is enhanced by agonist occupancy (21). This is analogous to the cAMP-dependent protein kinase-mediated phosphorylation of the β -adrenergic receptor (18). A classical feedback loop thus presumably operates whereby diacylglycerol, generated by agonist stimulation of phosphatidylinositol turnover, activates protein kinase C, which phosphorylates and desensitizes the α_1 -adrenergic receptor. The cAMP-dependent protein kinase will also directly phosphorylate the α_1 -adrenergic receptor *in vitro*, although agonists do not promote this reaction (21).

The situation for the muscarinic cholinergic receptor seems quite analogous to that of the α_1 -adrenergic receptor. Desensitization of muscarinic receptor-stimulated phosphatidylinositol hydrolysis and receptor down-regulation has been observed in response to cholinergic agonist or phorbol ester stimulation (46–50). Furthermore, Kwatra and Hosey (51) have recently demonstrated muscarinic receptor phosphorylation in cardiac tissue in response to agonist activation. The protein kinase(s) involved in this reaction has not yet been identified.

An additional G protein-coupled receptor system which demonstrates well characterized adaptive phenomena is the rhodopsin system in retinal rod outer segments. As reviewed in detail elsewhere (22, 52), this system is structurally and functionally analogous to hormone-activated adenylate cyclase. The analogous components are a photon of light instead of hormone, rhodopsin instead of receptor, a GTP-binding and hydrolyzing protein termed transducin instead of G_s , and a cyclic GMP (cGMP) phosphodiesterase, which controls retinal concentrations of cGMP, instead of the catalytic unit of adenylate cyclase. Rhodopsin undergoes a phosphorylation reaction that is catalyzed by a specific kinase termed rhodopsin kinase (53–57). Phosphorylation by rhodopsin kinase requires the light (agonist) activated form of rhodopsin and occurs on multiple serine and threonine residues within the carboxy terminus of rhodopsin (58). Phosphorylation of rhodopsin results in

an impaired ability of rhodopsin to interact with transducin. This system is thus desensitized by an agonist-promoted phosphorylation reaction that uncouples the receptor from its GTP-binding regulatory protein. Although phosphorylation of rhodopsin will reduce its interaction with transducin, an additional protein is required for complete uncoupling of this system. This protein, referred to as 48K protein, S antigen, or arrestin, only binds to the phosphorylated form of rhodopsin (59, 60). This observation raises the interesting hypothesis that there may be an analogous 48K protein in the adenylate cyclase system whose action is necessary to express the full functional effects of receptor phosphorylation.

The picture that emerges from these findings is of a variety of pathways by which G protein-coupled receptors are regulated. There are classical feedback regulatory loops, such as the protein kinase A phosphorylation of the β -adrenergic receptor or protein kinase C phosphorylation of the α_1 -adrenergic receptor. The rates of these reactions are enhanced by agonist occupancy of the receptors. Cross-talk pathways also exist by which, for example, protein kinase C can phosphorylate the adenylate cyclase-coupled β -adrenergic receptor or protein kinase A the α_1 -adrenergic receptor. These reactions are not promoted by agonist occupancy of the substrate receptor. Although their physiological regulatory significance is less clear, these reactions could play an important amplification role in tissues where a physiological or biochemical response of the target cell is reciprocally controlled by hormones using two distinct signal transduction pathways. Finally, there are specific receptor kinases, such as rhodopsin kinase and β ARK, which phosphorylate and desensitize specific receptors in a completely agonist-dependent fashion. Such kinases lead only to agonist-specific or homologous desensitization since agonist-promoted conformational changes in the receptor are required to transform them into substrates for the regulatory enzyme.

Insulin Receptors

Insulin induces a wide variety of biological effects in target cells leading to alterations in cellular metabolism and growth. These actions are mediated by specific high affinity cell surface receptors. Insulin receptors are integral membrane glycoproteins comprised of two α -subunits ($M_r \approx 130,000$) and two β -subunits ($M_r \approx 90,000$) linked by disulfide bonds (reviewed in Refs. 61 and 62). Insulin appears to interact with the α -subunit of the receptor which results in enhanced tyrosine kinase activity which is intrinsic to the β -subunit. Both the α - and β -subunits are known to be derived from a single glycosylated precursor protein $M_r \approx 190,000$. Recently, com-

plementary DNAs (cDNAs) encoding the precursor have been cloned which predict a polypeptide consisting of either 1,370 (63) or 1,382 (64) amino acids. The α -subunit comprises the N-terminal portion of the precursor, followed by an enzyme cleavage site and then the β -subunit containing a single transmembrane spanning sequence. The β -subunit also contains a protein kinase domain which exhibits homology with the epidermal growth factor (EGF) receptor and the src family of tyrosine kinases. A schematic representation of the insulin receptor is shown in Fig. 4.

Although the physiological effects of insulin receptor stimulation are well known, the initial biochemical signals have been difficult to elucidate. One of the earliest measurable responses to insulin stimulation, however, is that of receptor phosphorylation. Kasuga *et al.* (65, 66) initially demonstrated that insulin stimulates phosphorylation of the β -subunit of the receptor in ^{32}P -labeled lymphocytes and hepatoma cells. Similar results have been observed using isolated adipocytes (67) and rat

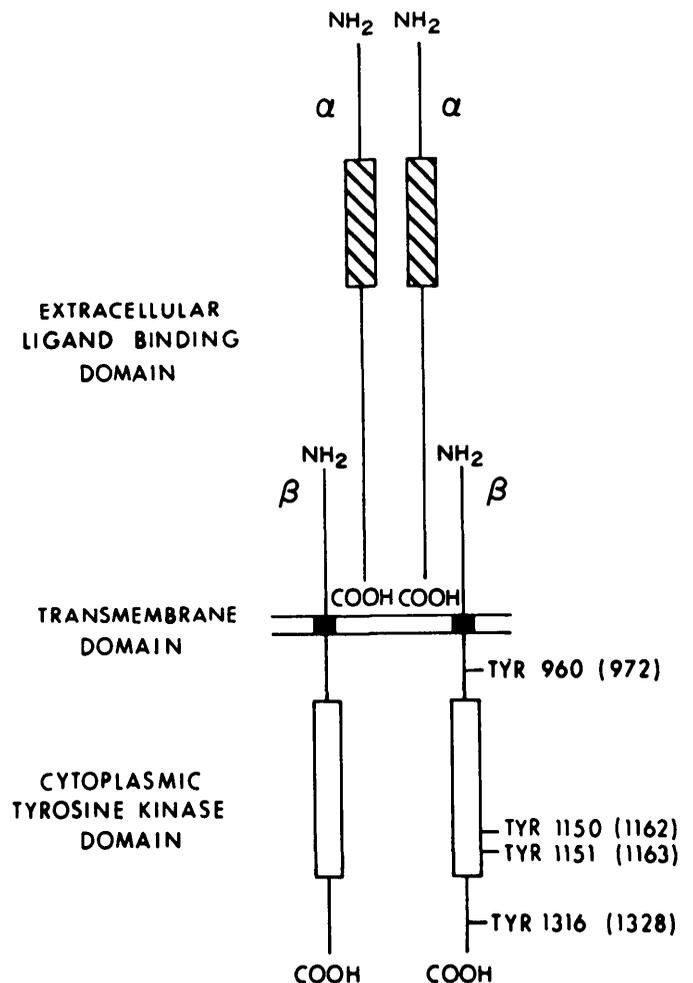


FIG. 4. Schematic representation of the insulin receptor. Regions of high cysteine concentration are shown as hatched boxes, transmembrane domains as solid boxes, and tyrosine kinase domains as open boxes.

hepatocytes (68). In most cells, under basal conditions the β -subunit contains predominantly phosphoserine and to a lesser extent phosphothreonine. Upon addition of insulin to the cells, increased levels of phosphoserine and phosphothreonine are observed in addition to phosphorylation of tyrosine residues in the β -subunit (67, 68–75). Interestingly, Pang *et al.* (71) have shown that the insulin-stimulated tyrosine phosphorylation temporally precedes the increased serine phosphorylation of the β -subunit. Peptide mapping of the phosphorylated β -subunits from intact cells indicates the presence of multiple sites of serine, and to a lesser extent threonine, phosphorylation whereas the tyrosine phosphorylation occurs on two to three major distinct sites (72–74). Recently, Stadtmauer and Rosen (76) have presented evidence suggesting that tyrosine 1,150¹ is one of the phosphorylated residues in insulin-stimulated cells (Fig. 4). In intact cells, the α -subunit does not appear to be phosphorylated.

In contrast to intact cells, when insulin-stimulated receptor phosphorylation is examined under cell-free conditions, using [γ -³²P]ATP, phosphorylation occurs exclusively on tyrosine residues. A large number of laboratories have characterized this reaction using solubilized insulin receptors in various stages of purity from a variety of tissues (see Refs. 61, 62, and 77 for review and references). It has been well documented that the cell-free phosphorylation is a result of autophosphorylation of the β -subunit and that the insulin receptor itself is a tyrosine kinase. Autophosphorylation of the β -subunit appears to be intramolecular as dilution of insulin receptors does not decrease the rate of β -subunit phosphorylation (78, 79). The β -subunit possesses an ATP binding site (80–82), and the cloned sequence predicts a protein kinase domain with homology to other tyrosine kinases (63, 64). Tyrosine phosphorylation of the α -subunit has also been observed under cell-free conditions (83, 84); however, as this subunit appears to be extracellular (Fig. 4), this reaction may not be physiologically significant.

Reports on the stoichiometry of insulin receptor phosphorylation indicate that *in vitro* the β -subunit is phosphorylated up to 2 mol phosphate/mol receptor (78, 79). Peptide mapping of the *in vitro* phosphorylated β -subunit indicates that there are two to three distinct sites of tyrosine phosphorylation (72, 74, 78, 79, 85). In the study of White *et al.* (72), two of the *in vitro* phosphorylation sites corresponded to the two tyrosine phosphorylation sites which were observed in intact cells.

Evidence is now available which indicates that the

activity of the insulin receptor tyrosine kinase is enhanced by tyrosine phosphorylation of its β -subunit. Rosen *et al.* (86) initially showed that autophosphorylation of the receptor results in increased tyrosine kinase activity as measured with exogenous substrates. Moreover, after autophosphorylation, the activated tyrosine kinase is rendered insulin independent. Dephosphorylation of the receptor with alkaline phosphatase restores the insulin dependency of the tyrosine kinase. Other laboratories have shown that autophosphorylation of the receptor both *in vitro* (85, 87) and in intact cells (74, 88) leads to enhanced tyrosine kinase activity of the β -subunit. In the studies of Yu and Czech (85) and Kwok *et al.* (87) the enhanced activity correlated with the phosphorylation of a single tryptic peptide site.

Using antibodies directed to peptides derived from the known sequence of the β -subunit, Herrera and Rosen (89) have provided evidence that tyrosines 1150 and 1316 (Fig. 4) are autophosphorylated *in vitro*. It was further shown that autophosphorylation of Tyr-1150, which is also phosphorylated in intact cells (76), is best correlated with kinase activation. Interestingly, antibodies directed against the sequence containing Tyr-960 block autophosphorylation and thus exogenous kinase activity (90), yet this residue does not appear to be autophosphorylated *in vitro* (76). Using microsequencing techniques, Tornqvist *et al.* (91) have also demonstrated *in vitro* autophosphorylation of tyrosine residues 1150 and 1316 as well as tyrosines 1146, 1151, and 1322. Recently, Ellis *et al.* (92) have demonstrated that point mutations in tyrosines 1150 and 1151 will reduce insulin-stimulated autophosphorylation and tyrosine kinase activity in parallel with a decrease in insulin-stimulated 2-deoxyglucose uptake when expression is performed in CHO cells. Taken together, these results argue strongly for a role of autophosphorylation of tyrosines 1150 and 1151 in activation of tyrosine kinase activity, although the molecular mechanism by which this activation occurs is not yet understood.

The observation that in intact cells insulin receptors undergo phosphorylation on serine and threonine residues in addition to tyrosine residues suggests that other protein kinases are involved in regulating insulin receptor function. A role for the cAMP-dependent protein kinase has been suggested by Pessin *et al.* (93) who showed that incubation of adipocytes with β -adrenergic agonists, which raise intracellular cAMP levels, inhibit high affinity binding of insulin to its receptor. Insulin-stimulated glucose transport is also inhibited, and these effects are mimicked by incubation with dibutyryl-cAMP. Haring *et al.* (94) have reported similar findings and further showed that β -adrenergic agonist treatment of cells reduces the tyrosine kinase activity of the receptor by increasing its Michaelis-Menten constant (K_m)

¹ The two reported amino acid sequences for the insulin receptor precursor differ in that the sequence of Ebina *et al.* (64) contains an insertion of 12 amino acids in the carboxy terminus of the α -subunit when compared to that of Ullrich *et al.* (63). In this review, we will refer to the sequence of Ullrich *et al.* (63) when designating amino acid residues.

for ATP. Similarly, Stadtmauer and Rosen (75) demonstrated that increasing the cAMP of IM-9 cells diminishes insulin receptor tyrosine kinase activity and that this is correlated with increased serine and threonine phosphorylation on the β -subunit. Although these data suggest that cAMP leads to attenuation of insulin action by altering the phosphorylation state of the insulin receptor, it is not clear whether the cAMP-dependent protein kinase directly phosphorylates the receptor. Inspection of the amino acid sequence of the β -subunit indicates the absence of classic consensus recognition sites for this enzyme (63, 64) suggesting that multiple kinases may be involved in this modification.

Protein kinase C also appears to be involved in regulating insulin receptor function. Treatment of cells with phorbol esters, which activate protein kinase C, results in increased phosphorylation of serine and threonine residues in the β -subunit (69, 73, 95, 96). In some cells, but not others, this modification results in a reduction in affinity of insulin for binding to the receptor. Takayama *et al.* (69) and Haring *et al.* (96) have shown that phorbol ester-induced receptor phosphorylation additionally results in a reduction of tyrosine kinase activity of the β -subunit. Recently, Bollag *et al.* (97) demonstrated that protein kinase C can directly phosphorylate the β -subunit of the insulin receptor *in vitro* to stoichiometric levels. This phosphorylation is accompanied by a 65% reduction in the tyrosine kinase activity of the receptor.

As noted earlier, treatment of intact cells with insulin results in enhanced receptor phosphorylation on serine and threonine residues. Peptide mapping experiments indicate that these residues appear, for the most part, to be distinct from those phosphorylated in response to protein kinase C activation (69, 73). This may suggest that insulin treatment leads to the activation of other serine/threonine kinases or that insulin occupancy of the receptor renders it a better substrate for those kinases. Recent results have indicated that long term insulin treatment of cells can lead to reductions in receptor tyrosine kinase activity (98, 99) which may be linked to the observed insulin-induced increase in serine/threonine phosphorylation.

Insulin receptors thus appear to be under both positive and negative control through phosphorylation of their β -subunits. One of the earliest events in insulin activation is autophosphorylation of the β -subunit leading to enhanced tyrosine kinase activity toward exogenous substrates. Tyr-1150 and 1151 appear to be intimately involved in this phosphorylation although other tyrosine residues cannot be ruled out as yet. In contrast, phosphorylation of the β -subunit on serine and perhaps threonine residues results in decreased autophosphorylation and tyrosine kinase activity of the receptor. The serine

and threonine residues involved in this modification have not yet been identified. A number of serine/threonine protein kinases may be involved in this phosphorylation including the cAMP-dependent protein kinase and protein kinase C. Whether or not insulin occupancy results in receptor phosphorylation by additional kinases remains to be determined.

Insulin-Like Growth Factor (IGF) Receptors I and II

IGF-1 or somatomedin C is a polypeptide growth factor with an amino acid sequence which is highly homologous to insulin. IGF-I initiates its mitogenic response in target cells by binding to specific receptors on the plasma membrane. Like the insulin receptor, the IGF-I receptor is a disulfide-linked heterotetramer that can be resolved into $M_r \cong 130,000$ α -subunits which bind IGF-I and $M_r \cong 98,000$ β -subunits which exhibit tyrosine kinase activity (100). In analogy with the insulin receptor, IGF-I receptor α - and β -subunits are encoded with a single $M_r \cong 180,000$ glycosylated receptor precursor for which cDNAs have been recently cloned and sequenced (101).

As with the receptor for insulin, the IGF-I receptor is a tyrosine protein kinase capable of autophosphorylation. Incubation of ^{32}P -labeled IM-9 and Hep-G2 cells with IGF-I promotes phosphorylation on tyrosine residues of the IGF-I receptor β -subunit (73). In addition, in both partially and highly purified preparations of IGF-1 receptors, IGF-I stimulates phosphorylation of tyrosine residues on the β -subunit of the receptor (95, 102-107). Evidence has been presented that this is an intramolecular reaction (105, 107). As with insulin receptors, autophosphorylation of the IGF-I receptor is believed to enhance its tyrosine kinase activity. IGF-I receptor-mediated phosphorylation of exogenous tyrosine-containing substrates is enhanced by prior autophosphorylation, and this enhancement is reversed by phosphatase treatment of the receptor (105, 107).

In intact cells, the β -subunits of the IGF-I receptor are constitutively phosphorylated on serine and threonine residues (73). Incubation of cells with biologically active phorbol esters increase this serine and threonine phosphorylation by about 4-fold (73, 95). In IM-9 cells, IGF-I also promotes serine and threonine phosphorylation on the β -subunit, although apparently on different sites than those observed with phorbol esters (73). The functional significance of these serine and threonine phosphorylations is at present unclear; however, in some cells phorbol esters have been shown to reduce IGF-I binding activity (108).

Like IGF-I, IGF-II is a peptide hormone which is structurally homologous with insulin. The receptor for IGF-II is distinct from the IGF-1 receptor and is com-

posed of a single polypeptide with $M_r \cong 250,000$ (109). The IGF-II receptor appears to be functionally different from insulin and IGF-I receptors in that binding of IGF-II to its receptor does not directly trigger acute metabolic or growth-permissive effects. Moreover, the IGF-II receptor does not appear to possess tyrosine kinase activity (110). Rather it has been suggested that internalization and recycling of the IGF-II receptor may be closely linked to IGF-II action (111). Interestingly, Oka *et al.* (112, 113) have observed that insulin treatment of adipocytes leads to increased numbers of IGF-II receptors in the plasma membrane. In addition, there is a concomitant decrease in IGF-II receptors located in a low density microsomal membrane compartment.

Corvera and Czech (114) have investigated the molecular basis of this phenomenon and demonstrated a role for IGF-II receptor phosphorylation. They found that under basal conditions about 80–90% of the total cellular IGF-II receptors reside in a microsomal fraction with 10–20% being located in the plasma membrane. Moreover, the receptors in the plasma membrane are stoichiometrically phosphorylated possessing 2- to 3-fold more phosphate than those receptors in the microsome fraction. Insulin treatment produces a specific and dramatic decrease in the phosphorylation state of the plasma membrane receptors which is correlated with the appearance of receptors on the cell surface. These data suggest a model in which the IGF-II receptor is persistently phosphorylated on the cell surface leading to internalization into a microsomal compartment where dephosphorylation can occur. Insulin perturbs this process by inhibiting the IGF-II receptor kinase or activating a phosphatase or both which leads to decreased internalization and increased IGF-II receptor expression on the cell surface. Interestingly, this model is analogous to that describing the role of phosphorylation in β -adrenergic receptor sequestration and recycling (see above).

The nature of the protein kinase(s) involved in IGF-II receptor phosphorylation is at present not known. Corvera *et al.* (110), however, have demonstrated that the IGF-II receptor can be phosphorylated by a tyrosine kinase in adipocyte membranes although, as mentioned above, this is not an autophosphorylation event.

The EGF Receptor

EGF is a polypeptide mitogen which is capable of stimulating the proliferation of a variety of epidermal and epithelial cells (115). The receptor for EGF is a 170,000 dalton glycosylated transmembrane protein which exhibits intrinsic tyrosine kinase activity (115, 116). Activation of the receptor with EGF leads to enhanced intracellular protein phosphorylation on tyrosine residues (see Refs. 77, 117, and 118 for reviews). Recent

molecular cloning of the EGF receptor indicates that it consists of 1,186 amino acids, contains a single membrane-spanning domain, and exhibits an extracellular amino terminus and a cytosolic carboxy terminus (119–121). The tyrosine kinase domain of the receptor is in the cytoplasmic region and demonstrates high homology to the transforming protein v-erb B of avian erythroblastosis virus (122). A schematic representation of the EGF receptor is shown in Fig. 5. In intact cells, the EGF receptor is constitutively phosphorylated on serine, threonine, and tyrosine residues (123–125). Evidence has accumulated suggesting that these phosphorylation sites may be intimately linked with regulating receptor function.

Stimulation of the EGF receptor with EGF rapidly leads to receptor autophosphorylation on tyrosine residues (reviewed in Refs. 77 and 115–118). Downward *et al.* (126) have shown that tyrosine residues 1,068, 1,148, and 1,173 in the carboxy terminus are involved in this autophosphorylation with residue 1,173 being the most heavily phosphorylated (Fig. 5). This reaction was shown by Weber *et al.* (127) to be intramolecular in nature. Gill and colleagues (128, 129) have suggested that this auto-

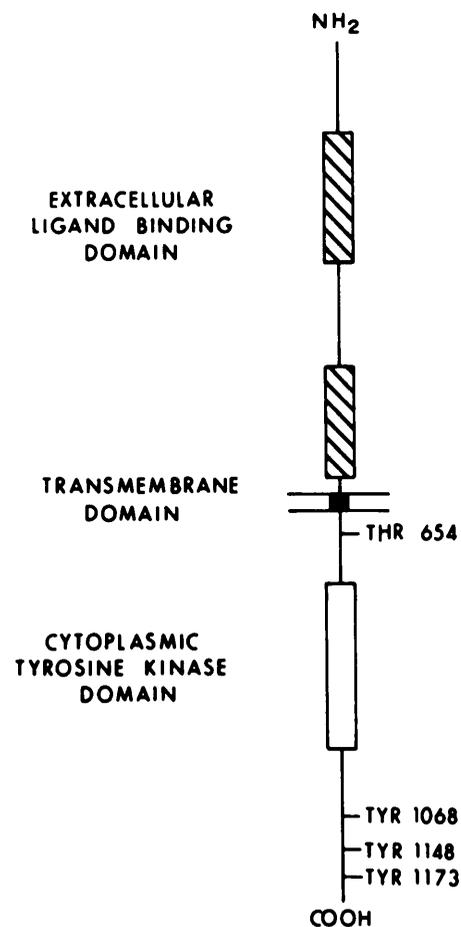


FIG. 5. Schematic representation of the EGF receptor. Regions of high cysteine concentration are shown as hatched boxes, the transmembrane domain as a solid box, and the tyrosine kinase domain as an open box.

phosphorylation enhances the tyrosine kinase activity of the receptor. When phosphorylation of exogenous peptide substrates is assayed as a function of receptor autophosphorylation, tyrosine kinase activity is enhanced by up to 3-fold at a phosphate/receptor stoichiometry of 1–2 mol/mol. High concentrations of peptide substrates can inhibit phosphorylation of both the receptor and the peptides, but this inhibition can be relieved by initially autophosphorylating the receptor. These results suggest then that autophosphorylation of the carboxy terminus may remove an inhibitory steric constraint, thus allowing access of exogenous substrates to the active site and increased tyrosine kinase activity.

In contrast to the above studies, an early report by Cassel *et al.* (130) indicated that EGF-induced autophosphorylation of the EGF receptor in A431 cell membranes does not affect its tyrosine kinase activity. In addition, the rate of EGF-induced exogenous peptide phosphorylation does not show a lag during the time period in which receptor phosphorylation was submaximal. Similarly, Downward *et al.* (131) have provided evidence that variation in the extent of receptor autophosphorylation from 0.1–2.8 mol phosphate/mol receptor does not influence receptor kinase activity either in purified or membrane-bound receptor preparations. Moreover, Gullick *et al.* (132) have shown that antibodies raised against synthetic peptides containing the EGF receptor tyrosine autophosphorylation sites can inhibit autophosphorylation of the native receptor without affecting EGF-stimulated tyrosine kinase activity toward exogenous substrates. In addition, Yarden and Schlessinger (133) have presented evidence favoring an intermolecular rather than an intramolecular mechanism of EGF receptor activation. It is thus not yet clear whether receptor autophosphorylation on tyrosine residues results in enhanced tyrosine kinase activity of the EGF receptor. It is expected that experiments involving site-directed mutagenesis of the relevant tyrosine residues in the EGF receptor will resolve this important issue.

In addition to phosphorylation on tyrosine residues, treatment of intact cells with EGF results in enhanced phosphorylation of serine and threonine residues in the receptor (123–125, 134). Since EGF has been shown to promote phosphatidylinositol turnover (135, 136) generating diacylglycerol which activates protein kinase C (137), it was proposed that this kinase phosphorylates the receptor. Indeed, a number of studies have demonstrated that treatment of cells with phorbol esters, which directly activate protein kinase C, results in attenuation of EGF binding to its receptor (138–144). Moreover, phorbol esters induce phosphorylation of serine and threonine residues in the EGF receptor in intact ³²P-labeled cells (124, 125, 134, 145, 146). Although treatment of cells with phorbol esters enhances phosphorylation of

both serine and threonine residues, peptide mapping experiments indicate the appearance of unique peptides containing only phosphothreonine (124, 134, 145, 146). Hunter *et al.* (145) and Davis and Czech (147) have identified threonine 654 in the EGF receptor as the unique site of phosphorylation in response to phorbol esters in intact cells. This residue is located on the cytoplasmic side of the receptor nine residues away from the plasma membrane (Fig. 5). It is thus in an ideal location to affect interaction between the extracellular and intracellular domains of the receptor. Protein kinase C added directly to A431 cell membranes will also phosphorylate the EGF receptor on the same sites as those promoted by phorbol esters (134).

Phosphorylation of EGF receptors by protein kinase C appears to inhibit receptor tyrosine kinase activity. Cells exposed to phorbol esters exhibit decreased phosphotyrosine levels in EGF receptors and other proteins (124, 134, 148). These studies also demonstrated that EGF-induced autophosphorylation of the receptor on tyrosine residues is reduced after phorbol esters treatment. Recently, Downward *et al.* (131) have also shown that phosphorylation of the EGF receptor by protein kinase C results in decreased tyrosine kinase activity toward exogenous substrates.

As discussed previously, phosphorylation of the EGF receptor by protein kinase C (via phorbol ester treatment) results in functional alterations in EGF binding. In some cells this is apparent as a reduction in the high affinity component of EGF-receptor binding, whereas in others the result is a decrease in the number of EGF receptors at the cell surface. Davis and Czech (149) have shown that reduction of the apparent affinity of the EGF receptor by protein kinase C correlates with phosphorylation of threonine 654 but not with phosphorylation of other residues on the receptor. Lin *et al.* (150) have further shown that mutating threonine 654 to an alanine residue blocks phorbol ester-induced EGF receptor internalization in a cellular expression system. These data suggest that phosphorylation of threonine 654 by protein kinase C regulates the affinity of the receptor for EGF, the activity of the tyrosine kinase domain, as well as the intracellular location of the receptor.

It should be noted that the mechanisms by which EGF induces internalization of its receptor may be distinct from those mediated by the actions of phorbol esters. First, phorbol esters appear to promote internalization of EGF receptors in the absence of receptor degradation (151, 152), whereas EGF promotes both internalization and degradation (153). Moreover, in the study of Lin *et al.* (150) the alanine 654 mutant receptors were still internalized and degraded in response to EGF. There thus appears to be two independent mechanisms by which EGF receptors are internalized. One of these in-

volves phosphorylation on threonine 654 of the receptor and can be promoted by both phorbol esters and EGF (154). The other, which is promoted only by EGF, also leads to receptor degradation and may or may not involve phosphorylation. In this regard, it is interesting that monoclonal antibodies to the EGF receptor can promote receptor internalization in the apparent absence of phosphorylation (155).

Although EGF stimulation leads to rapid autophosphorylation on tyrosine residues which may result in enhanced kinase activity. EGF treatment also results in the phosphorylation of threonine 654 (154) leading to eventual receptor internalization and tyrosine kinase inhibition (156). In addition to this negative feedback loop induced by EGF, the protein kinase C-induced receptor phosphorylation may also represent a mechanism of heterologous regulation by other hormone ligands which activate protein kinase C. Platelet-derived growth factor (PDGF), fibroblast growth factor, bombesin, and vasopressin, all of which stimulate phosphatidylinositol turnover, decrease EGF binding to its receptor (157-161). Davis and Czech (162) have further shown that PDGF will induce phosphorylation on threonine 654 of the EGF receptor. Moreover, synthetic derivatives of diacylglycerol, the endogenous activator of protein kinase C, will promote EGF receptor phosphorylation (163-165).

The EGF receptor is also potentially regulated through phosphorylation by the cAMP-dependent protein kinase. This kinase has been shown to phosphorylate membrane-bound and purified EGF receptor on serine and threonine residues (166, 167). Evidence that this phosphorylation may be physiologically significant has come from studies of Pessin *et al.* (93). They observed that treatment of adipocytes with both β -adrenergic agonists, which increase intracellular cAMP levels, and with dibutyryl-cAMP leads to a decreased affinity of the receptor for EGF. Phosphorylation of the EGF receptor by the cAMP-dependent protein kinase may thus lead to inhibition of EGF action.

The PDGF Receptor

PDGF is one of the major mitogens found in serum which stimulates DNA and protein synthesis as well as amino acid uptake and phosphatidylinositol turnover in a number of cells (168). Interestingly, PDGF is structurally homologous with the product of the *v-sis* oncogene (169). The PDGF receptor has been identified by affinity cross-linking experiments as a $M_r \cong 180,000$ single polypeptide glycoprotein (170, 171). Like the receptors for insulin, EGF and IGF-I, the PDGF receptor possesses tyrosine kinase activity. Initial studies using fibroblast membranes demonstrated that PDGF promotes the in-

corporation of phosphate into tyrosine residues of a $M_r \cong 180,000$ protein (172-174). Pike *et al.* (175) and Heldin *et al.* (170) subsequently provided evidence suggesting that the phosphoprotein observed in fibroblast membranes is the PDGF receptor. It was also observed that the $M_r \cong 180,000$ protein contains phosphoserine and phosphothreonine which are enhanced upon PDGF treatment (175). The PDGF receptor has been recently purified using a novel antiphosphotyrosine antibody (176-178). These studies indicate that the $M_r \cong 180,000$ substrate of the PDGF-stimulated tyrosine kinase is, in fact, the PDGF receptor and like the other tyrosine kinase receptors is capable of autophosphorylation. Very recently, the primary structure of the PDGF receptor has been obtained through molecular cloning techniques (179). The single polypeptide contains an extracellular ligand binding domain, a single membrane spanning region, and an intracellular tyrosine kinase domain which is homologous with other tyrosine kinases (179).

Since the functional consequences of PDGF receptor autophosphorylation are not yet known, it will be interesting to investigate whether this covalent modification is linked to tyrosine kinase activation as suggested for the other tyrosine kinase receptors. Similarly, it will be important to establish a role for the phosphorylation of the PDGF receptor on serine and threonine residues (175) and to identify the kinase(s) involved. Since PDGF promotes phosphatidylinositol turnover in several cell types, it is tempting to speculate that protein kinase C may somehow be involved in these phosphorylation events. However, a recent report by Sturani *et al.* (180) has suggested that phorbol esters do not affect PDGF receptor function, at least in fibroblasts.

The Nicotinic Acetylcholine Receptor

The nicotinic acetylcholine receptor is an integral membrane protein that functions as a neurotransmitter-dependent ion channel at the vertebrate neuromuscular junction (181). The nicotinic receptor has been most extensively studied in the *Torpedo* electric organ and exists as an oligomer of four polypeptide chains with masses of 40 (α), 50 (β), 60 (γ), and 65 (δ) kilodaltons (kD) with a stoichiometry of $\alpha_2\beta\gamma\delta$ (182). Phosphorylation of the nicotinic acetylcholine receptor has been shown to occur both in intact cells and *in vitro* with as many as nine phosphoserines being identified, distributed 1, 1, 2, and 5 among the α , β , γ , and δ subunits, respectively (183). Postsynaptic membranes rich in the nicotinic receptor appear to contain both endogenous protein kinase (184-186) and protein phosphatase activities (187).

The endogenous protein kinases include protein kinase C which phosphorylates the α - and δ -subunits of the

receptor on serine residues (188). In addition, an endogenous tyrosine protein kinase which phosphorylates the β -, γ -, and δ -subunits of the receptor has been reported (188). This kinase phosphorylates a single tyrosine residue on each of these subunits with stoichiometries of approximately 0.5 mol phosphate/mol subunit attained in postsynaptic membranes. It has also been reported that purified pp60^{src} of Rous sarcoma virus as well as A431 cell membranes, which are rich in EGF receptor, both specifically phosphorylate purified nicotinic acetylcholine receptor on the β -, γ -, and δ -subunits (188).

The cAMP-dependent protein kinase (protein kinase A) also phosphorylates the γ - and δ -subunits of the nicotinic receptor on serine residues (189, 190). This phosphorylation can be completely blocked by the specific inhibitor protein of the cAMP-dependent protein kinase. Haganir and Greengard (189) have demonstrated that the γ - and δ -subunits of the purified receptor are phosphorylated by the catalytic subunit of protein kinase A to stoichiometries of 1.0 and 0.89 mol phosphate/mol receptor, respectively. Based on amino acid sequences and subunit and substrate specificities, Haganir *et al.* (188) proposed the phosphorylation sites for protein kinase A as serine 354 (γ -subunit) and serine 361 (δ -subunit). Moreover, antibodies raised against synthetic peptides encompassing the proposed phosphorylation sites inhibit phosphorylation of the receptor by protein kinase A (191).

Several recent studies have provided evidence for a functional alteration of the acetylcholine receptor when phosphorylated by protein kinase A (192–194). Two groups have demonstrated that treatment of rat skeletal muscle with forskolin, a potent activator of adenylate cyclase, leads to desensitization of nicotinic receptor function (192–194). Moreover, Haganir *et al.* (193) have provided direct evidence in a reconstituted system that phosphorylation of the receptor by protein kinase A increases the rate of agonist-induced desensitization of the receptor by 7- to 8-fold. These results provide the first direct evidence that phosphorylation regulates the function of an ion channel receptor protein.

Intracellular Transport Receptors

Eukaryotic cells internalize many macromolecules and nutrients by receptor-mediated endocytosis. The intracellular pathways of this process have been investigated in a variety of receptor systems (reviewed in Refs. 195 and 196). Upon binding of ligand, the receptors move to and cluster in specialized regions of the plasma membrane termed coated pits. These structures contain the protein clathrin which may be involved in trapping of the receptor-ligand complex. Subsequently, the coated pits invaginate to form coated vesicles which rapidly give

rise to uncoated vesicles referred to as endosomes or receptosomes. The receptosome is a low pH compartment which promotes ligand-receptor dissociation. At this point the pathways of endocytotic receptors may diverge, with some classes of receptors being recycled back to the cell surface while others are degraded in lysosomes. The transreticular Golgi system may play a role in this sorting process (153). One of the major questions concerning receptor-mediated endocytosis is what signals are involved in triggering receptor internalization, sorting, and receptor recycling.

Recent attention has focused on phosphorylation of the receptor proteins as a mechanism of regulating their intracellular distribution. One well characterized recycling receptor, the transferrin receptor, has indeed been shown to undergo phosphorylation/dephosphorylation reactions. The transferrin receptor is an integral membrane glycoprotein composed of two identical subunits of $M_r \approx 90,000$ which are linked by a disulfide bond to form a dimer (197, 198). This receptor mediates the endocytosis of transferrin, resulting in cellular uptake of Fe^{2+} . Recently, it has been reported that endocytosis of the transferrin receptor can be promoted by phorbol esters (199, 200). Phorbol ester treatment results in a redistribution in approximately 50% of the transferrin receptors from the plasma membrane to an endosome compartment with no loss in total receptor number. In HL60 cells this event correlates with an increase in the phosphorylation state of the transferrin receptor (200, 201). Although phorbol esters and transferrin induce internalization of transferrin receptors in a nonadditive fashion, transferrin itself does not promote receptor phosphorylation. This may suggest that although phosphorylation results in receptor internalization, it is not the primary signal for transferrin-induced receptor endocytosis. Recently, Davis *et al.* (202) have identified serine 24 as the site on the transferrin receptor phosphorylated by protein kinase C.

In contrast to the effects of phorbol esters, recent work has demonstrated that insulin and various growth factors can induce the redistribution of transferrin receptors from an intracellular endosome compartment to the plasma membrane (203, 204). In view of the observation that insulin promotes a similar redistribution of IGF-II receptors by reducing their phosphorylation state (see above), it is tempting to speculate that insulin and growth factors induce dephosphorylation of transferrin receptors leading to their enhanced cell surface expression.

Other recycling receptors may be regulated by phosphorylation events. The asialoglycoprotein receptor is phosphorylated on serine residues (205, 206) although the functional significance of this modification is not known. Phorbol esters have been shown to reduce the

binding activity of low density lipoprotein (LDL) receptors (207); however receptor phosphorylation was not examined. In contrast, Kishimoto *et al.* (208) have demonstrated phosphorylation of the LDL receptor on serine 833 in the cytoplasmic domain by a casein kinase II-like enzyme purified from adrenal cortex. The physiological significance of this modification is unknown as mutating this serine to an alanine has no effect on the rate of LDL receptor internalization (209).

Receptors Mediating Immune Function

T Lymphocyte Receptors

The T cell antigen receptor is found on T lymphocytes where it mediates the activation of these cells by specific antigens. Clonotypic antibodies and cDNA cloning by subtractive screening methods have shown that the main core of the receptor is formed of an α (40–50 kD)- and β (43 kD)-subunit heterodimer (reviewed in Ref. 210). However, immunoprecipitation and cross-linking experiments have suggested that the functioning receptor may involve several additional protein components. A complex of proteins termed the T_3 complex has been characterized both in the human and murine systems (211–214). This complex is comprised of two glycoproteins of 20–22 kD (gp21) and 26 kD (gp26), and two nonglycosylated peptides of 25–26 kD (p25) and 16 kD (p16). An additional nonglycosylated subunit of 21 kD (p21) has recently been identified in the murine system (215). Activation of the T cell antigen receptor complex by clonotypic antibodies or specific antigens leads to the increased hydrolysis of polyphosphoinositides, suggesting that activation of protein kinase C may at least in part mediate T cell activation (216, 217). Although a biochemical role for each of the putative components of the receptor complex has not yet been established, several studies have suggested that many of these polypeptides are phosphorylated in response to T cell activation by specific antigens or mitogens. In human T lymphocytes, Cantrell *et al.* (218) have reported phosphorylation of two peptides of the T_3 complex ($M_r = 26,000$ and to a lesser extent $M_r = 21,000$) upon activation of cells with phorbol esters. Samuelson *et al.* (215) have shown in murine T cell hybridomas that phorbol esters lead to phosphorylation of p25 and gp21 on serine residues. Antigen activation of these cells leads to the phosphorylation of the same gp21 subunit and a previously unidentified 21 kD polypeptide on tyrosine residues. It has been speculated that phosphorylation of the various T cell receptor complex subunits might be involved in the process of down-regulation or desensitization of the T cell receptor, a process induced by phorbol esters (218). Recently, the suggestion has been made (219) that the phosphorylation pattern of one of these subunits (p21,

tyrosine phosphorylation) may be abnormally constitutive in a mouse model of lymphoproliferative disease. Thus, these data suggest that phosphorylation of the T cell antigen receptor complex may modulate several of its functions.

Another T lymphocyte-specific receptor is that which binds T cell growth factor (TCGF) or interleukin-2 (IL-2), a $M_r = 14,800$ glycopeptide hormone necessary for antigen- or mitogen-mediated activation of T cell proliferation (220). The receptor for IL-2 is a glycoprotein of $M_r \cong 55,000$ with a nonglycosylated amino acid core of $M_r = 28,428$ (220). The mechanisms of signal transduction for this hormone effector system are still not completely understood. However, IL-2 has been shown to induce a redistribution of protein kinase C activity (221), a property which is shared by hormones that use the phosphatidylinositol/ Ca^{++} pathway as a transducing mechanism.

Several studies have documented that the IL-2 receptor is a phosphoprotein. Leonard *et al.* (222) showed that human IL-2 receptors isolated from mitogen-stimulated peripheral lymphocytes and leukemic HUT 102B2 T cells are constitutively phosphorylated. Shackelford and Trowbridge (223) have shown that treatment of activated T cells or leukemic cells with phorbol esters induces the phosphorylation of the IL-2 receptor which is associated with increased expression of IL-2 receptors on the surface of these cells. More recently, Gaulton and Eardley (224) have shown that exposure of mitogen-activated splenic T cells to physiological concentrations of IL-2 causes rapid phosphorylation of several membrane proteins amongst which is the IL-2 receptor protein itself. By site-directed mutagenesis, Gallis *et al.* (225) have shown that cytoplasmic serine 247 is likely to be the site of phosphorylation of the IL-2 receptor. From these data it has been suggested that phosphorylation of the IL-2 receptor may be involved in the expression and maintenance of the receptor at the cell surface.

Immunoglobulin (Ig) receptors

Mast cells and basophils express, on their surface, specific receptors for IgE. Binding of IgE to these receptors initiates the noncytotoxic release and synthesis of a variety of chemical mediators of inflammation such as histamine and leukotrienes from these cells (226). The IgE receptor is heterotetramer composed of one α (45 kD)-, one β (33 kD)-, and two γ -subunits (9 kD) (226). The α -subunit is thought to be extracellular and serves as the ligand binding site for IgE whereas the β -subunit appears to be transmembrane. The two γ -subunits interact with the β -subunit within the cytoplasmic domain (226).

The biochemical signalling mechanisms by which IgE

initiates its effects are still poorly understood; nonetheless, phosphorylation of the IgE receptor has been examined as a possible control mechanism for IgE-mediated effects. Hempstead *et al.* (227, 228) have reported that in normal rat mast cells, the α - and β -subunits of the receptor are constitutively phosphorylated. Antigen activation of these cells results in increased phosphorylation of the α -subunit whereas the β -subunit phosphorylation is unaltered. The increase in α -subunit phosphorylation occurs within the time scale of cell activation leading to the hypothesis that this covalent modification is a primary event in receptor action. In contrast, Metzger and colleagues (229, 230), using basophilic leukemia cells, observed phosphorylation of the β - and γ -subunits of the IgE receptor but no phosphorylation of the α -subunit. In these cells, antigen activation results in increased phosphorylation of the β -subunit whereas the γ -subunit phosphorylation is decreased. In light of these controversial findings, it is clear that more work will be necessary to define a functional role for IgE receptor phosphorylation.

Polymeric IgA, the major immunoglobulin in external secretions, is synthesized in plasma cells of various mucosal tissues as well as exocrine glands (231). Receptors for IgA are synthesized by a variety of epithelial cells, including hepatocytes, and mediate the secretion of poly-IgA via receptor-mediated transport across the epithelia (232). In hepatocytes, the mature form of the receptor (120 kD) arises from two intermediate precursors, a 105 kD initial precursor which becomes glycosylated to a 116 kD secondary form. The final maturation of the 116 kD precursor to the mature 120 kD form is associated with phosphorylation of the intracellular domain of the receptor on serine residues (233). Whereas this active form of the IgA receptor appears to be phosphorylated, the biological significance of this phosphorylation is still not understood. One possibility is that it is involved in the sorting patterns and intracellular processing of the receptor protein (233).

Conclusions

A rapidly expanding body of literature now indicates that the functionality and/or cellular distribution of cell surface receptors for hormones, drugs, neurotransmitters, and growth factors can be regulated by phosphorylation. In many instances where phosphorylation directly alters receptor function, this appears to be in a negative direction. Thus, receptor phosphorylation may result in decreased agonist ligand binding or a diminished ability of the receptor to interact with its biochemical effector. In some cases, the enzymatic activity of a receptor molecule may be inhibited. In contrast, autophosphorylation of the tyrosine kinase receptors may result

in increased enzymatic activity and enhanced receptor function. In those receptor systems which exhibit internalization and recycling phenomena, phosphorylation appears to result in enhanced receptor internalization. The biological role for this internalization event may be quite varied and system specific. In some cases, the consequence of receptor internalization may be dephosphorylation and resensitization, whereas in others the result is enhanced endocytosis of a specific ligand. Future work in this rapidly growing field will undoubtedly focus on identifying a role for phosphorylation in other receptor systems, characterizing the protein kinases and phosphatases involved, and elucidating the molecular mechanisms by which phosphorylation produces its functional effects.

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