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Topical Review

Targeting G Protein-coupled Receptor Kinases to Their Receptor Substrates

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Introduction

G protein-coupled receptors (GPCRs) are integral membrane proteins containing seven putative transmembrane domains. In response to agonist occupancy, these proteins mediate signals to the interior of the cell via activation of heterotrimeric G proteins. GPCRs respond to a vast array of agonists including catecholamines, chemokines, small peptides, photons of light and specialized ligands such as taste and odorant molecules. Agonist binding leads to a change in receptor conformation which facilitates G protein activation; the exchange of GDP for GTP on the G protein α subunit. Activation of a heterotrimeric G proteins is accompanied by its dissociation into an α subunit and a $\beta\gamma$ dimer both of which subsequently interact with and activate various effector proteins. Effector activation alters cellular second messenger levels, which ultimately results in a physiological response.

GPCR desensitization, the loss of receptor responsiveness following continued or repeated agonist occupancy is mediated, at least in part, by receptor phosphorylation. Two classes of kinases phosphorylate GPCRs, the second messenger dependent kinases (cAMP-dependent protein kinase and protein kinase C) and a family of kinases termed G protein-coupled receptor kinases (GRKs). The GRKs are distinguished by their substrate specificity. In contrast to the second messenger dependent kinases, the GRKs phosphorylate only agonist-occupied GPCRs. Thus, GRK-mediated phosphorylation of GPCRs serves to specifically desensitize active receptors which is termed homologous desensitization.

Other reviews have focused on G protein-coupled receptors (Schwinn, Caron & Lefkowitz, 1992) and on the role played by the GRKs in homologous desensitization of receptor signaling. (Freedman & Lefkowitz 1996, Inglese et al., 1993; Premont, Inglese & Lefkowitz, 1995). In this review we concentrate on one aspect of GRK function, the various mechanisms employed to recruit GRKs to the plasma membrane. The localization of the GRKs to the membrane is required for their efficient function and as such is a highly regulated process.

The Family of G Protein-Coupled Receptor Kinases

To date six members of the GRK family have been identified, GRK1 (Rhodopsin kinase), GRK2 and GRK3 (also termed β-adrenergic receptor kinase 1 and 2), GRK4 (or IT11), GRK5 and GRK6. GRK1 and GRK2 were originally identified and subsequently purified based on their ability to phosphorylate respectively, rhodopsin and the β_2-adrenergic receptor. Oligonucleotides, designed using peptide fragments from purified protein, were used to screen a retinal (Lorenz et al., 1991) and a bovine brain cDNA library (Benovic et al., 1989) to obtain the full length cDNAs for, respectively, GRK1 and GRK2. The cDNA for GRK3 was subsequently obtained by screening a bovine brain cDNA library with the catalytic domain of GRK2 (Benovic et al., 1991). The GRK4 gene was identified by positional cloning in the search for the Huntington's disease locus (Ambrose et al., 1992) and later shown to undergo alternative splicing to yield four distinct GRK4 mRNAs (Sal-

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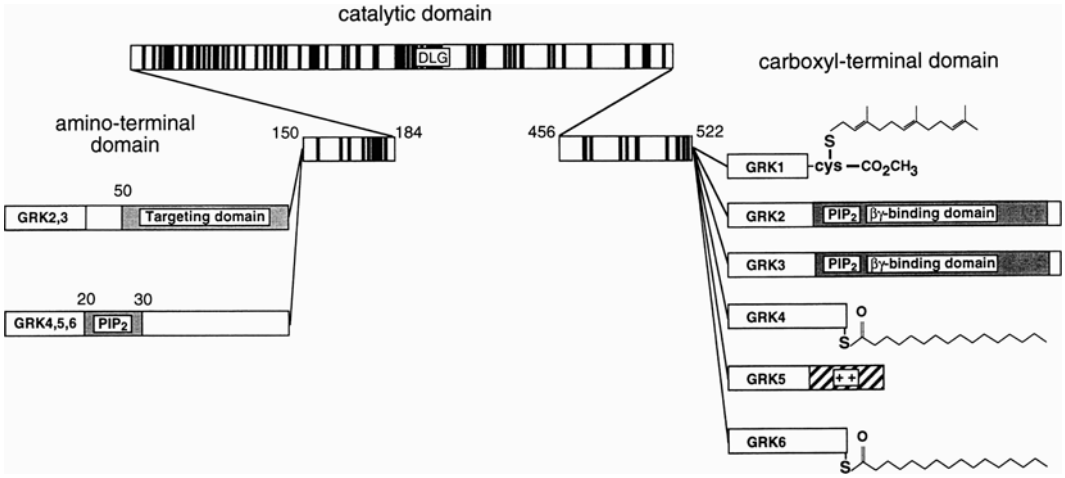

Fig. 1. Domain organization of the GRKs depicting protein and lipid binding sites as well as lipid modifications. Conserved residues are indicated by straight bars. GRK1 is farnesylated. GRK2 and 3 bind βγ and PIP2 in their carboxyl-terminal domain. GRK4 and 6 are palmitoylated. GRK5 binds lipids in its carboxyl-terminal domain. GRK4, 5 and 6 bind PIP2 in their amino-terminal domain and GRK2 binds a microsomal membrane protein in its amino terminus.

lese, Lombardi & DeBlasi, 1994; Premont et al., 1996). GRK5 and GRK6 were identified by polymerase chain reaction (PCR) using oligonucleotides designed to encode highly conserved domains in the GRK sequence (Kunapuli, Gurevich & Benovic, 1993; Haribabu & Snyderman, 1993; Benovic et al., 1993; Premont et al., 1994).

Structurally the GRKs are composed of three domains; a central highly conserved catalytic domain flanked by a conserved amino-terminal domain and a variable carboxyl-terminal domain (Fig. 1). The amino-terminal domain is approximately 187 amino acids in length and has been implicated in receptor recognition (Palczewski et al., 1993). In contrast, the carboxyl-terminal domain varies in length and appears to be principally involved in mediating the membrane association of these enzymes. The autophosphorylation sites of GRK1 (Palczewski et al., 1992), GRK5 (Premont et al., 1994) and the putative autophosphorylation sites of GRK4 (Premont et al., 1996) are located in the carboxyl-terminal domain.

Functionally, the GRKs exhibit several key regulatory features, elucidated principally from the study of GRK1 and GRK2. First, these enzymes preferentially phosphorylate agonist-occupied or activated receptor substrates. Second, interaction of the GRKs with their receptor substrates leads to a direct activation of GRK catalytic activity (Palczewski et al., 1991; Chen et al., 1993; Haga, Haga & Kameyama, 1994) and the regions on the receptor responsible for GRK activation are distinct from the sites of GRK-mediated phosphorylation. Finally, membrane association of the GRKs is required for GRK-mediated GPCR phosphorylation.

Membrane Targeting of the GRKs

The GRKs utilize distinct mechanisms for effecting membrane association. GRK1, 2 and 3 are primarily cytosolic proteins (Freedman & Lefkowitz, 1996) which upon receptor stimulation, translocate from the cytosol to the plasma membrane (Strasser et al., 1986; Inglese et al., 1992a). In contrast, GRK4, 5 and 6, do not exhibit agonist-dependent membrane association. GRK5 appears to be constitutively associated with the membrane (Premont et al., 1995; Premont et al., 1996) and this observation could potentially explain, as compared to GRK2, the higher basal (agonist-independent) activity of this kinase (Premont et al., 1994). Despite the differences between the three GRK subfamilies, membrane association of all the kinases appears to be mediated, at least in part, by determinants in their carboxyl-terminal domains. The various post-translational modifications, protein-protein and protein-lipid interactions responsible for localizing the GRKs to their membrane incorporated receptor substrates are discussed in detail below.

Isoprenylation

The amino acid sequence of GRK1 terminates in a CAAX motif, which directs the isoprenylation and carboxylmethylation of this kinase (Inglese et al., 1992*a*) (Fig. 1). Isoprenylation is a cotranslational modification in which the amino acid sequence of the CAAX motif specifies which isoprenoid homologue is attached to the protein, either farnesyl (C15) or geranylgeranyl (C20). For GRK1 the sequence is CVLS, which directs farnesylation of the kinase. The farnesylated kinase is predominantly a cytosolic protein that translocates in a light dependent manner to the rod outer segment membrane (Inglese et al., 1992*a*). Removal of the farnesyl group by mutation of the CVLS motif (cysteine (C) is mutated to serine (S)) results in a kinase that is unable to translocate in a light-dependent fashion and has a reduced ability to

phosphorylate rhodopsin (Inglese et al., 1992*b*). In contrast, mutation of the serine of the CVLS motif to leucine yields a kinase that is modified by geranylgeranylation and is constitutively associated with the membrane (Inglese et al., 1992*b*). The geranylgeranylated kinase although impaired in its ability to translocate to rod outer segment membranes in a light-dependent fashion, phosphorylates rhodopsin at a comparable rate to wild type (farnesylated) GRK1. Isoprenylation is required for the membrane association and activity of GRK1. Furthermore, the specific modification found in vivo, farnesylation, confers on GRK1 the ability to associate with membranes in a stimulus (light) dependent fashion.

Similar studies with isoprenylated mutants of GRK2 have shown that both kinase activity and degree of membrane association increase with isoprenylation status (Inglese et al., 1992*b*). Nonisoprenylated kinase does not bind to rod outer segment membranes. In contrast, a mutant farnesylated form of GRK2 is partially associated with the membrane and a mutant geranylgeranylated form of GRK2 is constitutively associated with the membrane (Inglese et al., 1992*b*). Notably, the GRK2 isoprenylation mutants, unlike GRK1, fail to display light-dependent translocation indicating that determinants other than isoprenylation participate in mediating the agonist-dependent translocation of this enzyme. As compared to the native enzyme, the isoprenylated mutants of GRK2 display an enhanced ability to phosphorylate GPCR substrates, demonstrating the importance of membrane localization for GRK2 function.

GRK2 and GRK3 lack a CAAX motif but still rely, interestingly, on isoprenylation for membrane association. The carboxyl-termini of these enzymes bind Gβγ and it is through this protein-protein interaction that GRK2 and GRK3 associate with the membrane (Pitcher et al., 1992) (Fig. 1). The gamma subunit of Gβγ is isoprenylated with the isoprenoid geranylgeranyl, a modification that anchors the Gβγ complex to the plasma membrane. The Gβγ binding region of GRK2 has been mapped to residues 546–670 and a glutathione-s-transferase (GST) fusion protein containing this sequence as well as a 28 amino acid peptide composed of residues 643–670 inhibit the interaction of Gβγ with GRK2 in vitro (Koch et al., 1993).

Originally, Gβγ was shown to enhance the activity of a partially purified preparation of a kinase with similar properties to GRK2 against purified reconstituted muscarinic cholinergic receptors (Haga et al., 1992). In subsequent experiments Gβγ was also shown to enhance GRK2-mediated phosphorylation of the purified reconstituted β2-adrenergic receptor (Pitcher et al., 1992). Notably, Gβγ binding to GRK2 does not enhance the activity of this enzyme towards a soluble peptide substrate (Pitcher et al., 1992; Kim, Dion & Benovic, 1993). These results suggest that the principal mechanism by which Gβγ enhances GRK2-mediated GPCR phosphorylation is by promoting the membrane association of GRK2, thus placing the kinase and its receptor substrate in close proximity (Pitcher et al., 1992). In addition, Gβγ-mediated translocation of GRK3 has also been demonstrated in permeabilized preparations of rat olfactory cilia, a system expressing endogenous levels of kinase (Boekhoff et al., 1994). Agonist-stimulated translocation of GRK3 was blocked by Gβγ sequestrants, a GST fusion protein encompassing the C-terminus of GRK3 (residues 467–688) and a peptide derived from this region (residues 664–670). For GRK2 and 3, Gβγ plays an integral role in mediating the translocation and membrane association of these kinases.

Gβγ may however play an additional role, that of facilitating the interaction of GRK2 and 3 with their receptor substrates. Activated GPCRs, synthetic receptor peptides and mastoparan enhance GRK2-mediated phosphorylation of soluble peptide substrates. That is, the interaction of GRK2 with an activated receptor substrate or an activated receptor substrate analogue results in allosteric activation of the kinase (Haga et al., 1994; Kim et al., 1993). Gβγ synergistically enhances GPCR-mediated activation of GRK2. Although Gβγ binding *per se* does not lead to direct activation of GRK2, Gβγ appears to facilitate the interaction between the GPCR and the GRK promoting the GPCR-mediated allosteric activation of this enzyme.

GRK2 is predominantly a soluble protein that has been shown to translocate in an agonist-dependent manner (Strasser et al., 1986; Daaka et al., 1997). The Gβγ-mediated membrane association of GRK2 observed in vitro provides a model to explain this agonist-dependent translocation. In this model agonist-occupancy of a GPCR leads to the activation of heterotrimeric G proteins and the release of free Gβγ dimer. The Gβγ subsequently interacts with GRK2 and/or GRK3 and serves to target these enzymes to their membrane incorporated receptor substrates. This model has been demonstrated in intact cells using Cos7 cells transiently overexpressing GRK2 (Daaka et al., 1997). Activation of β-adrenergic receptors promotes membrane association of GRK2, a process which is inhibited by overexpressing a carboxyl terminal GRK2 peptide encompassing the Gβγ binding domain. Furthermore, agonist-stimulated formation of a GRK2/Gβγ complex can be directly demonstrated by co-immunoprecipitation (Daaka et al., 1997). Although agonist occupancy of β2-adrenergic and lysophosphatidic acid receptors promotes GRK/Gβγ complex formation for both GRK2 and GRK3, thrombin receptor stimulation specifically induces GRK3/Gβγ complex formation (Daaka et al., 1997). These results suggest differences in Gβγ binding specificity between GRK2 and GRK3.

Isoprenylation plays a central role in mediating the membrane association of GRK1, 2 and 3 either through direct covalent modification of the kinase (GRK1) or

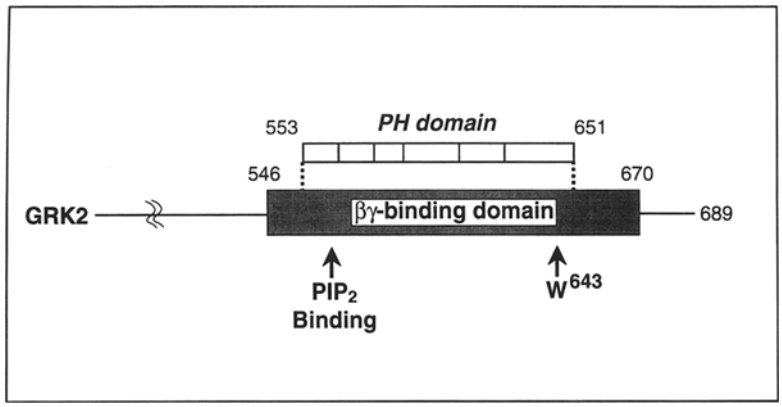

Fig. 2. Carboxyl-terminus of GRK2 mapping the Pleckstrin homology domain (PH) and the βγ binding domain.

through a protein-protein interaction between the kinase and isoprenylated Gβγ (GRK2 and GRK3). Notably, the isoprenyl moiety plays a critical role in conferring agonist-dependence on the membrane association of all three GRKs.

Protein Acylation

GRK4 and GRK6 are not isoprenylated and do not bind Gβγ (Premont et al., 1996). These kinases are however palmitoylated (Stoffel et al., 1994; Premont et al., 1996) (Fig. 1). Palmitoylation is the acylation of a protein with palmitic acid through a thioester or an oxyester bond. Palmitic acid is a 16-carbon saturated fatty acid that affords modified proteins a higher avidity for membranes. The most prevalent form of palmitoylation is the modification of a cysteine residue through a thioester bond. GRK6 contains a cluster of cysteines in its carboxyl terminus at positions 561, 562 and 565 and mutation of these cysteines to serines abolishes the palmitoylation of the kinase (Stoffel et al., 1994). By analogy with GRK6, the probable sites of palmitoylation on GRK4 are the cysteines at residue 561 and 578 (the carboxyl-terminal cysteine residue) (Premont et al., 1996). Palmitoylated GRK4 and 6 are found exclusively associated with membranes in cellular systems (Stoffel, *unpublished;* Stoffel et al., 1994). These results are similar to those previously reported for other palmitoylated proteins (Linder et al., 1993; Robinson et al., 1995). The importance of membrane localization for GRK6 function is demonstrated by the observation that palmitoylated GRK6 is approximately 10-fold more active at phosphorylating the β2-adrenergic receptor in vitro than its nonpalmitoylated counterpart (R.H. Stoffel et al., *in preparation*).

The thioester bond of palmitoylated proteins is extremely labile and this characteristic allows for the removal of palmitic acid from the modified protein. Indeed, several proteins have been shown to undergo cycles of palmitoylation and depalmitoylation following agonist treatment of GPCRs. These include the β2-adrenergic receptor (Mouliac et al., 1992), the α2-adrenergic receptor (Kennedy & Limbird, 1994), nitric oxide synthetase (Robinson, Busconi & Michel, 1995) and the α subunits of heterotrimeric G proteins (Wedegaertner & Bourne, 1994; Degtyarev, Spiegel & Jones, 1993). In the case of $G\alpha_s$, the agonist-dependent loss of palmitic acid parallels the translocation of this protein from the membrane to the cytosol (Levis & Bourne, 1992). The palmitoylation of GRK4 and GRK6 may potentially provide a mechanism for the dynamic regulation of the membrane association of these enzymes.

Regulation of GRK Activity Via the Noncovalent Interaction with Lipid Ligands

The GRKs are lipid-dependent enzymes. Thus agonist-occupied GPCRs serve as GRK substrates only when presented in physiological membranes or alternatively when incorporated into lipid or detergent/lipid micelles (Pitcher et al., 1995; Onorato et al., 1995). A number of lipid ligands for the GRKs have been identified which functionally fall into two categories. The binding of any lipid ligand potently enhances GRK-mediated GPCR phosphorylation by targeting the GRK to the membrane and placing the enzyme and its receptor substrate in close proximity. Additionally, however a subset of lipid ligands directly enhance GRK catalytic activity.

GRK2 and 3 contain within their carboxyl termini an approximately 100 amino acid region of sequence homology termed the Pleckstrin homology (PH) domain (residues 553–651 in GRK2) (Touhara et al., 1994; Touhara et al., 1995) (Fig. 2). PH domains are found in numerous proteins where they form a distinct structural module (reviewed in Cohen, Ren & Baltimore, 1995). Notably, the Gβγ binding site on GRK2 includes and extends slightly beyond the carboxyl-terminus of its constituent PH domain (residues 546–670). As previously described GRK2/Gβγ complex formation promotes GRK2-mediated GPCR phosphorylation. The Gβγ-mediated translocation of GRK2 is observed in cellular

systems or in vitro using receptor substrates reconstituted in heterogeneous ('crude') lipid environments. However, purified GPCRs reconstituted into pure phosphatidylcholine (PC) vesicles fail to serve as GRK2 substrates even in the presence of Gβγ (Pitcher et al., 1995; DebBurman et al., 1996). Furthermore, Gβγ fails to promote the association of GRK2 with pure PC vesicles (Pitcher et al., 1995). These results suggest that GRK2/Gβγ complex formation requires the presence of a lipid cofactor. Indeed, low concentrations (<10 μM) of the lipid phosphatidylinositol-4,5-bisphosphate (PIP2) have been shown to specifically promote GRK2/Gβγ complex formation and thereby GRK2-mediated GPCR phosphorylation (Pitcher et al., 1995; DebBurman et al., 1996). PIP2 binds to the amino terminus of the GRK2 PH domain in vitro (Harlan et al., 1994). Thus the coordinated binding of two ligands (PIP2 and Gβγ) to respectively, the amino and carboxyl-termini of the PH domain of GRK2, is required for the effective membrane localization and function of this enzyme. The concentrations of PIP2 that promote GRK2/Gβγ-mediated GPCR phosphorylation approximate to those believed to be physiologically relevant and have no direct effect on GRK2 catalytic activity. Notably, high concentrations of PIP2 (>200 μM) directly inhibit the catalytic activity of GRK2. This inhibitory effect of PIP2 is however, unlikely to be of significance in vivo since the concentrations of PIP2 required are approximately 20-fold higher than those believed to be physiologically relevant. Additionally, the inhibition of GRK2 activity observed at these high concentrations is mediated, at least partially, via binding to the ATP binding site of GRK2 and not the PH domain (DebBurman et al., 1996).

Lipids other than PIP2 also play a role in regulating GRK2 activity. Thus negatively charged phospholipids such as phosphatidylserine (PS) enhance GRK2-mediated GPCR phosphorylation (DebBurman et al., 1995; Onorato et al., 1995). Several characteristics distinguish the enhanced GRK2 activity observed in the presence of these negatively charged phospholipids from that observed in the presence of PIP2. Firstly, PS-enhanced GRK2 activity requires the presence of approximately 10-20-fold higher lipid concentrations. Since the physiological mol fraction percent for PS is estimated at approximately 10% as compared to 1–3% for PIP2 however, both lipid regulators would be predicted to of physiological relevance. A second feature distinguishing regulation by these two lipids concerns their mechanism of action. Thus in contrast to PIP2, PS directly increases the catalytic activity of GRK2 (Onorato et al., 1995; DebBurman et al., 1996). PS has been shown to bind to the carboxyl-terminus of GRK2 however unlike PIP2, the PH domain of this enzyme has not been directly implicated as the site of interaction (DebBurman et al., 1996). In light of the differences between the binding characteristics of PS and PIP2 it is interesting to speculate that they bind to distinct carboxyl terminal sites. It should be noted, however, that as for PIP2 (although at approximately 10-20-fold higher lipid concentrations) a synergistic enhancement of GRK2-mediated GPCR phosphorylation has been reported in the presence of PS and Gβγ (DebBurman et al., 1996).

GRK5 does not contain a sequence for isoprenylation (Benovic et al., 1994) and does not bind Gβγ (Premont et al., 1994). GRK5 is however a membrane-associated enzyme. When transiently overexpressed in Cos7 cells GRK5 is found exclusively associated with the plasma membrane (Premont et al., 1994). Additionally, purified GRK5 binds to ROS membranes, an association that is not effected by light activation of rhodopsin (Premont et al., 1994). Thus GRK5 constitutively associates with the membrane in an agonist-independent fashion. In vitro, GRK5 associates with liposomes composed of crude lipid mixtures (Kunapuli et al., 1994), but not purified phosphatidylcholine (PC) (Pitcher et al., 1996). Thus, the association of GRK5 with the membrane is not dependent upon receptor activation or the presence of receptor but rather upon specific lipid binding determinants present on the kinase.

Two distinct lipid binding domains have been identified on GRK5, one in the amino and one in the carboxyl terminus of the enzyme. The amino-terminal lipid binding domain exhibits a high degree of specificity for PIP2. GPCRs reconstituted in pure PC vesicles fail to serve as substrates for GRK5 (Pitcher et al., 1996). However, the incorporation of physiological concentrations of PIP2 into the PC vesicles, restores the ability of these kinases to associate with membranes and phosphorylate GPCRs. As is the case for PIP2-mediated activation of GRK2, PIP2 has no direct effect on the catalytic activity of GRK5 (Pitcher et al., 1996). Binding of PIP2 to GRK5 thus enhances GPCR phosphorylation by promoting the membrane localization of this kinase. Mapping the PIP2 binding site localizes it to the amino terminus of GRK5 (Fig. 1), and specifically to a region that has homology with the PIP2 binding site of gelsolin. Notably, GRK5, 4, and 6 are highly homologous across this region of sequence and indeed all these enzymes display PIP2-mediated membrane association and enhanced GPCR phosphorylation (Pitcher et al., 1996). PIP2 thus plays a role in mediating the membrane association of GRK2, 3, 4, 5 and 6. However, the structure, location and regulation of the PIP2 binding site distinguishes GRK2 and 3 from GRK4, 5, and 6.

GRK5 exhibits phospholipid stimulated autophosphorylation to a stoichiometry of approximately 2 mole Pi/mole enzyme (Kunapuli et al., 1994). A GST fusion protein encompassing the carboxyl terminus of GRK5 (residues 489–590) blocks this lipid effect, indicating the presence of a lipid binding site in this region of the

kinase (Kunapuli et al., 1994). Although lipid binding promotes autophosphorylation of GRK5, autophosphorylation of GRK5 does not effect lipid binding. Thus an autophosphorylation mutant inhibits the phospholipid-stimulated autophosphorylation of the native enzyme (Kunapuli et al., 1994). The binding characteristics of the carboxyl terminal lipid binding site have been largely characterized using PS as the lipid ligand (Pitcher et al., 1997). In the absence of additional lipid ligands, i.e., using as substrates GPCRs reconstituted in vesicles composed of only PC and PS, PS promotes membrane association of GRK5 and GRK5-mediated GPCR phosphorylation. PS binding requires the presence of a number of basic residues in the carboxyl terminus of GRK5 (residues 547, 548, 553, 556 and 557), localizing the site of PS interaction and distinguishing it from that of PIP2 (J.A. Pitcher et al., *in preparation*). Notably, PS-mediated activation of GRK5 requires approximately 10-fold higher lipid concentrations than that required for PIP2-mediated activation of the enzyme. Furthermore, unlike PIP2, PS binding directly increases the catalytic activity of GRK5. Interestingly, the PS-dependent increase in the catalytic activity of the enzyme is dependent on the presence of intact autophosphorylation sites (J.A. Pitcher et al., in preparation). Thus an autophosphorylation mutant of GRK5 although capable of binding PS is not directly activated by this lipid. The observation that the catalytic activity of the autophosphorylation deficient mutant of GRK5 is not enhanced by PS may provide an explanation for the observations of Kunapuli et al., demonstrating an impaired ability of the autophosphorylation mutant of GRK5 to phosphorylate receptor substrates (Kunapuli et al., 1994). In the absence of lipid activators native GRK5 and the autophosphorylation deficient mutant exhibit equivalent abilities to phosphorylate a soluble peptide substrate (Kunapuli et al., 1994). The inability of PS to directly activate the autophosphorylation deficient mutant of GRK5 would specifically impair the ability of this kinase to phosphorylate lipid incorporated i.e., receptor substrates. Interestingly, synergistic enhancement of GRK5 activity is observed in the presence of PIP2 and PS (J.A. Pitcher et al., *in preparation*). Thus binding of PS to a carboxyl-terminal region of GRK5 facilitates binding of PIP2 to the amino terminus (J.A. Pitcher et al., *in preparation*). GRK5's mechanism for membrane association is clearly distinct from that of GRK1, 2 and 3. GRK5's constitutive association with the membrane creates a population of kinase in close proximity to its receptor substrates, a proximity that potentially allows for a more rapid kinase response following agonist-occupancy of G protein-coupled receptors.

N-terminal Interacting Proteins

Recently, a new feature of the GRKs has come to light. Significant amounts of GRK2 have been shown to be associated with several membrane fractions after differential centrifugation (Garcia-Higuera et al., 1994). The binding of GRK2 to these fractions is reversible. Membranes treated with proteinase K or boiling were unable to bind GRK2 indicating that the association was through a protein-protein interaction (Garcia-Higuera et al., 1994). GRK2 was found to bind to this protein with nanomolar affinity. The highest specific activity for this interacting protein was in the microsomal membrane fraction. The binding of GRK2 to microsomal membranes was blocked with the addition of a GST fusion protein containing residues 50–145 of the amino terminus of the kinase and not a fusion protein containing the carboxyl-terminus of the kinase (Murga et al., 1996). This excludes the possibility that the microsomal membrane binding protein is $G\beta\gamma$. Addition of microsomal membranes, containing this protein, inhibited the activity of GRK2 when rhodopsin was used as a substrate and the inhibition was relieved by addition of aluminum fluoride or $GTP\gamma S$ plus mastoparan. This finding allows for the potential regulation of GRK2 activity through a GTP dependent mechanism; either through the release of G protein $\beta\gamma$ subunits after activation of $G\alpha$ or potentially through GTP binding to the inhibitory anchoring protein. These findings raise the possibility that additional as yet unidentified proteins direct the GRKs to distinct subcellular compartments and potentially to different receptor substrates.

Conclusion

Membrane association is essential for GRK function and because of this the GRKs have evolved complex regulatory mechanisms for associating with the membrane. Although the GRKs are highly homologous, each kinase utilizes a distinct mechanism for associating with the membrane, which makes it unique within the family. Initially, the carboxyl terminus of the GRKs was identified as the "membrane association domain" but recent evidence suggests that the amino terminus may also play a critical role in localizing the kinases to the membrane (Murga et al., 1996; Pitcher et al., 1996). It is within these two domains that the GRKs are most variable at the amino acid level. The GRKS exhibit an absolute requirement for phospholipids not only for association with the membrane but also for activity. There are differences in preference and binding sites for the phospholipids within the GRK family, which may reflect differential targeting of the GRKs to G protein-coupled receptors situated in different lipid environments. There are hundreds of G protein-coupled receptors and only six known GRKs. All the GRKs appear to phosphorylate the same receptor substrates in vitro (Sterne-Marr & Benovic, 1995; Premont et al., 1995). Receptor specificity, in a cellular

environment could be mediated via targeting proteins, specific lipid concentrations or direct lipid modifications that target the GRKs to different subcellular compartments and different G protein-coupled receptors.

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