

Mitogenic Signaling via G Protein-Coupled Receptors

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I. Introduction

RECEPTORS coupled to heterotrimeric GTP-binding proteins (G proteins) comprise the largest known family of cell surface receptors and mediate cellular responses to a diverse array of signaling molecules, including peptide and glycopeptide hormones, neurotransmitters, phospholipids, odorants, and photons. The basic unit of G protein-coupled receptor (GPCR) signaling is comprised of three parts; receptor, which detects ligand in the extracellular milieu; heterotrimeric G protein, which is dissociated into active $G\alpha$ -GTP and $G\beta\gamma$ -subunits after interaction with the liganded receptor; and effector, which interacts with dissociated $G\alpha$ -GTP and $G\beta\gamma$ -subunits to mediate the intracellular effects of ligand binding. In a given cell type, the responsiveness to stimulus, as well as the nature of the response, is dictated by the available complement of receptor, G protein, and effector.

Despite the diverse array of ligands with which they interact, GPCRs share a conserved predicted tertiary structure characterized by seven transmembrane domains joined by intracellular and extracellular loops. G protein coupling specificity is dictated by the intracellular domains, particu-

larly the second and third intracellular loops and the C-terminal tail. Indeed, it is possible to alter the G protein coupling specificity of GPCRs by exchanging relatively small regions within the third intracellular loop (1).

Heterotrimeric G proteins, whose crystal structure has recently been reported (2–5), consist of three heterologous subunits (α , β , and γ) that are stably associated only in the inactive, GDP-bound, state. Noncovalent interaction between the G protein and an agonist-occupied receptor leads to the exchange of GDP for GTP on the α -subunit and its subsequent dissociation from the stable $\beta\gamma$ -dimer, resulting in the generation of two units capable of regulating intracellular signaling, $G\alpha$ -GTP, and $G\beta\gamma$. The receptor-G protein activation is catalytic; that is, one receptor may activate many G proteins before it is itself inactivated by phosphorylation-dependent desensitization. The intrinsic GTPase activity of the $G\alpha$ -subunit mediates the rate-limiting hydrolysis of GTP to GDP and the subsequent reassociation of the $G\alpha\beta\gamma$ -complex, thereby inactivating the G protein.

G protein α -subunits have been divided into subgroups based in part on their effector interactions. Gs proteins stimulate adenylyl cyclase; Gi/o family members are sensitive to inactivation by pertussis toxin (PTX) and often mediate inhibition of adenylyl cyclase. Gq/11 proteins are PTX insensitive and frequently couple receptors to activation of phospholipase C (PLC) isoforms.

In addition to $G\alpha$ -GTP, G protein $\beta\gamma$ -subunits are involved in effector regulation, although little is known about the specificity of $G\beta\gamma$ -effector interactions. Examples of effectors that have been shown to be regulated by $G\alpha$ -GTP and/or $G\beta\gamma$ -subunits include adenylyl cyclase, phospholipase C and A_2 isoforms, serine/threonine kinases, protein tyrosine kinases (6, 7), and many others.

II. G Proteins and GPCRs in Disease

While the role of activating mutations in tumor promoter genes, and inactivating mutations in tumor suppressor genes, has been the subject of intense investigation for some time, the clinical relevance of GPCR-signaling systems in regulating cell growth and differentiation has only recently been appreciated.

Our current understanding of the processes regulating cell growth, division, and differentiation has been derived largely from the study of transforming viral oncogenes and their cellular homologs, as well as the related pathways that regulate fruitfly, yeast, and nematode development. Much of

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the work has focused on the role of receptor and nonreceptor protein tyrosine kinases as regulators of low molecular weight G proteins, such as p21ras and its relatives (8, 9). The low molecular weight G proteins, like their heterotrimeric counterparts, are signaling intermediates whose activation is achieved by the exchange of GDP for GTP. Unlike the heterotrimeric G proteins, the low molecular weight G proteins lack the intrinsic ability to catalyze GDP release and GTP hydrolysis and are therefore dependent on exogenous proteins, GEFs (guanine nucleotide exchange factors), GDIs (GDP dissociation inhibitors), and GAPs (GTPase activating proteins), for their regulation (10). Recruitment of these regulators of low molecular weight G protein function is, in many cases, directed by specific protein-protein interactions controlled by tyrosine phosphorylation.

Nonetheless, naturally occurring activating and inactivating mutations of both GPCRs and heterotrimeric G proteins are associated with several disease states (11, 12). Mutations resulting in the constitutive activation of the TSH receptor result in hyperfunctioning thyroid adenomas with hyperthyroidism (13). Similarly, a rare autosomal disease known as familial precocious puberty results from an activating mutation in the receptor for LH (14). The discovery of these naturally occurring mutations were anticipated by early findings that constitutively active α_{1B} , β_2 , and α_{2A} -adrenergic receptors generated *in vitro* lead to an increase in the basal activity of G protein-mediated signaling pathways (15–17). An activated mutant of the α_{1B} -adrenergic receptor has been shown to exhibit agonist-independent transforming properties in NIH 3T3 cells, thus predicting the oncogenic potential of constitutively activated GPCRs (18).

The function of heterotrimeric G proteins as oncogenes has recently been reviewed in detail (19). Activating mutations of G α s, which increase the basal activity of downstream effectors (20, 21), have been recovered from human pituitary and thyroid adenomas (20, 22–26). Constitutively active mutants of Gs are associated with McCune-Albright syndrome, a disease characterized by polyostotic fibrous dysplasia and hyperfunction of multiple endocrine glands (14) and have been found as somatic mutations in pituitary somatotrophs and thyroid cells. Many of these gain-of-function mutations localize to the GTPase domain of the G α -subunit where they inhibit the ability of G α to hydrolyze GTP to GDP, preventing its inactivation and reassociation with the $\beta\gamma$ -complex.

III. Growth-Promoting Effects of GPCRs

Several ligands that signal via GPCRs have been shown to elicit mitogenic responses, including stimulation of DNA synthesis, expression of nuclear oncogenes, and cell proliferation. In Chinese hamster lung fibroblasts, DNA synthesis stimulated by thrombin, but not by platelet-derived growth factor (PDGF), is blocked by PTX treatment, implicating PTX-sensitive Gi/o proteins in mitogenic signaling (27). The proliferative effects of serum have been shown to be due primarily to PDGF and lysophosphatidic acid (LPA), the simplest naturally occurring phospholipid (28). PDGF activates a receptor tyrosine kinase (RTK), while LPA mediates

intracellular signaling via both PTX-sensitive and -insensitive G proteins (28). Both PTX-sensitive and -insensitive proliferative responses have been observed in cells treated with adenosine (29), substance P and substance K (30), thrombin (30–32), and bombesin (33, 34).

The ability of LPA to stimulate DNA synthesis in Rat-1 fibroblasts is completely inhibited by PTX treatment, demonstrating the involvement of Gi/o proteins in cellular mitogenesis. In NIH 3T3 cells, the serotonin 5HT1b receptor mediates a PTX-sensitive increase in the rate of DNA synthesis (35), and serotonin 5HT1c receptors increase focus formation in an agonist-dependent manner (36). Cells isolated from 5HT1c-transformed foci form tumors when injected into nude mice, indicating that these receptors function as ligand-dependent protooncogenes in fibroblasts. In contrast, the Gi/o-coupled m2 and m4 acetylcholinergic receptors (AChRs) fail to induce transformation in these cells (37). The mitogenic effects of serotonin are synergistic with those of PDGF (38).

In human diploid fibroblasts, the Gq/11-coupled bradykinin receptor does not elicit a proliferative response (28). In contrast, the m1, m3, and m5 subtypes of the AChRs, which also couple to PTX-insensitive Gq/11 proteins, increase the rate of formation of transformed foci in an agonist-dependent manner in NIH 3T3 cells (37). Diversity must therefore exist both within and between cell types, in the mechanisms whereby GPCRs transduce mitogenic signals.

IV. Regulation of Mitogen-Activated Protein (MAP) Kinases by GPCRs

The ubiquitous MAP kinases comprise a family of serine/threonine kinases that are involved in the transduction of externally derived signals regulating cell growth, division, and differentiation. Depending upon the cellular context, activated MAP kinases may mediate pleiotropic responses in the membrane, cytoplasm, nucleus, or cytoskeleton. Upon activation, MAP kinases translocate to the nucleus, where they phosphorylate and activate nuclear transcription factors involved in DNA synthesis and cell division (39).

The first detected and best studied of the MAP kinases are the extracellular signal-regulated kinases (ERK) 1 and 2 (p44^{mapK} and p42^{mapK}). Regulation of ERK1 and ERK2 occurs via an evolutionarily conserved kinase cascade related to signaling pathways found in organisms as divergent as fruit-flies and yeast (40). The proximal kinases in the mammalian ERK1/2 pathway, Raf-1 and B-Raf [MAP kinase kinase (MAPKKK)], phosphorylate and activate MEK1 and MEK2 [MAP kinase kinase (MAPKK)]. The dual function threonine/tyrosine MEKs, in turn, mediate the activation of ERK1 and ERK2 (MAPK).

Other members of the mammalian MAP kinase family include two ERK3 isozymes, ERK5 (41), three distinct Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPK), two p38 MAP kinases, and p57 MAP kinases (40). Although all of the intermediates are not yet fully characterized, regulation of each of these kinases occurs via a MAPKKK, MAPKK, MAPK phosphorylation cascade that reprises the ERK1/2 paradigm. Thus, cells contain a series of parallel

MAP kinase-signaling cascades, potentially under independent regulation, each of which can initiate a pattern of regulated gene expression.

A. Extracellular-signal regulated kinases (ERKs)

Activation of the ERK1/2 cascade by peptide growth factors such as epidermal growth factor (EGF), PDGF, and fibroblast growth factor (FGF) has been studied extensively (9). The EGF, PDGF, and FGF receptors are single transmembrane domain proteins that possess intrinsic ligand-stimulated tyrosine kinase activity. Upon ligand binding, these RTKs dimerize and transphosphorylate on their cytoplasmic domains. The resulting phosphotyrosine residues serve as docking sites to recruit components of the mitogenic signaling complex to the receptor.

Recruitment of adapter proteins, such as Shc and Grb2, to the phosphorylated RTK serves to assemble a multiprotein complex for regulating the low molecular weight G protein p21ras. The p21ras guanine nucleotide exchange factor Sos1 is stably associated with Grb2; thus recruitment of Grb2 brings Sos1 to the cytoplasmic surface of the membrane where it activates p21ras by catalyzing GDP for GTP exchange. GTP-bound p21ras initiates the ERK1/2 kinase cascade by activating the Raf-1 serine/threonine kinase, followed by the sequential phosphorylation and activation of the MEK and ERK1/2 kinases (Fig. 1).

The assembly of this membrane-associated mitogenic signaling complex is dependent upon modular domains in the component proteins, which dictate the specificity of protein-protein interactions. Src-homology 2 (SH2) domains, which are present in both the Shc and Grb2 adapter proteins, mediate high-affinity binding to specific phosphotyrosine (pTyr) residues. In addition to its C-terminal SH2 domain, Shc has a second structurally unrelated pTyr-binding (PTB)

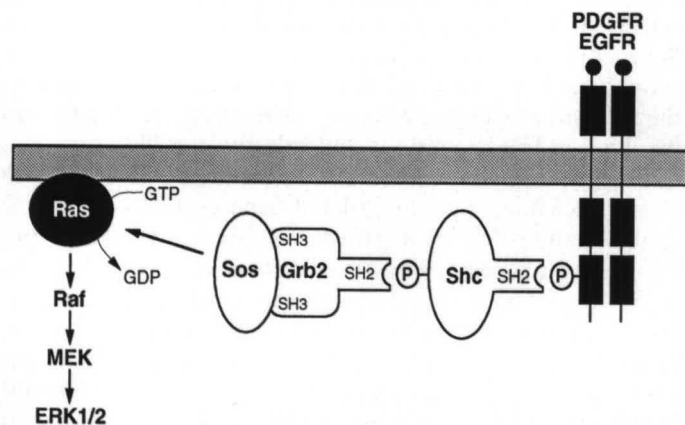


FIG. 1. The activation of the p21ras/ERK1/2 signaling pathway by RTKs. The binding of ligand to the extracellular domain of RTKs, such as the EGF and PDGF receptors, leads to receptor dimerization and autophosphorylation. The resulting phosphotyrosine residues on the intracellular domain of the RTK serve as high-affinity binding sites for SH2 domain-containing proteins such as the Shc and Grb2 adapter molecules. Grb2, in turn, recruits the Sos1 guanine nucleotide exchange factor into the signaling complex via an SH3 domain-mediated interaction. Sos1 catalyzes the exchange of GDP for GTP on p21ras, leading to its activation. GTP-bound p21ras binds to and activates Raf kinase, thereby initiating a kinase cascade resulting ultimately in the stimulation of ERK1/2 activity.

domain at its N terminus, which dictates receptor-Shc interaction. SH3 domains bind with high affinity to proline-rich regions of proteins. The SH3 domain of Grb2 mediates its binding to the C terminus of the Sos1 guanine-nucleotide exchange factor. Other components of the complex, including Sos and Ras-GAP, contain pleckstrin-homology (PH) domains, which have been shown to bind to both $G\beta\gamma$ -subunits (42) and phospholipids (43–45).

As with the RTKs, activation of the ERK1/2 cascade by GPCRs signaling via PTX-sensitive and -insensitive G proteins has been reported for a broad array of ligands. These include angiotensin II (46), somatostatin (47), endothelin-1 (48–51), thromboxane A₂/prostaglandin H₂ (52, 53), interleukin-8 (54), platelet-activating factor (PAF) (55), bombesin (56), formyl-methionyl peptide (fMLP) (57), C5a peptide (58), TRH (59), sphingosine-1-phosphate (60–62), oxytocin (63), and LHRH (64). Despite observations that GPCRs can mediate growth factor-like effects, the mechanisms by which these receptors mediate ERK activation were not studied in detail until recently. Since GPCR signals are transduced via heterotrimeric G proteins, it is useful to consider the mechanisms whereby the different $G\alpha$ - and $G\beta\gamma$ -subunits communicate with the ERK1/2 cascade. The mechanism employed by each receptor is determined by the G protein(s) with which it interacts and the available effectors in a particular cell type.

1. *G_s*. Constitutively active mutants of the *G_s*-coupled TSH receptor have been isolated from hyperfunctioning thyroid adenomas (13), suggesting a role for *G_s* in the regulation of cell growth. In COS-7 cells, stimulation with isoproterenol, a β -adrenergic agonist, results in activation of cAMP-dependent protein kinase A and ERK1/2 (65). Similar effects were reported for transiently expressed LHRH receptor and constitutively activated *G_s* in these cells (66).

Despite these observations, the role of *G_s* in regulation of cell growth is complex and apparently cell type-specific. In fibroblasts, cAMP inhibits RTK-mediated ERK1/2 activation (67–69). Forskolin-treated NIH 3T3 cells show decreased responsiveness to EGF and LPA (70), reflecting reduced activation of Raf, MEK, and ERK, but not p21ras (71–73). Increased cAMP levels are associated with increased phosphorylation of Ser-43 of the Raf-regulatory domain, resulting in reduced affinity of Raf for p21ras (67). ERK activity in p21ras-transformed Rat-1 fibroblasts is inhibited by treatment with 8-bromo-cAMP, consistent with cAMP-mediated inhibition at a step downstream of p21ras (68). In contrast, Faure and Bourne (74) have observed a lack of forskolin-mediated ERK inhibition in Swiss 3T3 and COS-7 cells while others have reported an activating role for cAMP on ERK activation in PC12 cells (75, 76). This lack of inhibition apparently results from differential sensitivity of Raf-1 and B-Raf to cAMP-mediated inhibition and suggests the existence of a Raf-1 independent, cAMP-insensitive pathway in these cells.

Crespo *et al.* (65) have proposed that, in COS-7 cells, *G_s*-coupled receptors mediate simultaneous, opposing effects. They suggest that the $G\beta\gamma$ -subunits derived from *G_s* mediate ERK1/2 activation, while cAMP generated by *G_s*-mediated activation of adenylyl cyclase opposes this effect. Thus, the

nature of the cellular response to β -adrenergic agonists in these cells is determined by the balance between two opposing signals.

2. Gi

Constitutively active Gai2, gip2: A direct role for Gai in oncogenesis was first postulated when a constitutively active, GTPase-deficient mutant of Gai2, gip2, was isolated from several human endocrine tumors and subsequently shown to induce neoplastic transformation of Rat-1 fibroblasts (77). NIH 3T3 cells stably transfected with gip2 demonstrate an increased rate of DNA synthesis (78), but gip2 does not cause complete transformation of NIH 3T3 fibroblasts, suggesting that its oncogenic potential is tissue specific (77, 79).

The mechanism by which gip2 transmits mitogenic signals remains unclear. p21ras Activation is apparently not required for gip2-mediated cellular proliferation (79, 80), suggesting that the mitogenic signaling pathway differs from that mediated by RTKs. The most readily detectable effect of gip2 expression is a sustained inhibition of adenylyl cyclase activity (81), resulting in a decreased basal level of cAMP.

Since cAMP opposes ERK1/2 activation in many cell types, the observation that the gip2 oncogene transforms Rat-1 fibroblasts may reflect its constitutive inhibition of adenylyl cyclase. This model predicts that decreased intracellular levels of cAMP would relieve cAMP-mediated inhibition of Raf and permit increased ERK activity.

Agonist-stimulated mitogenic signaling through Gi: p21ras-Dependent activation of ERK1/2 via Gi-coupled receptors was initially described by three laboratories (82–85). The receptors for LPA and α -thrombin mediate activation of p21ras (82), Raf-1, and ERK1/2 (83). The signals are PTX-sensitive, indicating the involvement of a Gi/o family heterotrimeric G protein. Stimulation of the α_{2A} -adrenergic receptor (α_{2A} AR), which is specifically coupled to Gi2 and Gi3, leads to rapid and transient activation of p21ras and ERK in Rat-1 fibroblasts (84). These responses are unrelated to inhibition of adenylyl cyclase and dissociable from PLC activation. Carbachol stimulation of Rat-1 cells expressing the Gi-coupled M₂AChR leads to the PTX-sensitive activation of p21ras, Raf, MEK, and ERK (85). The p21ras dependence of these signals distinguishes them from gip2-mediated cell transformation (79, 80), suggesting, instead, the existence of a distinct pathway with at least some features in common with RTK-mediated mitogenic signaling.

The analogy to RTK signaling was strengthened by the finding that LPA-mediated p21ras and ERK activation is sensitive to inhibition by low concentrations of the tyrosine kinase inhibitor genistein (82). Subsequent experiments revealed that LPA stimulation of Rat-1 cells increases tyrosine phosphorylation of several proteins (86). TRH (59), endothelin (87), angiotensin II (88), thrombin (89), LPA, and α_{2A} -adrenergic receptors (90, 91) stimulate tyrosine phosphorylation of the Shc adapter protein and its subsequent association with Grb2. These data suggest that RTK- and Gi-mediated mitogenic signals converge at or above the level of the pTyr-dependent Shc-Grb2 interaction.

Chemoattractant receptors, such as the fMLP receptor, also activate the Gi-mediated p21ras-dependent ERK pathway

(57). In human polymorphonuclear leukocytes, fMLP activates p21ras, Raf, and ERK in a PTX-sensitive manner. Consistent with the findings in fibroblasts and Chinese hamster ovary (CHO) cells, treatment of neutrophils with inhibitors of protein kinase C does not affect fMLP receptor-mediated ERK1/2 activation. Recently, fMLP was also shown to stimulate PTX-sensitive tyrosine phosphorylation of Shc as well as the association of Shc with Lyn, a Src family non-RTK (92).

The mounting evidence that Gi-coupled receptors can mediate increases in tyrosine protein phosphorylation and p21ras-dependent activation of ERK1/2 contrasts with the lack of effect on these pathways in cells expressing the activated Gai mutant, gip2. Indeed, Gi-coupled receptor-mediated ERK1/2 activation is not attributable to known effectors of G proteins, such as inhibition of adenylyl cyclase, PLC activation, or modulation of ion channels (82, 84). The resolution of this apparent paradox seems to reside in a novel signaling pathway regulated by G $\beta\gamma$ -subunits.

3. G protein $\beta\gamma$ -subunits. G protein $\beta\gamma$ -subunits were originally thought to function as structural proteins required for the membrane localization and inactivation of G α -subunits. Appreciation of the role of G $\beta\gamma$ -subunits as regulators of GPCR effector molecules has developed more recently (6). G $\beta\gamma$ -subunits are now known to directly regulate an array of effector molecules, among them β -adrenergic receptor kinase (β ARK1) (93, 94), PLC- β (95–105), phospholipase A₂ (106), types II and IV adenylyl cyclases (107, 108), phosducin (109), muscarinic K⁺ channels (110–112), PH domain-containing tyrosine kinases (7, 113), phosphatidylinositol-3 kinase (PI3K) (114), and N-type Ca⁺⁺ channels (115, 116).

Evidence that G-protein $\beta\gamma$ -subunits are capable of transmitting mitogenic signals initially came from three laboratories (66, 117, 118). In COS-7 cells, Crespo *et al.* (117) found that ERK1/2 activation via both the PTX-sensitive G protein-coupled M₂AChR and PTX-insensitive G protein-coupled M₁AChR receptors was attenuated by coexpression of the α -subunit of transducin, which acts to sequester G $\beta\gamma$ -subunits released upon stimulation from endogenous G proteins. Koch *et al.* (118) performed similar experiments using the carboxy-terminal fragment of the β ARK1 enzyme (β ARKct), which contains the G $\beta\gamma$ -binding domain of the kinase (119), as a sequestrant of free G $\beta\gamma$ -subunits. Expression of β ARKct significantly inhibits ERK1/2 activation by the Gi-coupled α_{2A} AR and M₂AChR in transiently transfected COS-7 cells. Similarly, β ARKct overexpression antagonizes activation of both p21ras and ERK1/2 via the endogenous LPA receptor. Since a constitutively active mutant of Gai2 did not increase ERK activity in COS-7 cells (66), the mitogenic signals mediated by Gi-coupled receptors appear to be entirely dependent on a G $\beta\gamma$ -mediated pathway. Furthermore, transient transfection of G β and G γ cDNAs into COS-7 cells results in a sustained increase in the basal level of ERK activity, indicating that G $\beta\gamma$ -subunits alone are sufficient for ERK activation (66, 117).

The $\beta\gamma$ -stimulated ERK activation pathway. Expression of constitutively active G α_q , unlike the Gai mutant, is sufficient to induce activation of ERK1/2 in COS-7 cells (66), indicating that Gq-coupled receptors activate additional signaling pathways. To differentiate the Gi- and Gq-mediated mitogenic

signaling pathways in COS-7 cells, Hawes *et al.* (120) compared the effects of Gi- and Gq-coupled receptors on both PI hydrolysis and ERK1/2 activation. The Gi-coupled α_{2A} AR signals, but not those mediated by the Gq-coupled α_{1B} AR and M₁AChR, were sensitive to inhibition by the β ARKct peptide, suggesting that the Gq/11-coupled receptors mediate ERK1/2 activation, as well as PI hydrolysis, via a predominantly G α -mediated mechanism.

Coexpression of combinations of G β and G γ cDNAs in COS-7 cells revealed that those pairs known to form functional G $\beta\gamma$ -complexes ($\beta 1\gamma 1$, $\beta 1\gamma 2$, $\beta 1\gamma 3$, and $\beta 2\gamma 2$) (121–124) stimulated both ERK1 and PI hydrolysis (120). Both processes were abrogated by coexpressed β ARKct peptide. Dominant negative mutants of p21ras (N17Ras) and Raf (N Δ Raf) specifically inhibited G $\beta\gamma$ -mediated ERK activation, without affecting PI hydrolysis. Thus, expression of G $\beta\gamma$ -subunits was sufficient to mimic Gi-coupled receptor-mediated activation of p21ras, Raf, and ERK (82, 84, 85).

Shc-Grb2-Sos complex formation. The effect of G $\beta\gamma$ -subunits on ERK1 activation can be differentiated from their effect on PI hydrolysis using inhibitors of protein tyrosine kinases (PTKs) (120). The PTK inhibitors genistein and herbimycin A attenuate G $\beta\gamma$ -stimulated ERK activation in a dose-dependent manner, with no effect on G $\beta\gamma$ -stimulated inositol phosphate production. The PTK inhibitors have no effect on ERK activation mediated by a constitutively active mutant of p21ras (T24Ras) (125), indicating that the inhibitor-sensitive step lies upstream of p21ras. Thus, activation of PLC by G $\beta\gamma$ -subunits is not sufficient to account for activation of the ERK pathway. Rather, G $\beta\gamma$ -subunits appear to require the activity of a PTK to mediate ERK activation, supporting the hypothesis that Gi-coupled receptors activate mitogenesis by elevating the intracellular level of tyrosine kinase activity (86).

In the RTK-mediated ERK pathway, tyrosine phosphorylation of the Shc adapter protein allows it to serve as a docking protein for the assembly of the mitogenic signaling complex that leads to p21ras activation. The G protein-coupled endothelin (87), LPA (90), α_{2A} AR (42), angiotensin II (88), thrombin (89), and TRH (59) receptors mediate an agonist-dependent increase in the tyrosine phosphorylation of the Shc adapter protein. LPA- and α_{2A} AR-mediated Shc tyrosine phosphorylation is sensitive to both PTX and the β ARKct peptide and can be mimicked by expression of G $\beta\gamma$ -subunits.

The tyrosine phosphorylation of Shc is accompanied by a simultaneous increase in complex formation between Shc and Grb2 (90, 88). Shc-Grb2 complex formation is rapid and transient, preceding the maximum level of ERK activation. Since Grb2 and Sos1 are constitutively associated, the G $\beta\gamma$ -dependent recruitment of Grb2 to tyrosine-phosphorylated Shc results in increased Shc-associated guanine-nucleotide exchange factor activity (90).

These findings suggest that GPCRs and RTKs can induce p21ras activation via convergent signaling pathways (Fig. 2). Dominant negative mutants of mSos1 demonstrate a requirement for Sos1 in G $\beta\gamma$ -mediated ERK activation (90). When expressed in COS-7 cells, a peptide derived from the proline-rich C terminus of mSos1, containing the Grb2 binding site (Sos-Pro), competitively inhibits the formation of functional

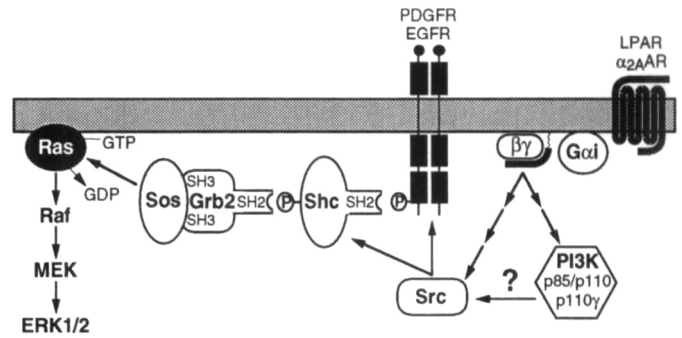


FIG. 2. ERK1/2 activation by G protein $\beta\gamma$ -subunits. Gi-coupled receptors, such as the LPAR and α_{2A} AR, stimulate ERK1/2 activity via a mechanism that is mediated entirely by G protein $\beta\gamma$ -subunits and which, in part, reprises the RTK paradigm (see Fig. 1). G $\beta\gamma$ stimulates c-Src kinase activity, resulting in the phosphorylation of a variety of cellular substrates, including the Shc adapter protein and RTKs, thereby activating the signaling pathway described in Fig. 1. The nature of the involvement of RTKs in G $\beta\gamma$ -mediated ERK1/2 activation remains unclear. Furthermore, c-Src activation by G $\beta\gamma$ occurs via a poorly understood mechanism that appears to involve PI3K activity and possibly an as yet unidentified effector protein.

Shc-Grb2-Sos1-signaling complexes. In cells stimulated by EGF, G $\beta\gamma$, or the Gi-coupled α_{2A} AR, Sos-Pro expression completely inhibited ERK activation, whereas mitogenic signaling by constitutively active T24Ras was unaffected (90). Thus, G $\beta\gamma$ -subunits apparently direct the assembly of a p21ras-activation complex, at the plasma membrane, which contains many of the intermediates employed by the RTKs and which is dependent upon G $\beta\gamma$ -subunit-mediated tyrosine phosphorylation.

PTKs. This early convergence of signaling pathways represents a previously unappreciated degree of cross-talk between the RTK and GPCR families of receptors. In each case, receptor activation leads to increased tyrosine phosphorylation. The intrinsic tyrosine kinase activity of the RTKs is required for their ability to signal. The identity of the tyrosine kinase(s) involved in GPCR signaling remains unclear, although data suggest that both RTKs and non-RTKs may be involved.

Recently, Linseman *et al.* (126) demonstrated that angiotensin II and PDGF activate a common mitogenic signaling cascade in vascular smooth muscle cells. Treatment of vascular smooth muscle cells with either angiotensin II or PDGF resulted in a rapid increase in Shc tyrosine phosphorylation and Shc-Grb2 complex formation. Furthermore, a 180-kDa protein coprecipitating with Shc-Grb2 complexes also demonstrated increased tyrosine phosphorylation in response to angiotensin II. This coprecipitating protein was identified as the PDGF receptor, suggesting that angiotensin II mediates mitogenic signaling by inducing phosphorylation of the PDGF-RTK (126). This effect is sensitive to inhibition by an antagonist of the angiotensin II type I receptor and does not involve autocrine activation of the PDGF receptor. Comparable results were reported by Daub *et al.* (127), who found increased tyrosine phosphorylation of the EGF-RTK in response to the GPCR agonists endothelin-1, LPA, and thrombin. EGF-RTK phosphorylation occurred concomitantly with increased binding of both Shc and EGF-RTK to a GST fusion protein containing the Grb2 SH2 domain, suggesting that the

mitogenic signaling complex is assembled on the cytoplasmic surface of the EGF-RTK (127). The EGF-RTK-specific tyrosine kinase inhibitor, tyrphostin AG1478, blocked GPCR-mediated EGF-RTK phosphorylation and ERK activation, suggesting that the receptor's intrinsic tyrosine kinase activity was required for GPCR-mediated mitogenic signaling. A dominant negative mutant of the EGF-RTK, HERCD533, had identical effects. The authors suggest that the intrinsic tyrosine kinase activity of RTKs may be modulated in a ligand-independent manner by GPCR-mediated signals and that the RTKs themselves may function as scaffolds in GPCR signaling (127).

GPCR-mediated tyrosine phosphorylation of the insulin growth factor 1 (IGF-1) receptor, insulin receptor substrate (IRS-1) (128), and focal adhesion kinase (FAK) (86) have also been reported. Like the EGF-RTK, PDGF-RTK, and Shc, each of these molecules can function as a docking protein when phosphorylated, and each participates in mitogenic signaling via RTKs or integrins. Collectively, these data suggest that GPCRs mediate a program of tyrosine protein phosphorylation that is required for mitogenic signaling. Other receptors that lack intrinsic tyrosine kinase activity, such as the GH or erythropoietin receptors and the antigen receptors of T cells and B cells, accomplish this via the recruitment and activation of non-RTKs of the Src and/or Janus kinase families (129–132).

Several lines of evidence suggest a role for the ubiquitous nonreceptor Src-family tyrosine kinases in signaling via GPCRs. Activation of the Src family kinases, c-Src, Fyn, or Yes, by the thrombin (133), endothelin (134), and angiotensin II (126, 135, 136, 137) receptors has been reported. Similarly, the N-formyl peptide chemoattractant receptor of polymorphonuclear leukocytes has been shown to mediate an agonist-dependent increase in complex formation between tyrosine-phosphorylated Shc and Lyn, a Src-family tyrosine kinase (92). In COS-7 cells, GPCR-dependent Shc tyrosine phosphorylation is dependent upon the c-Src tyrosine kinase (138). Stimulation of several Gi-coupled receptors leads to a rapid increase in c-Src autophosphorylation, c-Src activity, and the formation of a c-Src-Shc complex (133, 138). These effects are sensitive to PTX and β ARKct, indicating that the signal is mediated by G $\beta\gamma$ -subunits derived from PTX-sensitive G proteins. Transfection of cells with Csk (C-terminal Src Kinase), an inhibitor of Src-family tyrosine kinases (139–142), results in the inhibition of Shc tyrosine phosphorylation and ERK activation (138), indicating that c-Src activity is required for G $\beta\gamma$ -mediated ERK activation (Fig. 2). Nuclear signaling by endothelin-1 also appears to require Src-family tyrosine kinases (143). Activation of *c-fos* transcription by endothelin-1 was inhibited by both Csk and a dominant negative mutant of c-Src, indicating that nonreceptor protein-tyrosine kinases play a vital role in GPCR-mediated cell growth and development.

Cellular expression of mutationally activated c-Src results in Shc phosphorylation, Grb2 recruitment, and p21ras-dependent activation of ERK1/2. Regulation of Src-family kinases by GPCRs might also account for the phosphorylation of RTKs mediated by some GPCRs. Src kinases have been shown to associate with the EGF-RTK and ErbB2 (p185neu) in mammary tumor cell lines (144), and with Shc in *v-src*-

transformed cells (145). Furthermore, a kinase-inactive mutant of the EGF-RTK retained its ability to stimulate DNA synthesis upon EGF stimulation (146). The authors postulate that this mutant was phosphorylated on tyrosine in response to EGF by an associated tyrosine kinase. Activated c-Src directly phosphorylates several non-autophosphorylation sites on the cytoplasmic surface of the EGF-RTK, which then serve as high-affinity binding sites for both c-Src and the p85 subunit of PI3K (147, 148). Whether or not this phosphorylation of the receptor affects its intrinsic tyrosine kinase activity is unknown. However, c-Src and p85 exhibit a higher affinity for c-Src-phosphorylated EGF-RTK than for autophosphorylated EGF-RTK (147). The angiotensin II-mediated mitogenic signal (126) also involves the phosphorylation and recruitment of c-Src into a complex containing the PDGF-RTK, Shc, and Grb2.

Janus kinases (JAK) mediate the phosphorylation and activation of the STAT (signal transducers and activators of transcription) transcription factors (149). JAK2 also plays a role in ERK1/2 activation via the GH receptor, where JAK2 recruitment and activation are required for Shc phosphorylation and p21ras-activation (132, 150). Little is known about the involvement of JAK kinases in GPCR signaling. The angiotensin II type I receptor mediates direct activation of the JAK/STAT pathway (151), but as yet there is no evidence supporting a role for JAK in ERK activation pathways.

FAK is a tyrosine kinase involved in signaling via the integrin receptors, which convey information from the cellular substratum (152). Activated FAK autophosphorylates, and forms signaling complexes at focal adhesions that contain c-Src, paxillin, dynamin, and Grb2. Increased FAK phosphorylation after stimulation of endothelin I, bombesin (153), and M1 muscarinic (154), adrenergic, and thromboxane A2 GPCRs has been reported. The mechanism of FAK activation by GPCRs is unclear. Phosphorylation of FAK by LPA receptors in Rat-1 cells is mediated by PTX-insensitive G proteins, suggesting that GPCR-induced FAK phosphorylation is independent of the PTX-sensitive ERK1/2 activation pathway (86). Botulinum C3 toxin blocks bombesin and endothelin-induced FAK and paxillin phosphorylation, implicating the low molecular weight G protein Rho in the process. Still, the recruitment of the Grb2 adapter protein into focal adhesion complexes after GPCR stimulation suggests a mechanism for GPCR-mediated p21ras activation that might employ the focal adhesion complex as a scaffold.

PI3K. The p85/p110 isoform of PI3K (PI3K α) is a heterodimeric enzyme composed of a regulatory subunit (p85) and a catalytic subunit (p110), which catalyzes the 3'-phosphorylation of inositol phospholipids. PI3K α is regulated by SH2-mediated binding to tyrosine-phosphorylated proteins such as RTKs, Shc, and IRS-1. PI3K α may function as a downstream effector of p21ras (155–157) in the activation of the small molecular weight G proteins Rho and Rac. PI3Ks may also function upstream of p21ras (158, 159), perhaps as a regulator of growth signals (158, 160–163). Hu *et al.* (160) reported that expression of a constitutively active mutant of PI3K α increased both p21ras and ERK activity in *Xenopus* oocytes, indicating that PI3K α can function as a protooncogene. Using antibodies against the p110 subunit of PI3K α , Roche *et al.* (162) demonstrated a requirement for PI3K ac-

tivity in PDGF- and RTK-mediated entry of quiescent cells into S phase. The p85 subunit of PI3K α is known to bind with high affinity to several mitogenic signaling intermediates, including Shc-Grb2 (158) and RTKs such as the EGF-RTK (147) and PDGF-RTK (164–166). Activation of *c-fos* transcription by insulin is sensitive to inhibition by transfected p85 subunit, whereas insulin-mediated p21ras and Raf activation are unaffected (159). These data suggest a potential role for PI3K activity in growth factor-mediated mitogenic signaling.

Recently, G $\beta\gamma$ -mediated ERK activation in COS-7 and CHO cells was shown to be sensitive to inhibition by two specific inhibitors of PI3Ks, wortmannin and LY294002 (167, 168). These drugs also impaired LPA-mediated activation of p21ras (167) and G $\beta\gamma$ -stimulated phosphorylation of Shc (91). In contrast, expression of a constitutively activated mutant form of PI3K α (160) in COS-7 cells failed to induce ERK1/2 activation (T. van Biesen and R. J. Lefkowitz, unpublished observations) while thrombin-induced mitogenic signals in Swiss 3T3 cells were shown to occur in the absence of PI3K activation (158). Thus, while PI3K activity is apparently involved in mitogenic signaling in some cell types, it is not sufficient to mediate p21ras-dependent ERK1/2 activation.

A possible clue to the role of PI3Ks in mitogenic signaling upstream of p21ras comes from Rameh *et al.* (169), who found that the product of PI3K activity, phosphatidylinositol-3,4,5-trisphosphate (PIP₃), has a high binding affinity for the SH2 domains of various proteins, including Src and the p85 subunit of PI3K. Competition between PIP₃ and the tyrosine-phosphorylated SH2 binding site for p85 on IRS-1 apparently provides feedback-inhibitory control of PI3K activity after insulin stimulation. The authors postulate that similar interactions between PIP₃ and the c-Src SH2 domain might activate c-Src by displacing the interaction between the c-Src SH2 domain and its tyrosine-phosphorylated C terminus.

A novel isoform of PI3K, p110 γ or PI3K γ , has recently been cloned (114, 170). PI3K γ is a monomeric enzyme that undergoes conditional stimulation by G $\beta\gamma$ -subunits *in vitro* and is sensitive to inhibition by wortmannin. Whether PI3K γ functions as a proximate effector of G $\beta\gamma$ -subunits in the ERK1/2 activation pathway remains unclear, since wortmannin and LY294002 cannot distinguish between the p85/p110 and PI3K γ -mediated signals.

Protein tyrosine phosphatases. A role for protein-tyrosine-phosphatases such as SHP2 has been described for mitogenic signaling mediated by growth factors such as insulin (171–173) and PDGF (174, 175) and appears to be required for early *Xenopus* development (176). One of its functions may be to mediate dephosphorylation of the C-terminal regulatory tyrosine residue of c-Src family kinases, thereby stabilizing the kinase in the active state. SHP2 forms active signaling complexes containing Grb2 and PI3K (177) and may be required for PDGF-mediated p21ras activation (175). Recently, Rivard *et al.* (178) reported the involvement of SHP2 in fibroblast proliferation mediated by both PDGF and thrombin, suggesting a role for GPCR-mediated activation of protein-tyrosine-phosphatases in mitogenic signaling. Similarly, phosphotyrosine phosphatase activity is detectable after stimulation of the somatostatin SSTR1 receptor in CHO-K1 cells (179) and a human pancreatic cell line (180).

PH domains in mitogenic signaling: The β ARKct peptide used as a sequestrant of G $\beta\gamma$ contains the region of the BARK1 enzyme shown to bind reversibly to purified G $\beta\gamma$ -subunits *in vitro* (93, 119). This region of the kinase contains a PH domain (119), a common structural motif found in more than 90 proteins (181, 182), including guanine-nucleotide exchange factors such as Sos1, GTPase-activating proteins such as Ras-GAP, phospholipases, cytoskeletal proteins, and PTKs.

PH domains of several proteins reversibly bind with high affinity to G $\beta\gamma$ -subunits *in vitro*, including those of Ras-GRF, Ras-GAP, PLC- γ , IRS-1, Dbl (42, 183), and Btk (184). Transient expression of these PH domains inhibits G $\beta\gamma$ -subunit-mediated PI hydrolysis and the activation of p21ras and ERKs (118, 185), suggesting that the ability to sequester free G $\beta\gamma$ -subunits is a shared property. However, binding of G $\beta\gamma$ -subunits is not a universal property of PH domains. The PH domain of spectrin, for example, does not detectably bind G $\beta\gamma$ *in vitro* (42). Furthermore, many G $\beta\gamma$ -binding proteins including G α -subunits, G protein-gated K⁺ channel (186), Raf-1 (187), and phosducin (109, 188) lack PH domains. Phosducin, like the β ARKct peptide, competitively inhibits G $\beta\gamma$ -mediated functions (189).

The structures of several PH domains have been reported (190–195) and consist of seven antiparallel β -strands forming a β -barrel with an amino-terminal α -helix. Binding of G $\beta\gamma$ -subunits to the β ARK1ct requires the C-terminal portion of the PH domain plus a short sequence distal to it. The N-terminal β -barrel portion of PH domains apparently mediates phospholipid binding, with highest affinity for phosphatidylinositols such as phosphatidylinositol-4,5-bisphosphate (PIP₂) (44, 196, 197), phosphatidylinositol-3,4,5-trisphosphate (PIP₃) (198), and inositol trisphosphate (45). These observations suggest that PH domains may coordinate interactions between inositol phospholipids, notably the products of PI3K activity, and membrane proteins, such as G $\beta\gamma$ -subunits, thereby providing a unique mechanism of inducible membrane-protein interaction (169, 199). Indeed, optimal translocation and activation of β ARK *in vitro* requires both PIP₂ and G $\beta\gamma$ -subunits (44). Mutational disruption of either the β -barrel or α -helical portions of the PH domain compromises function (43).

Among the PH domain-containing proteins is a family of PTKs, including Btk, Tsk, Tec, Itk, and Bmx (200, 201). Expression of these Btk family kinases is generally limited to the hematopoietic system where they play a crucial role in B cell activation and development. X-linked agammaglobulinemia results from mutations in the Btk gene involving the PH domain (202, 203) or SH3 domain (204) of the kinase, among others (205).

The isolated PH domain of Btk can bind G $\beta\gamma$ -subunits *in vitro* and antagonizes G $\beta\gamma$ -dependent signaling when transiently overexpressed in intact cells (42, 43, 185). Interestingly, both Btk and Tsk are activated by the addition of exogenous G $\beta\gamma$ -subunits in an *in vitro* kinase assay (7). These data suggest that tyrosine kinases may be regulated in a manner analogous to β ARK and possibly provide a direct link between G $\beta\gamma$ -subunits and tyrosine phosphorylation. Further, Btk appears to interact with, and be regulated by, c-Src (206–209), another kinase implicated in GPCR-medi-

ated mitogenic signals. Thus far, however, there are no data to suggest that Btk family kinases are involved in regulation of p21ras or MAP kinase pathways. Moreover, limited tissue distribution makes it unlikely that they could provide a general mechanism for G $\beta\gamma$ -mediated mitogenic signaling.

Recently, the PTB domain of IRS-1 was shown to share extensive structural similarity with the PLC δ PH domain, despite their affinities for unrelated ligands and their divergent primary amino acid sequences (210, 211). Like SH2 domains, with which they share no primary or tertiary structural similarity, PTB domains bind with high affinity to context-specific phosphotyrosine residues. Functionally, the PTB, PH, and SH2 domains are each involved in recruiting signaling molecules to the cell surface by binding to different ligands (210). The structural similarity of PH and PTB domains suggests that ligands common to both domains might exist, although none have been identified.

The mechanism by which G $\beta\gamma$ activates the pTyr-dependent signaling cascade to p21ras and ERK remains unclear, although the overall similarities to the classical model of RTK-mediated signaling are striking (Fig. 2). The emerging roles of c-Src, PI3K, SHP2, and RTKs in this pathway may help to define the sequence of events leading to p21ras activation. These studies are complicated by the fact that many of the proteins which form the mitogenic signaling complex, such as PI3K and SHP2, have dual roles, functioning both as enzymes and as substrates for tyrosine phosphorylation, which creates docking sites for other SH2 domain-containing proteins. Further, concomitant activation of additional effectors, such as adenylyl cyclase and PLC, by G α - and G $\beta\gamma$ -subunits, may have indirect effects on the regulation of the mitogenic signal.

4. *Gq/11*. The best characterized effector of the PTX-insensitive Gq/11 family of G proteins is PLC. When activated, PLC hydrolyzes phosphatidylinositol, yielding diacylglycerol and inositol phosphates, which in turn regulate intracellular Ca⁺⁺ (212–215). Ca⁺⁺ and diacylglycerol together activate protein kinase C (PKC) enzymes, which regulate a broad array of cellular signals (216). Treatment of cells with tumor-

promoting phorbol esters that directly activate PKC results in the potent activation of ERK. The mechanism by which PKC activates the ERK1/2 kinase cascade is unclear, although it has been suggested that PKC directly phosphorylates and activates Raf in a p21ras-independent manner (217).

In contrast to Gi-mediated mitogenic signaling, ERK activation by Gq/11-coupled receptors is mostly insensitive to inhibition by the G $\beta\gamma$ -sequestrants β ARKct and α -transducin (66, 118, 120), indicating that the signal is mediated predominantly by the α -subunit of Gq/11. Since the magnitude of ERK activation by Gq-coupled and Gi-coupled receptors is similar, it is unclear why a larger contribution by G $\beta\gamma$ -subunits to the Gq-mediated signal is not detected. One laboratory has reported that a significant portion of the Gq-mediated M1AChR signal in COS-7 cells is G $\beta\gamma$ -dependent (117).

The regulation of mitogenic signals by receptors coupled to Gq/11 appears to be mediated by two or more parallel signaling pathways, either p21ras-dependent or -independent. Early observations revealed that mitogenic signals mediated by thrombin and bradykinin were Gq-dependent and involved PLC activation (218). Furthermore, cellular expression of PLC β 2 or a GTPase-deficient mutant of G α q is sufficient to activate ERK (66).

In COS-7 and CHO cells, the stimulation of ERK activity by Gq-coupled receptors occurs independently of p21ras activation (118) and is insensitive to inhibition by a dominant negative mutant of p21ras (120). However, Gq-mediated signals are inhibited by a dominant negative mutant of Raf (120), indicating that G α q activates ERK via a p21ras-independent, but Raf-dependent, pathway. The down-regulation of PKC activity in CHO cells by chronic exposure to phorbol ester completely blocks Gq-mediated mitogenic signals, whereas Gi- and RTK-mediated signals are unaffected (120). These observations reveal a Gq-specific mitogenic signaling pathway involving the PLC-mediated activation of PKC and Raf, but not p21ras (Fig. 3).

Recently, a novel Ca⁺⁺-dependent PTK, PYK2, was cloned

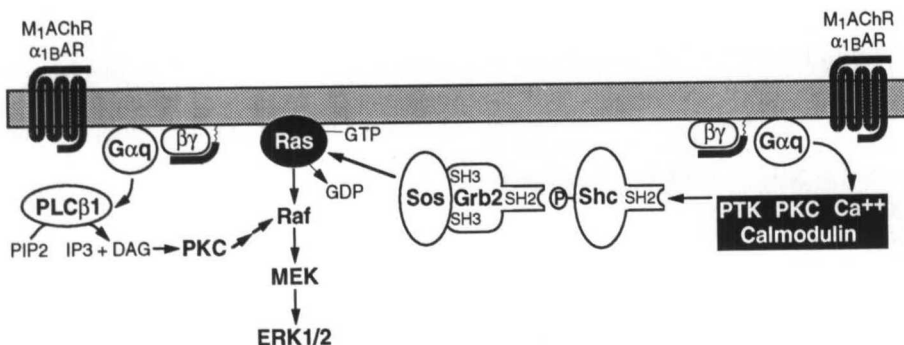


FIG. 3. ERK1/2 activation by GPCRs coupled to PTX-insensitive G proteins occurs via two distinct signaling pathways. GPCRs that signal via PTX-insensitive G proteins (shown in the Fig. collectively as Gq), such as the M₁AChR and α_{1B} AR, activate ERK1/2 via either a p21ras-dependent (right part of figure) or p21ras-independent (left part of figure) mechanism, depending primarily on cell type (see text). Left, The GTP-bound α -subunit of Gq activates PLC β 1, resulting in the conversion of PIP₂ to IP₃ and diacylglycerol. Diacylglycerol, in turn, contributes to the activation of PKC, which activates Raf kinase via a poorly understood mechanism. Thus, this pathway leads to potent ERK1/2 activation in the apparent absence of p21ras involvement. Right, The GTP-bound α -subunit of Gq activates a series of intracellular signals, including activation of PTKs, PKC, calmodulin, and Ca⁺⁺ release. Via an as yet undefined mechanism, these signals contribute to the phosphorylation of the Shc adapter molecule and the subsequent activation of the p21ras/ERK pathway similar to the RTK paradigm (see Fig. 1). ERK1/2 activation by Gq-coupled receptors may be further modulated by the contribution of the G protein $\beta\gamma$ -subunits to the signal (see Fig. 2).

from human brain (219). PYK2, also known as $\text{CAK}\beta$ (220) or RAFTK (221), is a member of the FAK family of non-RTKs, which are known to function in integrin signaling. PYK2, which is highly expressed only in cells of neuronal origin, can be activated either by PKC or elevated levels of intracellular Ca^{++} . Stimulation of Gq-coupled bradykinin receptors in PC12 neuroblastoma cells activates PYK2. The activated kinase in turn mediates p21ras-dependent ERK activation via tyrosine-phosphorylation of Shc and Shc-Grb2-Sos1 complex formation. These observations define a Gq- and Ca^{++} -mediated mitogenic signaling pathway that requires the same intermediates as the $\text{G}\beta\gamma$ - and RTK-mediated pathways (Fig. 3).

In cultured rat vascular smooth muscle cells, PTX-insensitive ERK activation via the endogenous angiotensin II receptor is blocked by intracellular Ca^{++} chelation (222). Interestingly, this Ca^{++} -dependent pathway is also completely blocked by the calmodulin inhibitor calmidazolium and the tyrosine kinase inhibitor genistein (223), suggesting that a Ca^{++} /calmodulin-sensitive tyrosine kinase might play a role in the regulation of mitogenic signaling. The cloning of a Ca^{++} /calmodulin-stimulated tyrosine kinase has been reported (224).

Mitogenic signaling via the Gq-coupled prostaglandin $\text{F}_2\alpha$ and M1 muscarinic receptors in NIH 3T3 cells involves ERK activation via a p21ras-dependent, PKC-independent mechanism (53, 225). In astrocytes, endothelin has been reported to activate Gq-dependent increases in Shc tyrosine-phosphorylation and Shc-Grb2 complex formation (87). The mechanism by which $\text{G}\alpha_q$ stimulates p21ras-dependent ERK activation remains unclear, although this pathway might depend on the activation of a PYK2-like kinase, which functions in a manner analogous to c-Src in Gi-mediated mitogenic signaling (138) (Fig. 3).

5. *Go*. *Go* is expressed at high levels in neuronal growth cones, where its activity is regulated by GAP-43, a guanine nucleotide-releasing protein known to be involved in the regulation of neuronal growth (226, 227). Several receptors have been shown to couple to *Go* *in vivo*, including D1 dopamine receptors (228), SSTR2 somatostatin receptor (229, 230), PAF receptor (231), M2 muscarinic receptors (230, 232), and human 5-HT_{1A} serotonin receptor (233). The ability of the PTX-sensitive *Go* protein to regulate mitogenic signaling was first reported by Kroll *et al.* (234, 235), who demonstrated that a constitutively activated, GTPase-deficient mutant of $\text{G}\alpha_o$ induced transformation of NIH 3T3 fibroblasts and maturation of *Xenopus* oocytes.

In CHO cells stably transfected with PAF receptor (55, 236, 237), stimulation leads to a rapid and transient activation of ERK. This signal is sensitive to inhibition by PTX, indicating the involvement of a Gi/o protein, and is abolished by PKC depletion. PAF stimulation does not mediate an increase in p21ras GTP loading (55), and ERK activation is insensitive to inhibition by a dominant negative mutant of p21ras (237). Sphingosyl phosphocholine (61) and LHRH (65) receptors also appear to employ this pathway.

In CHO cells expressing the M1AChR, carbachol stimulation results in PTX-sensitive, PKC-dependent ERK activation (237). Coexpression of a PTX-insensitive mutant of $\text{G}\alpha_o$,

but not such a mutated form of $\text{G}\alpha_{i1}$, -2, or -3, restores M1AChR-mediated ERK activation in PTX-treated cells. In COS-7 cells, which lack detectable $\text{G}\alpha_o$ expression, the M1AChR signal is transmitted entirely by PTX-insensitive G proteins, and the PAF receptor does not mediate ERK activation (237).

B. Other MAP kinases

JNK/SAPK is a member of a novel family of kinases structurally related to ERKs, although it appears to be differentially regulated (238, 244). Regulation of JNK is associated with the activation of the small molecular weight G proteins, Rac and cdc42 (239, 240), and proceeds via a kinase cascade involving MEKK and JNKK (241–243), whose function is analogous to Raf and MEK in the ERK pathway.

JNK is activated in response to several agonists for GPCRs, including carbachol (240, 241), PAF (245), and angiotensin II (246). Persistent JNK activity is detected in cells expressing GTPase-deficient mutants of $\text{G}\alpha_{12}$, $\text{G}\alpha_{13}$ (247), $\text{G}\alpha_{16}$, and $\text{G}\alpha_q$ (248). In several cases, JNK activation is reported to involve the activation of p21ras (247) and increased intracellular levels of calcium (246, 249). However, in NIH 3T3 cells, M1AChR-mediated increases in *c-jun* mRNA and AP-1 activity correlate with the activation of JNK, but not ERK (243), while stimulation with PDGF activates ERK without stimulating JNK activity. Thus the p21ras/ERK pathway and Rac, cdc42/JNK pathways can be independently regulated.

The low molecular weight G proteins Rac, Rho, and cdc42 are, like p21ras, integrally involved in cellular transformation (250–252). Rac, Rho, and cdc42 regulate the formation of stress fibers (actin polymerization), lamellipodia (membrane ruffling), and filopodia, respectively (252–255). These cellular and cytoskeletal rearrangements play an important role in growth factor-mediated transformation. Collectively, Rac, Rho, and cdc42 function to activate JNKs (239, 240), leading to the transcription of nuclear oncogenes.

In Swiss 3T3 cells, Rho proteins mediate the LPA- and bombesin-induced formation of focal adhesions and actin stress fibers (256). PDGF, insulin, bombesin, and phorbol ester activate Rac, resulting in actin polymerization at the membrane and the formation of membrane ruffles (256). Actin filament organization in activated mast cells requires the involvement of both heterotrimeric and low molecular weight G proteins (257).

The mechanism by which heterotrimeric G proteins and GPCRs activate Rac/Rho/cdc42-dependent signaling pathways is unclear. Rho-mediated stress fiber formation in response to LPA or bombesin is unaffected by changes in the levels of cAMP, intracellular calcium, or PKC activity, but significantly attenuated by PTK inhibitors (258), suggesting that novel signaling mechanisms similar to those regulating the p21ras/ERK pathway exist. The elucidation of the signaling pathways leading from RTKs and GPCRs to the events involved in cytoskeletal rearrangement is crucial to a complete understanding of cellular transformation.

V. Cross-Talk from RTKs to Heterotrimeric G Proteins

The ERK activation pathway employed by receptors that couple to PTX-sensitive G proteins is mediated by $G\beta\gamma$ -subunits and requires the activity of Src family PTKs, which directly or indirectly regulate the tyrosine phosphorylation state of adapter proteins such as Shc (59, 87–91), SHP2 (178), FAK (86), and possibly the RTKs themselves (126, 127). The prospect of such intimate cross-talk between GPCR and RTK signals raises not only the possibility that GPCRs signal via tyrosine phosphorylation cascades, but also that some aspects of RTK signaling might be dependent upon heterotrimeric G proteins (Fig. 2).

Previous work has suggested that in certain cell types, PTX-sensitive G proteins play a role in some aspects of signaling by the insulin receptor. PTX treatment abolishes insulin-mediated inhibition of lipolysis, attenuates insulin-stimulated glucose oxidation in adipocytes (259), and blocks insulin-mediated inhibition of adenylyl cyclase and insulin-stimulated activation of "dense vesicle" cAMP phosphodiesterase (260) in rat hepatocytes. Insulin-stimulated phosphatidylinositol (PI)-glycan hydrolysis and *de novo* phosphatidic acid synthesis involves activation of a Gi-requiring PI-glycan-specific PLC in BC3H-1 myocytes (261, 262), where PTX treatment also attenuates insulin-stimulated [³H]thymidine incorporation into DNA (262).

Insulin and IGF-1, in contrast to EGF, behave as relatively weak mitogens. Their receptors are closely related proteins that share an $\alpha\beta\gamma$ heterotetrameric structure and, like the EGF receptor, possess ligand-stimulated tyrosine kinase activity (263, 264). In contrast to the EGF receptor, ligand-induced autophosphorylation of the insulin and IGF-I receptors does not directly create recognition sites, on the receptor molecule, for SH2 domain-containing signaling intermediates. Rather, receptor-catalyzed tyrosine phosphorylation of exogenous adapter proteins, such as IRS-1, IRS-2 (19–21), and Shc (265) is one of the earliest steps in mitogenesis triggered by these receptors. Phosphorylation of these adapter proteins, therefore, provides the platform for assembly of the mitogenic signaling protein complex, which then proceeds after the EGF receptor paradigm.

In Rat 1 fibroblasts, ERK activation via the endogenous IGF-I receptor and Gi-coupled LPA receptor, but not via the EGF receptor, is sensitive both to PTX treatment and to cellular expression of the β ARKct peptide (266). In these cells, IGF-I stimulation results in tyrosine phosphorylation of Shc, without detectable IRS-1 phosphorylation. The IGF-I, LPA, and EGF receptor-mediated signals are sensitive to inhibitors of PTKs, require p21ras activation, and are independent of PKC. This apparent paradox suggests that in some tissues, the insulin receptor RTKs and Gi protein-coupled receptors employ a similar mechanism for mitogenic signaling that involves both tyrosine phosphorylation and $G\beta\gamma$ -subunits derived from PTX-sensitive G proteins.

The mechanism whereby the insulin or IGF-I receptor might promote the generation of free $G\beta\gamma$ -subunits is unclear, although some data suggest that a direct protein-protein interaction may occur between receptor and G protein. The α -subunits of G_o and G_i , but not G_t , can serve as sub-

strates for insulin receptor-mediated tyrosine phosphorylation *in vitro* (267). The stoichiometry of phosphorylation is about 1:1, and the presence of G_o or G_i enhances the rate of receptor autophosphorylation, suggesting a stable physical interaction (267). Insulin also inhibits PTX-catalyzed ADP-ribosylation of G_i by about 50% in isolated rat liver plasma membranes (268) and promotes guanine-nucleotide binding to BC3H-1 myocyte plasma membranes (269). In adipocyte plasma membranes (270), insulin stimulates the binding of GTP to a 40-kDa protein, and binding of GTP leads to a decrease in [¹²⁵I]insulin binding to the receptor, suggesting a feedback interaction between the insulin-stimulated GTP-binding site and the insulin receptor. Further, peptides derived from autophosphorylation sites of the insulin receptor have been shown to directly activate G_i in phospholipid vesicles (271), and this activation is modulated by tyrosine phosphorylation.

Recently, data obtained in a transgenic mouse model harboring inducible expression of RNA antisense to the gene encoding $G\alpha_2$ suggest an important physiological interaction between insulin and G protein-signaling pathways (272). These mice, which exhibit $G\alpha_2$ deficiency in adipose tissue and liver, display a runted phenotype, hyperinsulinemia, and impaired insulin-induced GLUT4 translocation, inhibition of lipolysis, and activation of glycogen synthase. Insulin-induced receptor autophosphorylation is unaffected, but insulin-induced IRS-1 phosphorylation is absent and phosphotyrosine phosphatase activity is increased. While these data do not provide a molecular mechanism for the regulation of G protein-signaling pathways by RTKs, they do indicate a physiologically relevant role for PTX-sensitive G proteins as positive regulators of insulin action.

VI. Conclusions

The involvement of GPCRs and G proteins in the regulation of MAP kinase activity adds a further level of complexity to the study of tumorigenesis, cell division, and differentiation. However, most mitogenic signals appear to be mediated by intermediates that are shared between the G proteins and RTKs, suggesting that local control of growth and differentiation is equally dependent on GPCR and RTK signaling.

The early convergence of mitogenic signaling pathways may facilitate the design of pharmaceuticals for the treatment of diseases involving uncontrolled cell proliferation. Thus, a complete understanding of mitogenic signaling mechanisms is vitally important to the study of the pathophysiology of diseases such as, for example, postangioplasty restenosis, tumorigenesis, atherosclerosis, and proliferative complications of diabetes.

References

1. Cotecchia S, Ostrowski J, Kjelsberg MA, Caron MG, Lefkowitz RJ 1992 Discrete amino acid sequences of the alpha 1-adrenergic receptor determine the selectivity of coupling to phosphatidylinositol hydrolysis. *J Biol Chem* 267:1633–1639
2. Wall MA, Coleman DE, Lee E, Iniguez-Lluhi JA, Posner BA,

- Gilman AG, Sprang SR 1995 The structure of the G protein heterotrimer Gi alpha 1 beta 1 gamma 2. *Cell* 83:1047-1058
3. Sondek J, Bohm A, Lambright DG, Hamm HE, Sigler PB 1996 Crystal structure of a G protein beta gamma dimer at 2.1 Å resolution. *Nature* 379:369-374
 4. Lambright DG, Sondek J, Bohm A, Skiba NP, Hamm HE, Sigler PB 1996 The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature* 379:311-319
 5. Noel JP, Hamm HE, Sigler PB 1993 The 2.2 Å crystal structure of transducin-alpha complexed with GTP gamma S. *Nature* 366:654-663
 6. Clapham DE, Neer EJ 1993 New roles for G-protein beta gamma dimers in transmembrane signalling. *Nature* 365:403-406
 7. Langhans-Rajasekaran SA, Wan Y, Huang XY 1995 Activation of Tsk and Btk tyrosine kinases by G protein beta gamma subunits. *Proc Natl Acad Sci USA* 92:8601-8605
 8. Medema RH, Bos JL 1993 The role of p21ras in receptor tyrosine kinase signaling. *Crit Rev Oncog* 4:615-661
 9. Pawson T 1995 Protein modules and signalling networks. *Nature* 373:573-580
 10. Boguski MS, McCormick F 1993 Proteins regulating Ras and its relatives. *Nature* 366:643-654
 11. Lefkowitz RJ 1993 G-protein-coupled receptors. Turned on to ill effect. *Nature* 365:603-604
 12. Clapham DE 1993 Mutations in G protein-linked receptors: novel insights on disease. *Cell* 75:1237-1239
 13. Parma J, Duprez L, Van Sande J, Cochaux P, Gervy C, Mockel J, Dumont J, Vassart G 1993 Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* 365:649-651
 14. Weinstein LS, Shenker A, Gejman PV, Merino MJ, Friedman E, Spiegel A M 1991 Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N Engl J Med* 325:1688-1695
 15. Kjelsberg MA, Cotecchia S, Ostrowski J, Caron MG, Lefkowitz RJ 1992 Constitutive activation of the alpha 1β-adrenergic receptor by all amino acid substitutions at a single site. *J Biol Chem* 267:1430-1433
 16. Samama P, Cotecchia S, Costa T, Lefkowitz RJ 1993 A mutation-induced activated state of the β2-adrenergic receptor. Extending the ternary complex model. *J Biol Chem* 268:4625-4636
 17. Ren Q, Kurose H, Lefkowitz RJ, Cotecchia S 1993 Constitutively active mutants of the α2-adrenergic receptor. *J Biol Chem* 268:16483-16487
 18. Allen LF, Lefkowitz RJ, Caron MG, Cotecchia S 1991 G-protein-coupled receptor genes as protooncogenes: constitutively activating mutation of the alpha 1B-adrenergic receptor enhances mitogenesis and tumorigenicity. *Proc Natl Acad Sci USA* 88:11354-11358
 19. Dhanasekaran N, Heasley LE, Johnson GL 1995 G protein-coupled receptor systems involved in cell growth and oncogenesis. *Endocr Rev* 16:259-270
 20. Landis CA, Masters SB, Spada A, Pace AM, Bourne HR, Vallar L 1989 GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. *Nature* 340:692-696
 21. Masters SB, Miller RT, Chi MH, Chang FH, Beiderman B, Lopez NG, Bourne HR 1989 Mutations in the GTP-binding site of Gs alpha alter stimulation of adenylyl cyclase. *J Biol Chem* 264:15467-15474
 22. O'Sullivan C, Barton CM, Staddon SL, Brown CL, Lemoine NR 1991 Activating point mutations of the gsp oncogene in human thyroid adenomas. *Mol Carcinog* 4:345-349
 23. Suarez HG, du Villard JA, Caillou B, Schlumberger M, Parmentier C, Monier R 1991 gsp Mutations in human thyroid tumours. *Oncogene* 6:677-679
 24. Lyons J, Landis CA, Harsh G, Vallar L, Grünwald K, Feichtinger H, Duh QY, Clark OH, Kawasaki E, Bourne HR, McCormick F 1990 Two G protein oncogenes in human endocrine tumors. *Science* 249:655-659
 25. Clementi E, Margaretti N, Meldolesi J, Taramelli R 1990 A new constitutively activating mutation of the Gs protein alpha subunit-gsp oncogene is found in human pituitary tumours. *Oncogene* 5:1059-1061
 26. Vallar L, Spada A, Giannattasio G 1987 Altered Gs and adenylyl cyclase activity in human GH-secreting pituitary adenomas. *Nature* 330:566-568
 27. Chambard JC, Paris S, L'Allemain G, Pouyssegur J 1987 Two growth factor signalling pathways in fibroblasts distinguished by pertussis toxin. *Nature* 326:800-803
 28. van Corven EJ, Groenink A, Jalink K, Eichholtz T, Moolenaar WH 1989 Lysophosphatidate-induced cell proliferation: identification and dissection of signaling pathways mediated by G proteins. *Cell* 59:45-54
 29. Meininger CJ, Granger HJ 1990 Mechanisms leading to adenosine-stimulated proliferation of microvascular endothelial cells. *Am J Physiol* 258:H198-206
 30. Nilsson J, von Euler AM, Dalsgaard CJ 1985 Stimulation of connective tissue cell growth by substance P and substance K. *Nature* 315:61-63
 31. Vouret-Craviari V, Van Obberghen-Schilling E, Scimeca JC, Van Obberghen E, Pouyssegur J 1993 Differential activation of p44 mapk (ERK1) by alpha-thrombin and thrombin-receptor peptide agonist. *Biochem J* 289:209-214
 32. Paris S, Pouyssegur J 1986 Pertussis toxin inhibits thrombin-induced activation of phosphoinositide hydrolysis and Na⁺/H⁺ exchange in hamster fibroblasts. *EMBO J* 5:55-60
 33. Rozengurt E, Sinnett-Smith J 1983 Bombesin stimulation of DNA synthesis and cell division in cultures of Swiss 3T3 cells. *Proc Natl Acad Sci USA* 80:2936-2940
 34. Letterio JJ, Coughlin SR, Williams LT 1986 Pertussis toxin-sensitive pathway in the stimulation of c-myc expression and DNA synthesis by bombesin. *Science* 234:1117-1119
 35. Seuwen K, Magnaldo I, Pouyssegur J 1988 Serotonin stimulates DNA synthesis in fibroblasts acting through 5-HT1B receptors coupled to a Gi-protein. *Nature* 335:254-256
 36. Julius D, Livelli TJ, Jessell TM, Axel R 1989 Ectopic expression of the serotonin 1c receptor and the triggering of malignant transformation. *Science* 244:1057-1062
 37. Gutkind JS, Novotny EA, Brann MR, Robbins KC 1991 Muscarinic acetylcholine receptor subtypes as agonist-dependent oncogenes. *Proc Natl Acad Sci USA* 88:4703-4707
 38. Nemecek GM, Coughlin SR, Handley DA, Moskowitz MA 1986 Stimulation of aortic smooth muscle cell mitogenesis by serotonin. *Proc Natl Acad Sci USA* 83:674-678
 39. Blenis J 1993 Signal transduction via the MAP kinases: proceed at your own RSK. *Proc Natl Acad Sci USA* 90:5889-5892
 40. Cobb MH, Goldsmith EJ 1995 How MAP kinases are regulated. *J Biol Chem* 270:14843-14846
 41. Zhou G, Bao ZQ, Dixon JE 1995 Components of a new human protein kinase signal transduction pathway. *J Biol Chem* 270:12665-12669
 42. Touhara K, Inglese J, Pitcher JA, Shaw G, Lefkowitz RJ 1994 Binding of G protein beta gamma-subunits to pleckstrin homology domains. *J Biol Chem* 269:10217-10220
 43. Touhara K, Koch WJ, Hawes BE, Lefkowitz RJ 1995 Mutational analysis of the pleckstrin homology domain of the beta-adrenergic receptor kinase. Differential effects on G beta gamma and phosphatidylinositol 4,5-bisphosphate binding. *J Biol Chem* 270:17000-17005
 44. Pitcher JA, Touhara K, Payne ES, Lefkowitz RJ 1995 Pleckstrin homology domain-mediated membrane association and activation of the beta-adrenergic receptor kinase requires coordinate interaction with G beta gamma subunits and lipid. *J Biol Chem* 270:11707-11710
 45. Ferguson KM, Lemmon MA, Schlessinger J, Sigler PB 1995 Structure of the high affinity complex of inositol trisphosphate with a phospholipase C pleckstrin homology domain. *Cell* 83:1037-1046
 46. Sadoshima J, Qiu Z, Morgan JP, Izumo S 1995 Angiotensin II and other hypertrophic stimuli mediated by G protein-coupled receptors activate tyrosine kinase, mitogen-activated protein kinase, and 90-kD S6 kinase in cardiac myocytes. The critical role of Ca(2⁺)-dependent signaling. *Circ Res* 76:1-15
 47. Sakanaka C, Ferby I, Waga I, Bito H, Shimizu T 1994 On the mechanism of cytosolic phospholipase A2 activation in CHO cells

- carrying somatostatin receptor: wortmannin-sensitive pathway to activate mitogen-activated protein kinase. *Biochem Biophys Res Commun* 205:18–23
48. Wang Y, Pouyssegur J, Dunn MJ 1993 Endothelin stimulates mitogen-activated protein kinase p42 activity through the phosphorylation of the kinase in rat mesangial cells. *J Cardiovasc Pharmacol* 22[Suppl 8]:S164–S167
 49. Wang Y, Simonson MS, Pouyssegur J, Dunn MJ 1992 Endothelin rapidly stimulates mitogen-activated protein kinase activity in rat mesangial cells. *Biochem J* 287:589–594
 50. Cazaubon S, Parker PJ, Strosberg AD, Couraud PO 1993 Endothelins stimulate tyrosine phosphorylation and activity of p42/mitogen-activated protein kinase in astrocytes. *Biochem J* 293:381–386
 51. Koide M, Kawahara Y, Tsuda T, Ishida Y, Shii K, Yokoyama M 1992 Endothelin-1 stimulates tyrosine phosphorylation and the activities of two mitogen-activated protein kinases in cultured vascular smooth muscle cells. *J Hypertens* 10:1173–1182
 52. Morinelli TA, Zhang LM, Newman WH, Meier KE 1994 Thromboxane A₂/prostaglandin H₂-stimulated mitogenesis of coronary artery smooth muscle cells involves activation of mitogen-activated protein kinase and S6 kinase. *J Biol Chem* 269:5693–5698
 53. Watanabe T, Waga I, Honda Z, Kurokawa K, Shimizu T 1995 Prostaglandin F₂ alpha stimulates formation of p21ras-GTP complex and mitogen-activated protein kinase in NIH-3T3 cells via G_q-protein-coupled pathway. *J Biol Chem* 270:8984–8990
 54. Knall C, Young S, Nick JA, Buhl AM, Worthen GS, Johnson GL 1996 Interleukin-8 regulation of the ras/raf/mitogen-activated protein kinase pathway in human neutrophils. *J Biol Chem* 271:2832–2838
 55. Honda Z, Takano T, Gotoh Y, Nishida E, Ito K, Shimizu T 1994 Transfected platelet-activating factor receptor activates mitogen-activated protein (MAP) kinase and MAP kinase kinase in Chinese hamster ovary cells. *J Biol Chem* 269:2307–2315
 56. Pang L, Decker SJ, Saltiel AR 1993 Bombesin and epidermal growth factor stimulate the mitogen-activated protein kinase through different pathways in Swiss 3T3 cells. *Biochem J* 289:283–287
 57. Worthen GS, Avdi N, Buhl AM, Suzuki N, Johnson GL 1994 FMLP activates Ras and Raf in human neutrophils. Potential role in activation of MAP kinase. *J Clin Invest* 94:815–823
 58. Buhl AM, Avdi N, Worthen GS, Johnson GL 1994 Mapping of the C5a receptor signal transduction network in human neutrophils. *Proc Natl Acad Sci USA* 91:9190–9194
 59. Ohmichi M, Sawada T, Kanda Y, Koike K, Hirota K, Miyake A, Saltiel AR 1994 Thyrotropin-releasing hormone stimulates MAP kinase activity in GH3 cells by divergent pathways. Evidence of a role for early tyrosine phosphorylation. *J Biol Chem* 269:3783–3788
 60. Goodemote KA, Mattie ME, Berger A, Spiegel S 1995 Involvement of a pertussis toxin-sensitive G protein in the mitogenic signaling pathways of sphingosine 1-phosphate. *J Biol Chem* 270:10272–10277
 61. Seufferlein T, Rozengurt E 1995 Sphingosylphosphorylcholine activation of mitogen-activated protein kinase in swiss 3T3 cells requires protein kinase C and a pertussis toxin-sensitive G protein. *J Biol Chem* 270:24334–24342
 62. Wu J, Spiegel S, Sturgill TW 1995 Sphingosine 1-phosphate rapidly activates the mitogen-activated protein kinase pathway by a G protein-dependent mechanism. *J Biol Chem* 270:11484–11488
 63. Ohmichi M, Koike K, Nohara A, Kanda Y, Sakamoto Y, Zhang ZX, Hirota K, Miyake A 1995 Oxytocin stimulates mitogen-activated protein kinase activity in cultured human puerperal uterine myometrial cells. *Endocrinology* 136:2082–2087
 64. Sim PJ, Wolbers WB, Mitchell R 1995 Activation of MAP kinase by the LHRH receptor through a dual mechanism involving protein kinase C and a pertussis toxin-sensitive G protein. *Mol Cell Endocrinol* 112:257–263
 65. Crespo P, Cachero TG, Xu N, Gutkind JS 1995 Dual effect of beta-adrenergic receptors on mitogen-activated protein kinase. Evidence for a beta gamma-dependent activation and a G alpha s-cAMP-mediated inhibition. *J Biol Chem* 270:25259–25265
 66. Faure M, Voyno-Yasenetskaya TA, Bourne HR 1994 cAMP and beta gamma subunits of heterotrimeric G proteins stimulate the mitogen-activated protein kinase pathway in COS-7 cells. *J Biol Chem* 269:7851–7854
 67. Wu J, Dent P, Jelinek T, Wolfman A, Weber MJ, Sturgill TW 1993 Inhibition of the EGF-activated MAP kinase signaling pathway by adenosine 3',5'-monophosphate. *Science* 262:1065–1069
 68. Severson BR, Kong X, Lawrence Jr JC 1993 Increasing cAMP attenuates activation of mitogen-activated protein kinase. *Proc Natl Acad Sci USA* 90:10305–10309
 69. Graves LM, Bornfeldt KE, Raines EW, Potts BC, Macdonald SG, Ross R, Krebs EG 1993 Protein kinase A antagonizes platelet-derived growth factor-induced signaling by mitogen-activated protein kinase in human arterial smooth muscle cells. *Proc Natl Acad Sci USA* 90:10300–10304
 70. Hordijk PL, Verlaan I, Jalink K, van Corven EJ, Moolenaar WH 1994 cAMP abrogates the p21ras-mitogen-activated protein kinase pathway in fibroblasts. *J Biol Chem* 269:3534–3538
 71. Li X, Zarinetchi F, Schrier RW, Nemenoff RA 1995 Inhibition of MAP kinase by prostaglandin E₂ and forskolin in rat renal mesangial cells. *Am J Physiol* 269:C986–991
 72. Cook SJ, McCormick F 1993 Inhibition by cAMP of Ras-dependent activation of Raf. *Science* 262:1069–1072
 73. Burgering BM, Pronk GJ, van Weeren PC, Chardin P, Bos JL 1993 cAMP antagonizes p21ras-directed activation of extracellular signal-regulated kinase 2 and phosphorylation of mSos nucleotide exchange factor. *EMBO J* 12:4211–4220
 74. Faure M, Bourne HR 1995 Differential effects on cAMP on the MAP kinase cascade: evidence for a cAMP-insensitive step that can bypass Raf-1. *Mol Biol Cell* 6:1025–1035
 75. Vaillancourt RR, Gardner AM, Johnson GL 1994 B-Raf-dependent regulation of the MEK-1/mitogen-activated protein kinase pathway in PC12 cells and regulation by cyclic AMP. *Mol Cell Biol* 14:6522–6530
 76. Frodin M, Peraldi P, Van Obberghen E 1994 Cyclic AMP activates the mitogen-activated protein kinase cascade in PC12 cells. *J Biol Chem* 269:6207–6214
 77. Pace AM, Wong YH, Bourne HR 1991 A mutant alpha subunit of G_{i2} induces neoplastic transformation of Rat-1 cells. *Proc Natl Acad Sci USA* 88:7031–7035
 78. Hermouet S, Merendino Jr JJ, Gutkind JS, Spiegel AM 1991 Activating and inactivating mutations of the alpha subunit of G_{i2} protein have opposite effects on proliferation of NIH 3T3 cells. *Proc Natl Acad Sci USA* 88:10455–10459
 79. Gupta SK, Gallego C, Lowndes JM, Pleiman CM, Sable C, Eisfelder BJ, Johnson GL 1992 Analysis of the fibroblast transformation potential of GTPase-deficient gip2 oncogenes. *Mol Cell Biol* 12:190–197
 80. Gupta SK, Gallego C, Johnson GL, Heasley LE 1992 MAP kinase is constitutively activated in gip2 and src transformed rat 1a fibroblasts. *J Biol Chem* 267:7987–7990
 81. Lowndes JM, Gupta SK, Osawa S, Johnson GL 1991 GTPase-deficient G alpha i2 oncogene gip2 inhibits adenylcyclase and attenuates receptor-stimulated phospholipase A₂ activity. *J Biol Chem* 266:14193–14197
 82. van Corven EJ, Hordijk PL, Medema RH, Bos JL, Moolenaar WH 1993 Pertussis toxin-sensitive activation of p21ras by G protein-coupled receptor agonists in fibroblasts. *Proc Natl Acad Sci USA* 90:1257–1261
 83. Howe LR, Marshall CJ 1993 Lysophosphatidic acid stimulates mitogen-activated protein kinase activation via a G-protein-coupled pathway requiring p21ras and p74raf-1. *J Biol Chem* 268:20717–20720
 84. Alblas J, van Corven EJ, Hordijk PL, Milligan G, Moolenaar WH 1993 Gi-mediated activation of the p21ras-mitogen-activated protein kinase pathway by alpha 2-adrenergic receptors expressed in fibroblasts. *J Biol Chem* 268:22235–22238
 85. Winitz S, Russell M, Qian NX, Gardner A, Dwyer L, Johnson GL 1993 Involvement of Ras and Raf in the Gi-coupled acetylcholine muscarinic m2 receptor activation of mitogen-activated protein (MAP) kinase kinase and MAP kinase. *J Biol Chem* 268:19196–19199
 86. Hordijk PL, Verlaan I, van Corven EJ, Moolenaar WH 1994 Protein tyrosine phosphorylation induced by lysophosphatidic acid in

- Rat-1 fibroblasts. Evidence that phosphorylation of map kinase is mediated by the Gi-p21ras pathway. *J Biol Chem* 269:645-651
87. Cazaubon SM, Ramos-Morales F, Fischer S, Schweighoffer F, Strosberg AD, Couraud PO 1994 Endothelin induces tyrosine phosphorylation and GRB2 association of Shc in astrocytes. *J Biol Chem* 269:24805-24809
 88. Sadoshima J, Izumo S 1996 The heterotrimeric Gq protein-coupled angiotensin II receptor activates p21 ras via the tyrosine kinase-Shc-Grb2-Sos pathway in cardiac myocytes. *EMBO J* 15:775-787
 89. Chen Y, Gral D, Salcini AE, Pelicci PG, Pouyssegur J, Van Obberghen-Schilling E 1996 Shc adaptor proteins are key transducers of mitogenic signaling mediated by the G protein-coupled thrombin receptor. *EMBO J* 15:1037-1044
 90. van Biesen T, Hawes BE, Luttrell DK, Krueger KM, Touhara K, Porfiri E, Sakae M, Luttrell LM, Lefkowitz RJ 1995 Receptor-tyrosine-kinase- and G beta gamma-mediated MAP kinase activation by a common signalling pathway. *Nature* 376:781-784
 91. Touhara K, Hawes BE, van Biesen T, Lefkowitz RJ 1995 G protein beta gamma subunits stimulate phosphorylation of Shc adapter protein. *Proc Natl Acad Sci USA* 92:9284-9287
 92. Ptasznik A, Traynor-Kaplan A, Bokoch GM 1995 G protein-coupled chemoattractant receptors regulate Lyn tyrosine kinase. Shc adapter protein signaling complexes. *J Biol Chem* 270:19969-19973
 93. Pitcher JA, Inglese J, Higgins JB, Arriza JL, Casey PJ, Kim C, Benovic JL, Kwatra MM, Caron MG, Lefkowitz RJ 1992 Role of beta gamma subunits of G proteins in targeting the beta-adrenergic receptor kinase to membrane-bound receptors. *Science* 257:1264-1267
 94. Inglese J, Koch WJ, Caron MG, Lefkowitz RJ 1992 Isoprenylation in regulation of signal transduction by G-protein-coupled receptor kinases. *Nature* 359:147-150
 95. Park D, Jhon DY, Lee CW, Lee KH, Rhee SG 1993 Activation of phospholipase C isozymes by G protein beta gamma subunits. *J Biol Chem* 268:4573-4576
 96. Katz A, Wu D, Simon MI 1992 Subunits beta gamma of heterotrimeric G protein activate beta 2 isoform of phospholipase C. *Nature* 360:686-689
 97. Camps M, Hou C, Sidiropoulos D, Stock JB, Jakobs KH, Gierschik P 1992 Stimulation of phospholipase C by guanine-nucleotide-binding protein beta gamma subunits. *Eur J Biochem* 206:821-831
 98. Carozzi A, Camps M, Gierschik P, Parker PJ 1993 Activation of phosphatidylinositol lipid-specific phospholipase C-beta 3 by G-protein beta gamma subunits. *FEBS Lett* 315:340-342
 99. Boyer JL, Graber SG, Waldo GL, Harden TK, Garrison JC 1994 Selective activation of phospholipase C by recombinant G-protein alpha- and beta gamma-subunits. *J Biol Chem* 269:2814-2819
 100. Blank JL, Shaw K, Ross AH, Exton JH 1993 Purification of a 110-kDa phosphoinositide phospholipase C that is activated by G-protein beta gamma-subunits. *J Biol Chem* 268:25184-25191
 101. Smrcka AV, Sternweis PC 1993 Regulation of purified subtypes of phosphatidylinositol-specific phospholipase C beta by G protein alpha and beta gamma subunits. *J Biol Chem* 268:9667-9674
 102. Lee SB, Shin SH, Hepler JR, Gilman AG, Rhee SG 1993 Activation of phospholipase C-beta 2 mutants by G protein alpha q and beta gamma subunits. *J Biol Chem* 268:25952-25957
 103. Blank JL, Brattain KA, Exton JH 1992 Activation of cytosolic phosphoinositide phospholipase C by G-protein beta gamma subunits. *J Biol Chem* 267:23069-23075
 104. Camps M, Carozzi A, Schnabel P, Scheer A, Parker PJ, Gierschik P 1992 Isozyme-selective stimulation of phospholipase C-beta 2 by G protein beta gamma-subunits. *Nature* 360:684-686
 105. Wu D, Katz A, Simon MI 1993 Activation of phospholipase C beta 2 by the alpha and beta gamma subunits of trimeric GTP-binding protein. *Proc Natl Acad Sci USA* 90:5297-5301
 106. Jelsema CL, Axelrod J 1987 Stimulation of phospholipase A2 activity in bovine rod outer segments by the beta gamma subunits of transducin and its inhibition by the alpha subunit. *Proc Natl Acad Sci USA* 84:3623-3627
 107. Lustig KD, Conklin BR, Herzmark P, Taussig R, Bourne HR 1993 Type II adenylyl cyclase integrates coincident signals from Gs, Gi, and Gq. *J Biol Chem* 268:13900-13905
 108. Federman AD, Conklin BR, Schrader KA, Reed RR, Bourne HR 1992 Hormonal stimulation of adenylyl cyclase through Gi-protein beta gamma subunits. *Nature* 356:159-161
 109. Hawes BE, Touhara K, Kurose H, Lefkowitz RJ, Inglese J 1994 Determination of the G beta gamma-binding domain of phosphodiacylglycerol kinase. A regulatable modulator of G beta gamma signaling. *J Biol Chem* 269:29825-29830
 110. Ito H, Tung RT, Sugimoto T, Kobayashi I, Takahashi K, Katada T, Ui M, Kurachi Y 1992 On the mechanism of G protein beta gamma subunit activation of the muscarinic K⁺ channel in guinea pig atrial cell membrane. Comparison with the ATP-sensitive K⁺ channel. *J Gen Physiol* 99:961-983
 111. Reuveny E, Slesinger PA, Inglese J, Morales JM, Iniguez-Lluhi JA, Lefkowitz RJ, Bourne HR, Jan YN, Jan LY 1994 Activation of the cloned muscarinic potassium channel by G protein beta gamma subunits. *Nature* 370:143-146
 112. Lim NF, Dascal N, Labarca C, Davidson N, Lester HA 1995 A G protein-gated K channel is activated via beta 2-adrenergic receptors and G beta gamma subunits in *Xenopus* oocytes. *J Gen Physiol* 105:421-439
 113. Tsukada S, Simon MI, Witte ON, Katz A 1994 Binding of beta gamma subunits of heterotrimeric G proteins to the PH domain of Bruton tyrosine kinase. *Proc Natl Acad Sci USA* 91:11256-11260
 114. Stoyanov B, Volinia S, Hanck T, Rubio I, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nürnberg B, Gierschik P, Seedorf K, Hsuan JJ, Waterfield MD, Wetzker R 1995 Cloning and characterization of a G protein-activated human phosphoinositide-3 kinase. *Science* 269:690-693
 115. Herlitze S, Garcia DE, Mackie K, Hille B, Scheuer T, Catterall WA 1996 Modulation of Ca²⁺ channels by G-protein beta-gamma subunits. *Nature* 380:258-262
 116. Ikeda SR 1996 Voltage-dependent modulation of N-type calcium channels by G-protein beta-gamma subunits. *Nature* 380:255-258
 117. Crespo P, Xu N, Simonds WF, Gutkind JS 1994 Ras-dependent activation of MAP kinase pathway mediated by G-protein beta gamma subunits. *Nature* 369:418-420
 118. Koch WJ, Hawes BE, Allen LF, Lefkowitz RJ 1994 Direct evidence that Gi-coupled receptor stimulation of mitogen-activated protein kinase is mediated by G beta gamma activation of p21ras. *Proc Natl Acad Sci USA* 91:12706-12710
 119. Koch WJ, Inglese J, Stone WC, Lefkowitz RJ 1993 The binding site for the beta gamma subunits of heterotrimeric G proteins on the beta-adrenergic receptor kinase. *J Biol Chem* 268:8256-8260
 120. Hawes BE, van Biesen T, Koch WJ, Luttrell LM, Lefkowitz RJ 1995 Distinct pathways of Gi- and Gq-mediated mitogen-activated protein kinase activation. *J Biol Chem* 270:17148-17153
 121. Ueda N, Iniguez-Lluhi JA, Lee E, Smrcka AV, Robishaw JD, Gilman AG 1994 G protein beta gamma subunits. Simplified purification and properties of novel isoforms. *J Biol Chem* 269:4388-4395
 122. Iniguez-Lluhi JA, Simon MI, Robishaw JD, Gilman AG 1992 G protein beta gamma subunits synthesized in Sf9 cells. Functional characterization and the significance of prenylation of gamma. *J Biol Chem* 267:23409-23417
 123. Pronin AN, Gautam N 1992 Interaction between G-protein beta and gamma subunit types is selective. *Proc Natl Acad Sci USA* 89:6220-6224
 124. Schmidt CJ, Thomas TC, Levine MA, Neer EJ 1992 Specificity of G protein beta and gamma subunit interactions. *J Biol Chem* 267:13807-13810
 125. Spandidos DA, Wilkie NM 1984 Malignant transformation of early passage rodent cells by a single mutated human oncogene. *Nature* 310:469-475
 126. Linseman DA, Benjamin CW, Jones DA 1995 Convergence of angiotensin II and platelet-derived growth factor receptor signaling cascades in vascular smooth muscle cells. *J Biol Chem* 270:12563-12568
 127. Daub H, Weiss FU, Wallasch C, Ullrich A 1996 Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* 379:557-560
 128. Myers MGJ, Sun XJ, White MF 1994 The IRS-1 signaling system. *Trends Biochem Sci* 19:289-293
 129. Sharfe N, Dadi HK, Roifman CM 1995 JAK3 protein tyrosine

- kinase mediates interleukin-7-induced activation of phosphatidylinositol-3' kinase. *Blood* 86:2077-2085
130. **Klingmuller U, Lorenz U, Cantley LC, Neel BG, Lodish HF** 1995 Specific recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of JAK2 and termination of proliferative signals. *Cell* 80:729-738
 131. **Wang YD, Wong K, Wood WI** 1995 Intracellular tyrosine residues of the human growth hormone receptor are not required for the signaling of proliferation or Jak-STAT activation. *J Biol Chem* 270:7021-7024
 132. **Winston LA, Hunter T** 1995 JAK2, Ras, and Raf are required for activation of extracellular signal-regulated kinase/mitogen-activated protein kinase by growth hormone. *J Biol Chem* 270:30837-30840
 133. **Chen YH, Pouyssegur J, Courtneidge SA, Van Obberghen-Schilling E** 1994 Activation of Src family kinase activity by the G protein-coupled thrombin receptor in growth-responsive fibroblasts. *J Biol Chem* 269:27372-27377
 134. **Simonson MS, Herman WH** 1993 Protein kinase C and protein tyrosine kinase activity contribute to mitogenic signaling by endothelin-1. Cross-talk between G protein-coupled receptors and pp60c-src. *J Biol Chem* 268:9347-9357
 135. **Schieffer B, Paxton WG, Chai Q, Marrero MB, Bernstein KE** 1996 Angiotensin II controls p21ras activity via pp60c-src. *J Biol Chem* 271:10329-10333
 136. **Kramer RM, Roberts EF, Strifler BA, Johnstone EM** 1995 Thrombin induces activation of p38 MAP kinase in human platelets. *J Biol Chem* 270:27395-27398
 137. **Marrero MB, Schieffer B, Paxton WG, Schieffer E, Bernstein KE** 1995 Electroporation of pp60c-src antibodies inhibits the angiotensin II activation of phospholipase C-gamma 1 in rat aortic smooth muscle cells. *J Biol Chem* 270:15734-15738
 138. **Luttrell LM, Hawes BE, van Biesen T, Luttrell DK, Lansing TJ, Lefkowitz RJ** 1996 Role of c-Src tyrosine kinase in G protein-coupled receptor- and G beta-gamma subunit-mediated activation of mitogen activated protein kinases. *J Biol Chem* 271:19443-19450
 139. **Koegl M, Courtneidge SA, Supertifurga G** 1995 Structural requirements for the efficient regulation of the Src protein tyrosine kinase by Csk. *Oncogene* 11:2317-2329
 140. **Superti-Furga G, Fumagalli S, Koegl M, Courtneidge SA, Draetta G** 1993 Csk inhibition of c-Src activity requires both the SH2 and SH3 domains of Src. *EMBO J* 12:2625-2634
 141. **Sabe H, Okada M, Nakagawa H, Hanafusa H** 1992 Activation of c-Src in cells bearing v-Crk and its suppression by Csk. *Mol Cell Biol* 12:4706-4713
 142. **Okada M, Nada S, Yamanashi Y, Yamamoto T, Nakagawa H** 1991 CSK: a protein-tyrosine kinase involved in regulation of src family kinases. *J Biol Chem* 266:24249-24252
 143. **Simonson MS, Wang Y, Herman WH** 1996 Nuclear signaling by endothelin-1 requires Src protein-tyrosine kinases. *J Biol Chem* 271:77-82
 144. **Luttrell DK, Lee A, Lansing TJ, Crosby RM, Jung KD, Willard D, Luther M, Rodriguez M, Berman J, Gilmer TM** 1994 Involvement of pp60c-src with two major signaling pathways in human breast cancer. *Proc Natl Acad Sci USA* 91:83-87
 145. **McGlade J, Cheng A, Pelicci G, Pelicci PG, Pawson T** 1992 Shc proteins are phosphorylated and regulated by the v-Src and v-Fps protein-tyrosine kinases. *Proc Natl Acad Sci USA* 89:8869-8873
 146. **Coker KJ, Staros JV, Guyer CA** 1994 A kinase-negative epidermal growth factor receptor that retains the capacity to stimulate DNA synthesis. *Proc Natl Acad Sci USA* 91:6967-6971
 147. **Stover DR, Becker M, Liebetanz J, Lydon NB** 1995 Src phosphorylation of the epidermal growth factor receptor at novel sites mediates receptor interaction with Src and p85 α . *J Biol Chem* 270:15591-15597
 148. **Wasilenko WJ, Payne DM, Fitzgerald DL, Weber MJ** 1991 Phosphorylation and activation of epidermal growth factor receptors in cells transformed by the src oncogene. *Mol Cell Biol* 11:309-321
 149. **Schindler C, Darnell Jr JE** 1995 Transcriptional responses to polypeptide ligands: the JAK-STAT pathway. *Annu Rev Biochem* 64:621-651
 150. **Silva CM, Lu H, Weber MJ, Thorner MO** 1994 Differential tyrosine phosphorylation of JAK1, JAK2, and STAT1 by growth hormone and interferon-gamma in IM-9 cells. *J Biol Chem* 269:27532-27539
 151. **Marrero MB, Schieffer B, Paxton WG, Heerdt L, Berk BC, Delafontaine P, Bernstein KE** 1995 Direct stimulation of Jak/STAT pathway by the angiotensin II AT1 receptor. *Nature* 375:247-250
 152. **Clark EA, Brugge JS** 1995 Integrins and signal transduction pathways: the road taken. *Science* 268:233-239
 153. **Rankin S, Morii N, Narumiya S, Rozengurt E** 1994 Botulinum C3 exoenzyme blocks the tyrosine phosphorylation of p125FAK and paxillin induced by bombesin and endothelin. *FEBS Lett* 354:315-319
 154. **Gutkind JS, Robbins KC** 1992 Activation of transforming G protein-coupled receptors induces rapid tyrosine phosphorylation of cellular proteins, including p125FAK and the p130 v-src substrate. *Biochem Biophys Res Commun* 188:155-161
 155. **Kodaki T, Woscholski R, Parker PJ** 1995 V-Ras activates phosphatidylinositol 3-kinase. *Biochem Soc Trans* 23:1955
 156. **Kodaki T, Woscholski R, Hallberg B, Rodriguez-Viciana P, Downward J, Parker PJ** 1994 The activation of phosphatidylinositol 3-kinase by Ras. *Curr Biol* 4:798-806
 157. **Rodriguez Viciana P, Warne PH, Dhand R, Vanhaesebroeck B, Gout I, Fry MJ, Waterfield MD, Downward J** 1994 Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature* 370:527-532
 158. **Saleem A, Kharbanda S, Yuan ZM, Kufe D** 1995 Monocyte colony-stimulating factor stimulates binding of phosphatidylinositol 3-kinase to Grb2.Sos complexes in human monocytes. *J Biol Chem* 270:10380-10383
 159. **Yamauchi K, Holt K, Pessin JE** 1993 Phosphatidylinositol 3-kinase functions upstream of Ras and Raf in mediating insulin stimulation of c-fos transcription. *J Biol Chem* 268:14597-14600
 160. **Hu Q, Klippel A, Muslin AJ, Fantl WJ, Williams LT** 1995 Ras-dependent induction of cellular responses by constitutively active phosphatidylinositol-3 kinase. *Science* 268:100-102
 161. **Kimura K, Hattori S, Kabuyama Y, Shizawa Y, Takayanagi J, Nakamura S, Toki S, Matsuda Y, Onodera K, Fukui Y** 1994 Neuretin outgrowth of PC12 cells is suppressed by wortmannin, a specific inhibitor of phosphatidylinositol 3-kinase. *J Biol Chem* 269:18961-18967
 162. **Roche S, Koegl M, Courtneidge SA** 1994 The phosphatidylinositol 3-kinase alpha is required for DNA synthesis induced by some, but not all, growth factors. *Proc Natl Acad Sci USA* 91:9185-9189
 163. **Jhun BH, Rose DW, Seely BL, Rameh L, Cantley L, Saltiel AR, Olefsky J M** 1994 Microinjection of the SH2 domain of the 85-kilodalton subunit of phosphatidylinositol 3-kinase inhibits insulin-induced DNA synthesis and c-fos expression. *Mol Cell Biol* 14:7466-7475
 164. **Kavanaugh WM, Klippel A, Escobedo JA, Williams LT** 1992 Modification of the 85-kilodalton subunit of phosphatidylinositol-3 kinase in platelet-derived growth factor-stimulated cells. *Mol Cell Biol* 12:3415-3424
 165. **Klippel A, Escobedo JA, Fantl WJ, Williams LT** 1992 The C-terminal SH2 domain of p85 accounts for the high affinity and specificity of the binding of phosphatidylinositol 3-kinase to phosphorylated platelet-derived growth factor beta receptor. *Mol Cell Biol* 12:1451-1459
 166. **Escobedo JA, Kaplan DR, Kavanaugh WM, Turck CW, Williams LT** 1991 A phosphatidylinositol-3 kinase binds to platelet-derived growth factor receptors through a specific receptor sequence containing phosphotyrosine. *Mol Cell Biol* 11:1125-1132
 167. **Hawes BE, Luttrell LM, van Biesen T, Lefkowitz RJ** 1996 Phosphatidylinositol 3-kinase is an early intermediate in the G beta-gamma-mediated mitogen-activated protein kinase signaling pathway. *J Biol Chem* 271:12133-12136
 168. **Pace AM, Faure M, Bourne HR** 1995 G(i2)-mediated activation of the map kinase cascade. *Mol Biol Cell* 6:1685-1695
 169. **Rameh LE, Chen CS, Cantley LC** 1995 Phosphatidylinositol (3, 4, 5)P3 interacts with SH2 domains and modulates PI 3-kinase association with tyrosine-phosphorylated proteins. *Cell* 83:821-830
 170. **Zhang J, Benovic JL, Sugai M, Wetzker R, Gout I, Rittenhouse SE** 1995 Sequestration of a G-protein beta-gamma subunit or ADP-ribosylation of Rho can inhibit thrombin-induced activation of platelet phosphoinositide 3-kinases. *J Biol Chem* 270:6589-6594
 171. **Noguchi T, Matozaki T, Horita K, Fujioka Y, Kasuga M** 1994 Role

- of SH-PTP2, a protein-tyrosine phosphatase with Src homology 2 domains, in insulin-stimulated Ras activation. *Mol Cell Biol* 14: 6674–6682
172. Xiao S, Rose DW, Sasaoka T, Maegawa H, Burke Jr TR, Roller PP, Shoelson SE, Olefsky JM 1994 Syp (SH-PTP2) is a positive mediator of growth factor-stimulated mitogenic signal transduction. *J Biol Chem* 269:21244–21248
 173. Yamauchi K, Milarski KL, Saltiel AR, Pessin JE 1995 Protein-tyrosine-phosphatase SHPTP2 is a required positive effector for insulin downstream signaling. *Proc Natl Acad Sci USA* 92:664–668
 174. Lechleider RJ, Sugimoto S, Bennett AM, Kashishian AS, Cooper JA, Shoelson SE, Walsh CT, Neel BG 1993 Activation of the SH2-containing phosphotyrosine phosphatase SH-PTP2 by its binding site, phosphotyrosine 1009, on the human platelet-derived growth factor receptor. *J Biol Chem* 268:21478–21481
 175. Bennett AM, Tang TL, Sugimoto S, Walsh CT, Neel BG 1994 Protein-tyrosine-phosphatase SHPTP2 couples platelet-derived growth factor receptor β to Ras. *Proc Natl Acad Sci USA* 91:7335–7339
 176. Tang TL, Freeman Jr RM, O'Reilly AM, Neel BG, Sokol SY 1995 The SH2-containing protein-tyrosine phosphatase SH-PTP2 is required upstream of MAP kinase for early *Xenopus* development. *Cell* 80:473–483
 177. Welham MJ, Dechert U, Leslie KB, Jirik F, Schrader JW 1994 Interleukin (IL)-3 and granulocyte/macrophage colony-stimulating factor, but not IL-4, induce tyrosine phosphorylation, activation, and association of SHPTP2 with Grb2 and phosphatidylinositol 3'-kinase. *J Biol Chem* 269:23764–23768
 178. Rivard N, McKenzie FR, Brondello JM, Pouyssegur J 1995 The phosphotyrosine phosphatase PTP1D, but not PTP1C, is an essential mediator of fibroblast proliferation induced by tyrosine kinase and G protein-coupled receptors. *J Biol Chem* 270:11017–11024
 179. Florio T, Rim C, Hershberger RE, Loda M, Stork PJ 1994 The somatostatin receptor SSTR1 is coupled to phosphotyrosine phosphatase activity in CHO-K1 cells. *Mol Endocrinol* 8:1289–1297
 180. Pan MG, Florio T, Stork PJ 1992 G protein activation of a hormone-stimulated phosphatase in human tumor cells. *Science* 256:1215–1217
 181. Inglese J, Koch WJ, Touhara K, Lefkowitz RJ 1995 G beta gamma interactions with PH domains and Ras-MAPK signaling pathways. *Trends Biochem Sci* 20:151–156
 182. Gibson TJ, Hyvonen M, Musacchio A, Saraste M, Birney E 1994 PH domain: the first anniversary. *Trends Biochem Sci* 19:349–353
 183. Mahadevan D, Thanki N, Singh J, McPhie P, Zangrilli D, Wang LM, Guerrero C, LeVine III H, Humblet C, Saldanha J, Gutkind JS, Najmabadi-Haske T 1995 Structural studies on the PH domains of Db1, Sos1, IRS-1, and β ARK1 and their differential binding to $G_{\beta\gamma}$ subunits. *Biochemistry* 34:9111–9117
 184. Tsukada S, Simon MI, Witte ON, Katz A 1994 Binding of beta gamma subunits of heterotrimeric G proteins to the PH domain of Bruton tyrosine kinase. *Proc Natl Acad Sci USA* 91:11256–11260
 185. Luttrell LM, Hawes BE, Touhara K, van Biesen T, Koch WJ, Lefkowitz RJ 1995 Effect of cellular expression of pleckstrin homology domains on Gi-coupled receptor signaling. *J Biol Chem* 270:12984–12989
 186. Krapivinsky G, Krapivinsky L, Wickman K, Clapham DE 1995 G beta gamma binds directly to the G protein-gated K^+ channel, IKACH. *J Biol Chem* 270:29059–29062
 187. Pumiglia KM, LeVine H, Haske T, Habib T, Jove R, Decker SJ 1995 A direct interaction between G-protein beta gamma subunits and the Raf-1 protein kinase. *J Biol Chem* 270:14251–14254
 188. Xu J, Wu D, Slepak VZ, Simon MI 1995 The N terminus of phosducin is involved in binding of beta-gamma subunits of G protein. *Proc Natl Acad Sci USA* 92:2086–2090
 189. Schröder S, Lohse MJ 1996 Inhibition of G-protein $\beta\gamma$ -subunit functions by phosducin-like protein. *Proc Natl Acad Sci USA* 93: 2100–2104
 190. Timm D, Salim K, Gout I, Guruprasad L, Waterfield M, Blundell T 1994 Crystal structure of the pleckstrin homology domain from dynamin. *Nature Struct Biol* 1:782–788
 191. Fushman D, Cahill S, Lemmon MA, Schlessinger J, Cowburn D 1995 Solution structure of pleckstrin homology domain of dynamin by heteronuclear NMR spectroscopy. *Proc Natl Acad Sci USA* 92:816–820
 192. Yoon HS, Hajduk PJ, Petros AM, Olejniczak ET, Meadows RP, Fesik SW 1994 Solution structure of a pleckstrin-homology domain. *Nature* 369:672–675
 193. Downing AK, Driscoll PC, Gout I, Salim K, Zvelebil MJ, Waterfield MD 1994 Three-dimensional solution structure of the pleckstrin homology domain from dynamin. *Curr Biol* 4:884–891
 194. Ferguson KM, Lemmon MA, Schlessinger J, Sigler PB 1994 Crystal structure at 2.2 Å resolution of the pleckstrin homology domain from human dynamin. *Cell* 79:199–209
 195. Macias MJ, Musacchio A, Ponstingl H, Nilges M, Saraste M, Oschkinat H 1994 Structure of the pleckstrin homology domain from beta-spectrin. *Nature* 369:675–677
 196. Harlan JE, Hajduk PJ, Yoon HS, Fesik SW 1994 Pleckstrin homology domains bind to phosphatidylinositol-4,5-bisphosphate. *Nature* 371:168–170
 197. Garcia P, Gupta R, Shah S, Morris AJ, Rudge SA, Scarlata S, Petrova V, McLaughlin S, Rebecchi MJ 1995 The pleckstrin homology domain of phospholipase C-delta 1 binds with high affinity to phosphatidylinositol 4,5-bisphosphate in bilayer membranes. *Biochemistry* 34:16228–16234
 198. Ferguson KM, Lemmon MA, Schlessinger J, Sigler PB 1995 Structure of the high affinity complex of inositol trisphosphate with a phospholipase C pleckstrin homology domain. *Cell* 83:1037–1046
 199. Liscovitch M, Cantley LC 1994 Lipid second messengers. *Cell* 77:329–334
 200. Tamagnone L, Lahtinen I, Mustonen T, Virtaneva K, Francis F, Muscatelli F, Alitalo R, Smith CI, Larsson C, Alitalo K 1994 BMX, a novel nonreceptor tyrosine kinase gene of the BTK/ITK/TEC/TKX family located in chromosome Xp22.2. *Oncogene* 9:3683–3688
 201. Desiderio S, Siliciano JD 1994 The Itk/Btk/Tec family of protein-tyrosine kinases. *Chem Immunol* 59:191–210
 202. Li T, Tsukada S, Satterthwaite A, Havlik MH, Park H, Takatsu K, Witte ON 1995 Activation of Bruton's tyrosine kinase (BTK) by a point mutation in its pleckstrin homology (PH) domain. *Immunity* 2:451–460
 203. Vihinen M, Zvelebil MJ, Zhu Q, Brooimans RA, Ochs HD, Zegers BJ, Nilsson L, Waterfield MD, Smith CI 1995 Structural basis for pleckstrin homology domain mutations in X-linked agammaglobulinemia. *Biochemistry* 34:1475–1481
 204. Zhu Q, Zhang M, Rawlings DJ, Vihinen M, Hagemann T, Saffran DC, Kwan SP, Nilsson L, Smith CI, Witte ON, Chen SH, Ochs HD 1994 Deletion within the Src homology domain 3 of Bruton's tyrosine kinase resulting in X-linked agammaglobulinemia (XLA). *J Exp Med* 180:461–470
 205. Conley ME, Rohrer J 1995 The spectrum of mutations in Btk that cause X-linked agammaglobulinemia. *Clin Immunol Immunopathol* 76:S192–7
 206. Mahajan S, Fargnoli J, Burkhardt AL, Kut SA, Saouaf SJ, Bolen JB 1995 Src family protein tyrosine kinases induce autoactivation of Bruton's tyrosine kinase. *Mol Cell Biol* 15:5304–5311
 207. Cheng G, Ye ZS, Baltimore D 1994 Binding of Bruton's tyrosine kinase to Fyn, Lyn, or Hck through a Src homology 3 domain-mediated interaction. *Proc Natl Acad Sci USA* 91:8152–8155
 208. Rawlings DJ, Saffran DC, Tsukada S, Largaespa DA, Grimaldi JC, Cohen L, Mohr RN, Bazan JF, Howard M, Copeland NG, Jenkins NA, Witte ON 1993 Mutation of unique region of Bruton's tyrosine kinase in immunodeficient XID mice. *Science* 261:358–361
 209. Rawlings DJ, Scharenberg AM, Park H, Wahl MI, Lin S, Kato RM, Fluckiger AC, Witte ON, Kinet JP 1996 Activation of BTK by a phosphorylation mechanism initiated by SRC family kinases. *Science* 271:822–825
 210. Lemmon MA, Ferguson KM, Schlessinger J 1996 PH domains: diverse sequences with a common fold recruit signaling molecules to the cell surface. *Cell* 85:621–624
 211. Eck MJ, Dhe-Paganon S, Trub T, Nolte RT, Shoelson SE 1996 Structure of the IRS-1 PTB domain bound to the juxtamembrane region of the insulin receptor. *Cell* 85:695–705
 212. Wu D, Katz A, Lee CH, Simon MI 1992 Activation of phospholipase C by alpha 1-adrenergic receptors is mediated by the alpha subunits of Gq family. *J Biol Chem* 267:25798–25802
 213. Wu DQ, Lee CH, Rhee SG, Simon MI 1992 Activation of phos-

- pholipase C by the alpha subunits of the Gq and G11 proteins in transfected Cos-7 cells. *J Biol Chem* 267:1811-1817
214. Taylor SJ, Chae HZ, Rhee SG, Exton JH 1991 Activation of the beta 1 isozyme of phospholipase C by alpha subunits of the Gq class of G proteins. *Nature* 350:516-518
 215. Hepler JR, Kozasa T, Smrcka AV, Simon MI, Rhee SG, Sternweis PC, Gilman AG 1993 Purification from Sf9 cells and characterization of recombinant Gq alpha and G11 alpha. Activation of purified phospholipase C isozymes by G alpha subunits. *J Biol Chem* 268:14367-14375
 216. Newton AC 1995 Protein kinase C: structure, function, and regulation. *J Biol Chem* 270:28495-28498
 217. Kolch W, Heidecker G, Kochs G, Hummel R, Vahidi H, Mischak H, Finkenzeller G, Marme D, Rapp UR 1993 Protein kinase C alpha activates RAF-1 by direct phosphorylation. *Nature* 364:249-252
 218. LaMorte VJ, Harootunian AT, Spiegel AM, Tsien RY, Feramisco JR 1993 Mediation of growth factor induced DNA synthesis and calcium mobilization by Gq and Gi2. *J Cell Biol* 121:91-99
 219. Lev S, Moreno H, Martinez R, Canoll P, Peles E, Musacchio JM, Plowman GD, Rudy B, Schlessinger J 1995 Protein tyrosine kinase PYK2 involved in Ca(2+)-induced regulation of ion channel and MAP kinase functions. *Nature* 376:737-745
 220. Sasaki H, Nagura K, Ishino M, Tobioka H, Kotani K, Sasaki T 1995 Cloning and characterization of cell adhesion kinase beta, a novel protein-tyrosine kinase of the focal adhesion kinase subfamily. *J Biol Chem* 270:21206-21219
 221. Avraham S, London R, Fu Y, Ota S, Hiregowdara D, Li J, Jiang S, Pasztor LM, White RA, Groopman JE, Avraham H 1995 Identification and characterization of a novel related adhesion focal tyrosine kinase (RAFTK) from megakaryocytes and brain. *J Biol Chem* 270:27742-27751
 222. Eguchi S, Matsumoto T, Motley ED, Utsunomiya H, Inagami T 1996 Identification of an essential signaling cascade for mitogen-activated protein kinase activation by angiotensin II in cultured rat vascular smooth muscle cells. *J Biol Chem* 271:14169-14175
 223. Eguchi S, Matsumoto T, Motley ED, Utsunomiya H, Inagami T 1996 Identification of an essential signaling cascade for mitogen-activated protein kinase activation by angiotensin II in cultured rat vascular smooth muscle cells. Possible requirement of Gq-mediated p21ras activation coupled to a Ca²⁺/calmodulin-sensitive tyrosine kinase. *J Biol Chem* 271:14169-14175
 224. Castoria G, Migliaccio A, Green S, Di Domenico M, Chambon P, Auricchio F 1993 Properties of a purified estradiol-dependent calf-uterus tyrosine kinase. *Biochemistry* 32:1740-1750
 225. Crespo P, Xu N, Daniotti JL, Troppmair J, Rapp UR, Gutkind JS 1994 Signaling through transforming G protein-coupled receptors in NIH 3T3 cells involves c-Raf activation. Evidence for a protein kinase C-independent pathway. *J Biol Chem* 269:21103-21109
 226. Strittmatter SM, Valenzuela D, Kennedy TE, Neer EJ, Fishman MC 1990 Go is a major growth cone protein subject to regulation by GAP-43. *Nature* 344:836-841
 227. Strittmatter SM, Valenzuela D, Sudo Y, Linder ME, Fishman MC 1991 An intracellular guanine nucleotide release protein for Go. GAP-43 stimulates isolated alpha subunits by a novel mechanism. *J Biol Chem* 266:22465-22471
 228. Kimura K, White BH, Sidhu A 1995 Coupling of human D-1 dopamine receptors to different guanine nucleotide binding proteins. Evidence that D-1 dopamine receptors can couple to both Gs and G(o). *J Biol Chem* 270:14672-14678
 229. Law SF, Yasuda K, Bell GI, Reisine T 1993 Gi alpha 3 and G(o) alpha selectively associate with the cloned somatostatin receptor subtype SSTR2. *J Biol Chem* 268:10721-10727
 230. Kleuss C, Hescheler J, Ewel C, Rosenthal W, Schultz G, Wittig B 1991 Assignment of G-protein subtypes to specific receptors inducing inhibition of calcium currents. *Nature* 353:43-48
 231. Yue TL, Stadel JM, Sarau HM, Friedman E, Gu JL, Powers DA, Gleason MM, Feuerstein G, Wang HY 1992 Platelet-activating factor stimulates phosphoinositide turnover in neurohybrid NCB-20 cells: involvement of pertussis toxin-sensitive guanine nucleotide-binding proteins and inhibition by protein kinase C. *Mol Pharmacol* 41:281-289
 232. Matesic DF, Manning DR, Luthin GR 1991 Tissue-dependent association of muscarinic acetylcholine receptors with guanine nucleotide-binding regulatory proteins. *Mol Pharmacol* 40:347-353
 233. Mulheron JG, Casanas SJ, Arthur JM, Garnovskaya MN, Gettys TW, Raymond JR 1994 Human 5-HT1A receptor expressed in insect cells activates endogenous G(o)-like G protein(s). *J Biol Chem* 269:12954-12962
 234. Kroll SD, Chen J, De Vivo M, Carty DJ, Buku A, Premont RT, Iyengar R 1992 The Q205LGo-alpha subunit expressed in NIH-3T3 cells induces transformation. *J Biol Chem* 267:23183-23188
 235. Kroll SD, Omri G, Landau EM, Iyengar R 1991 Activated alpha subunit of Go protein induces oocyte maturation. *Proc Natl Acad Sci USA* 88:5182-5186
 236. Mori M, Bito H, Sakanaka C, Honda Z, Kume K, Izumi T, Shimizu T 1994 Activation of mitogen-activated protein kinase and arachidonate release via two G protein-coupled receptors expressed in the rat hippocampus. *Ann NY Acad Sci* 744:107-125
 237. van Biesen T, Hawes BE, Raymond JR, Luttrell LM, Koch WJ, Lefkowitz RJ 1996 G(o)-protein alpha-subunits activate mitogen-activated protein kinase via a novel protein kinase C-dependent mechanism. *J Biol Chem* 271:1266-1269
 238. Davis RJ 1994 MAPKs: new JNK expands the group. *Trends Biochem Sci* 19:470-473
 239. Minden A, Lin A, Claret FX, Abo A, Karin M 1995 Selective activation of the JNK signaling cascade and c-Jun transcriptional activity by the small GTPases Rac and Cdc42Hs. *Cell* 81:1147-1157
 240. Coso OA, Chiariello M, Yu JC, Teramoto H, Crespo P, Xu N, Miki T, Gutkind JS 1995 The small GTP-binding proteins Rac1 and Cdc42 regulate the activity of the JNK/SAPK signaling pathway. *Cell* 81:1137-1146
 241. Minden A, Lin A, McMahon M, Lange-Carter C, Derijard B, Davis RJ, Johnson GL, Karin M 1994 Differential activation of ERK and JNK mitogen-activated protein kinases by Raf-1 and MEKK. *Science* 266:1719-1723
 242. Lin A, Minden A, Martinetto H, Claret FX, Lange-Carter C, Mercurio F, Johnson GL, Karin M 1995 Identification of a dual specificity kinase that activates the Jun kinases and p38-Mpk2. *Science* 268:286-290
 243. Coso OA, Chiariello M, Kalinec G, Kyriakis JM, Woodgett J, Gutkind JS 1995 Transforming G protein-coupled receptors potentially activate JNK (SAPK). Evidence for a divergence from the tyrosine kinase signaling pathway. *J Biol Chem* 270:5620-5624
 244. Derijard B, Hibi M, Wu IH, Barrett T, Su B, Deng T, Karin M, Davis R J 1994 JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* 76:1025-1037
 245. Squinto SP, Block AL, Braquet P, Bazan NG 1989 Platelet-activating factor stimulates a fos/jun/AP-1 transcriptional signaling system in human neuroblastoma cells. *J Neurosci Res* 24:558-566
 246. Zohn IE, Yu H, Li X, Cox AD, Earp HS 1995 Angiotensin II stimulates calcium dependent activation of c-Jun N-terminal kinase. *Mol Cell Biol* 15:6160-6168
 247. Prasad MV, Dermott JM, Heasley LE, Johnson GL, Dhanasekaran N 1995 Activation of Jun kinase/stress-activated protein kinase by GTPase-deficient mutants of G alpha 12 and G alpha 13. *J Biol Chem* 270:18655-18659
 248. Heasley LE, Storey B, Fanger GR, Butterfield L, Zamarripa J, Blumberg D, Maue RA 1996 GTPase-deficient G alpha 16 and G alpha q induce PC12 cell differentiation and persistent activation of cJun NH2-terminal kinases. *Mol Cell Biol* 16:648-656
 249. Mitchell FM, Russell M, Johnson GL 1995 Differential calcium dependence in the activation of c-Jun kinase and mitogen-activated protein kinase by muscarinic acetylcholine receptors in rat 1a cells. *Biochem J* 309:381-384
 250. Chrzanowska-Wodnicka M, Burrige K 1992 Rho, rac and the actin cytoskeleton. *Bioessays* 14:777-778
 251. Ridley AJ 1994 Signal transduction through the GTP-binding proteins Rac and Rho. *J Cell Sci Suppl* 18:127-131
 252. Downward J 1992 Signal transduction. Rac and Rho in tune. *Nature* 359:273-274
 253. Nobes CD, Hall A 1995 Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell* 81:53-62
 254. Ridley AJ, Paterson HF, Johnston CL, Diekmann D, Hall A 1992

- The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell* 70:401-410
255. **Ridley AJ, Hall A** 1992 The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell* 70:389-399
256. **Nobes CD, Hawkins P, Stephens L, Hall A** 1995 Activation of the small GTP-binding proteins rho and rac by growth factor receptors. *J Cell Sci* 108:225-233
257. **Norman JC, Price LS, Ridley AJ, Hall A, Koffer A** 1994 Actin filament organization in activated mast cells is regulated by heterotrimeric and small GTP-binding proteins. *J Cell Biol* 126:1005-1015
258. **Ridley AJ, Hall A** 1994 Signal transduction pathways regulating Rho-mediated stress fibre formation: requirement for a tyrosine kinase. *EMBO J* 13:2600-2610
259. **Goren HJ, Northup JK, Hollenberg MD** 1985 Action of insulin modulated by pertussis toxin in rat adipocytes. *Can J Physiol Pharmacol* 63:1017-1022
260. **Heyworth CM, Grey AM, Wilson SR, Hanski E, Houslay MD** 1986 The action of islet activating protein (pertussis toxin) on insulin's ability to inhibit adenylate cyclase and activate cyclic AMP phosphodiesterases in hepatocytes. *Biochem J* 235:145-149
261. **Vila MC, Milligan G, Standaert ML, Farese RV** 1990 Insulin activates glycerol-3-phosphate acyltransferase (*de novo* phosphatidic acid synthesis) through a phospholipid-derived mediator. Apparent involvement of Gi alpha and activation of a phospholipase C. *Biochemistry* 29:8735-8740
262. **Luttrell LM, Hewlett EL, Romero G, Rogol AD** 1988 Pertussis toxin treatment attenuates some effects of insulin in BC³H-1 murine myocytes. *J Biol Chem* 263:6134-6141
263. **Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M, Mason A, Seeburg PH, Grunfeld C, Rosen OM, Ramachandran J** 1985 Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. *Nature* 313:756-761
264. **Rosen OM** 1987 After insulin binds. *Science* 237:1452-1458
265. **Sasaoka T, Draznin B, Leitner JW, Langlois WJ, Olefsky JM** 1994 Shc is the predominant signaling molecule coupling insulin receptors to activation of guanine nucleotide releasing factor and p21ras-GTP formation. *J Biol Chem* 269:10734-10738
266. **Luttrell LM, van Biesen T, Hawes BE, Koch WJ, Touhara Y, Lefkowitz R J** 1995 G beta gamma subunits mediate mitogen-activated protein kinase activation by the tyrosine kinase insulin-like growth factor 1 receptor. *J Biol Chem* 270:16495-16498
267. **Krupinski J, Rajaram R, Lakonishok M, Benovic JL, Cerione R*** 1988 Insulin-dependent phosphorylation of GTP-binding protein α in phospholipid vesicles. *J Biol Chem* 263:12333-12341
268. **Rothenberg PL, Kahn CR** 1988 Insulin inhibits pertussis toxin-catalyzed ADP-ribosylation of G-proteins. Evidence for a novel interaction between insulin receptors and G-proteins. *J Biol Chem* 263:15546-15552
269. **Luttrell L, Kilgour E, Larner J, Romero G** 1990 A pertussis toxin-sensitive G-protein mediates some aspects of insulin action in BC³H-1 murine myocytes. *J Biol Chem* 265:16873-16879
270. **Kellerer M, Obermaier-Kusser B, Profrock A, Schleicher E, Seffer E, Mushack J, Ermel B, Haring HU** 1991 Insulin activates GTP binding to a 40 kDa protein in fat cells. *Biochem J* 276:103-108
271. **Okamoto T, Murayama Y, Hayashi Y, Ogata E, Nishimoto I** 1993 GTP-binding protein-activator sequences in the insulin receptor. *FEBS Lett* 334:143-148
272. **Moxham CM, Malbon CC** 1996 Insulin action impaired by deficiency of the G-protein subunit G i alpha2. *Nature* 379:840-844