The Pathways Connecting G Protein-coupled Receptors to the Nucleus through Divergent Mitogen-activated Protein Kinase Cascades

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Receptors coupled to heterotrimeric GTP-binding proteins (G proteins) are integral membrane proteins involved in the transmission of signals from the extracellular environment to the cytoplasm. The best known family of G protein-coupled receptors (GPCRs), currently comprising more than 1000 members, exhibits a common structural motif consisting of seven membrane-spanning regions (1) (Fig. 1). A diverse array of external stimuli including neurotransmitters, hormones, phospholipids, photons, odorsants, certain taste ligands, and growth factors can activate specific members of this receptor family and promote interaction between the receptor and the G protein on the intracellular side of the membrane. This causes the exchange of GDP for GTP bound to the G protein α subunit and apparently the dissociation of the βγ heterodimers. In turn, GTP-bound G protein α subunits or βγ complexes initiate intracellular signaling responses by acting on effector molecules such as adenylate cyclases or phospholipases and directly regulating ion channel or kinase function (Fig. 1, and see below). Sixteen distinct mammalian G protein α subunits have been molecularly cloned and are divided into four families based upon sequence similarity: α1, α2, α5, and α12. Similarly, eleven G protein γ subunits and five G protein β subunits have been identified. Thus, GPCRs are likely to represent the most diverse signal transduction systems in eukaryotic cells. The biochemical and biological consequences of such diversity in subunit composition and coupling specificity for each receptor have just begun to be elucidated. In this review, we will briefly describe the role of G proteins and their coupled receptors in normal growth control and tumorigenesis and then focus on current efforts to elucidate the signaling pathways connecting this class of cell surface receptors to nuclear events regulating gene expression.

Proliferative Signaling through G Protein-coupled Receptors

Proliferative signaling has generally been associated with polypeptide growth factor receptors that possess an intrinsic protein tyrosine kinase activity (2). A variety of oncogenes have been found to code for mutated forms of these receptors (3) and their ligands (4) or for molecules that participate in their growth-promoting pathways (5). On the other hand, GPCRs have been traditionally linked to tissue-specific, fully differentiated cell functions (1). However, GPCRs are also expressed in proliferating cells, and they have been implicated in embryogenesis, tissue regeneration, and growth stimulation (reviewed in Ref. 6). In this regard, many ligands acting via GPCRs, including thrombin, bombesin, bradykinin, substance P, endothelin, serotonin, acetylcholine, gastrin, prostaglandin F2α, and lysophosphatidic acid, are known to elicit a mitogenic response in a variety of cell types (reviewed in Refs. 6 and 7), and recent gene knock-out studies indicate that certain GPCRs are essential for cell growth under physiological conditions (8). Furthermore, accumulating evidence indicates that GPCRs and their signaling molecules can harbor oncogenic potential. For example, the mas oncogene, which encodes a putative GPCR, was initially cloned using standard transfection assays by virtue of its ability to induce tumors in mice (9). Subsequently, serotonin 1C (10), muscarinic m1, m3, and m5 (11), and adrenergic α1 (12) receptors were shown effectively to transform contact-inhibited cultures of rodent fibroblasts when persistently activated. Together these studies demonstrated that GPCRs can behave as agonist-dependent oncogenes and prompt several groups to explore the transforming potential of G protein α subunits. In recent studies, constitutively active mutants of Gα12, Gα13, and Gα13-12 were shown to behave as transforming genes in a variety of cell types (reviewed in Ref. 13).

The recent discovery of activating mutations in GPCRs and G proteins in several disease states, including cancer, further supports a role for GPCRs in normal and aberrant growth control. For example, mutationally activated Gαq results in hyperplasia of endocrine cells and has been found in human thyroid and pituitary tumors (reviewed in Ref. 13) and in the McCune-Albright syndrome, a disease in which multiple endocrine glands exhibit autonomous hyperproliferation (14). Interestingly, activated GPCRs also contribute significantly to hyperplasia only in tissues where cAMP stimulates proliferation, thus acting as an oncogene referred to as the gap oncogene (15). Activating mutations have also been identified for Gα12, referred to as the gip2 oncogene, in a subset of ovarian sex cord stromal tumors and adrenal cortical tumors (16). On the other hand, Gα13, referred as the gep oncogene (17, 18), was isolated as a transforming gene from a soft tissue sarcoma-derived cell (19), although its role in tumorigenesis remains unclear. Naturally occurring activated mutations in members of the Gαq family have not yet been described.

At the receptor level, the identification of constitutively active thyroid-stimulating hormone receptor mutations in 30% of thyroid adenomas (20) provided a direct link between this class of receptors and human cancer. Similarly, mutationally activated luteinizing hormone receptors have been identified in a form of familial male precocious puberty, which results from hyperplastic growth of Leydig cells (21). Perhaps more frequently than activating mutations, paracrine and autocrine stimulation of multiple GPCRs for neuropeptides and prostaglandins has been implicated in a number of human neoplasias, including small cell lung carcinoma (22), colon adenomas and carcinomas (23), and gastric hyperplasia and cancer (24). Sequences encoding functional GPCRs have also been found in the genome of transforming DNA viruses, including herpesvirus saimiri (25) and Kaposi's sarcoma-associated herpesvirus (26). Currently available evidence suggests that, at least for Kaposi's sarcoma-associated herpesvirus, these viral GPCRs are sufficient to subvert normal growth control.

The mechanism(s) whereby GPCRs regulate cell proliferation remain poorly understood. Although inhibition of adenyl cyclase has been observed in cells responding to growth-promoting agents acting on Gαi-coupled receptors, there is no formal proof that induction of DNA synthesis results from decreasing intracellular levels of cAMP. Conversely, several lines of investigation have implicated phosphatidylinositol bisphosphate (PIP2) hydrolysis as a critical component of mitogenesis (6). However, recent studies using mutant tyrosine kinase receptors suggested that PIP2 hydrolysis is neither necessary nor sufficient for mitogenesis (27, 28). Further-
Under identical experimental conditions, activated forms of \( \text{Go}_{G a}, \text{Go}_{G b}, \text{Go}_{G b}, \text{or Go}_{G c} \) were not able to induce MAPK activation (35).

The failure of activated \( \text{Go} \) subunits to mimic receptor stimulation of MAPK activity and the accumulating evidence supporting an active role for the \( G\beta \) dimers in signal transmission (37) prompted exploration of the role of \( G\beta \) complexes in signaling to the MAPK pathway. This led to the observation that membrane-bound forms of \( G\beta \) heterodimers can directly elicit signaling pathways leading to MAPK activation (35) and prompted the search for molecules acting downstream of \( G\beta \) in this biochemical route. In a variety of experimental conditions, it was shown that MAPK activation by \( G\beta \) subunits required neither PLC-\( \beta \) nor PKC activation but was blocked by dominant interfering mutants of the GTP-binding protein Ras (34, 35) and that \( G\beta \) subunits can induce the accumulation of Ras in the GTP-bound, active form (34). Taken together, these findings indicated that signaling from GPCRs to MAPK involves \( G\beta \) subunits of heterotrimeric G proteins acting on a Ras-dependent pathway and provided strong evidence that the GPCR signaling pathway converges at the level of Ras with that emerging from receptors of the tyrosine kinase class.

**The Pathway Linking GPCRs and \( G\beta \) to Ras: Tyrosine Kinases, Adaptor Molecules, Phosphoinositide 3-Kinases, PKC, and Novel Molecular Mediators**

The inhibitory effect of genistein on lysophosphatidic acid-induced MAPK activation provided the first indirect indication that tyrosine kinases might mediate the activation of MAPK by GPCRs (38). Furthermore, several groups observed that activation of GPCRs in a variety of cellular systems leads to the rapid phosphorylation of the adaptor protein Shc on tyrosine residues and the consequent formation of Shc-Ras complexes (39, 40). Searching for candidate tyrosine kinases, Luttrel et al. (41) have recently obtained evidence that Src, or a Src-like kinase, links \( G\beta \) to activation of the Ras-MAPK pathway through phosphorylation of Shc and the recruitment of GRB2 and SOS. That report was soon followed by several studies describing the implication of other non-receptor tyrosine kinases linking GPCRs to MAPK. These include Src-like kinases such as Fyn, Lyn, and Yes and the more distantly related Syk (42, 43) and a novel Ca\(^{2+}\) and PKC-dependent protein tyrosine kinase, Pyk2 (44–46). The latter is closely related to focal adhesion kinase, which is involved in the formation of focal complexes containing Src, paxillin, dynamin, and Grb2 after integrin binding. Focal adhesion kinase can also be activated by GPCRs (47, 48) and may possibly be involved in GPCR signaling to MAPK. Tyrosine kinases of the receptor class have also been implicated in GPCR signaling; both PDGF and epidermal growth factor receptors were recently shown to become phosphorylated in response to GPCR agonists (49, 50) and to play a role in MAPK activation by GPCRs by recruiting signaling complexes containing Shc and...
GRB2. In short, it is becoming increasingly clear that a number of non-receptor tyrosine kinases and tyrosine kinase receptors can link GPCRs to the Ras-MAPK pathway. However, the relative contribution of each of these kinases in GPCR signaling to MAPK is still unclear and under current investigation.

Additional potential links between Gβγ and the Ras-MAPK pathway have been recently identified. They include the protein tyrosine phosphatase SH-PTP1 (51) and Ras-GRF, a distinct Ras guanine nucleotide exchange factor expressed in neuronal cells, which can be activated in response to GPCR stimulation or upon coexpression of Gβγ (52). In addition, several groups observed that wortmannin, a phosphatidylinositol 3-kinase (PI3K) inhibitor, can diminish MAPK activation by GPCRs (see Ref. 53), and a novel PI3K isotype, termed PI3Kγ, that is activated by Gβγ complexes (54) was found to play a critical role in linking Gα-coupled receptors and Gβγ to the MAPK signaling pathway (55). In this case, PI3Kγ was found to act downstream from Gβγ and upstream of Src-like kinases, thus suggesting a potential mechanism whereby heterotrimeric G proteins can regulate non-receptor tyrosine kinases.

Ras-independent activation of MAPK by GPCRs has also been reported (56, 57), although it was defined as such primarily based on the failure to observe accumulation of Ras in the GTP-bound form in response to GPCR stimulation. However, as dominant interfering mutants of Ras can diminish MAPK activation, even in systems where GTP-bound Ras was not readily demonstrable (56), it is still possible that undetected amounts of Ras in the GTP-bound form might be sufficient to cooperate with other pathways to induce MAPK activation. Alternatively, in certain cellular backgrounds, GPCRs might be able to utilize pathways bypassing the requirement for Ras activation. One such potential Ras-independent pathway might help explain the activation of MAPK by constitutively active Gt2α, the gip2 oncogene, which can be observed in only a limited number of cell types (58). Another putative Ras-independent pathway might involve PKC, as direct activation of PKC by phorbol esters can induce MAPK in a Ras-dependent or Ras-independent fashion (59, 60). Consequently, in cells where PKC can directly activate signaling pathways leading to MAPK activation, it is expected that MAPK activation by Gα-coupled receptors would not strictly require Ras. In this line, Gαi-coupled receptor activation of MAPK has been shown to be PKC-dependent (60), fully PKC-independent (61), or partially PKC-dependent (62).

We can conclude that multiple molecules may mediate MAPK activation by GPCRs and Gβγ. The expression of some of these molecules follows a restricted tissue distribution (44, 52, 54), which might help explain the seemingly conflicting results obtained by different groups analyzing the relative contribution of each pathway in different cell lines and tissue culture systems. The nature of the biochemical routes utilized to communicate GPCRs to the MAPK pathway would then be expected to depend heavily on the repertoire of signaling molecules available in each particular tissue and cell type.

**G Protein-coupled Receptors Activate the Jun Kinase (JNK) Pathway by a Novel Biochemical Route**

The studies described above strongly suggest that both GPCRs and tyrosine kinase receptors can activate Ras, thereby initiating a cascade of events leading to MAPK activation and transcriptional regulation. However, activation of GPCRs was found to induce a clearly distinct pattern of expression of immediate early genes, including those of the *jun* and *fos* family (64). In particular, activation of GPCRs but not tyrosine kinase receptors for PDGF led, in NIH 3T3 cells, to a remarkable expression of c-Jun (64). This response did not correlate with MAPK activation (64), thus suggesting that GPCRs control a distinct biochemical route regulating gene expression. Furthermore, recent work demonstrated that a novel family of enzymes closely related to MAPK, named Jun kinases (JNKs) (65) or stress-activated protein kinases (SAPKs) (66), selectively phosphorylates and regulates the activity of the c-Jun protein. Based on those findings, the ability to signal to JNK by cell surface receptors was further investigated. Interestingly, in NIH 3T3 cells, GPCRs but not PDGF receptors were found potently to activate JNK (64), thus establishing that the GPCR signaling pathways diverge at the level of JNK from those utilized by tyrosine kinase receptors.

Although it was initially thought that JNKs were located downstream from Ras, this hypothesis was in conflict with the lack of activation of JNK by PDGF or by other agonists acting on receptors that are known to couple to the Ras pathway (64, 66). Soon, it was found that the Ras-related small GTP-binding proteins Rac1 and Cdc42 initiate an independent kinase cascade regulating JNK activity (67) and that Rac and Cdc42 are an integral part of the signaling route linking many cell surface receptors, including GPCRs, to JNK (68). More recent work has identified many components of this pathway and has shown that JNK is potently activated by several naturally occurring human oncogenes (reviewed in Ref. 69). Further examination of the G protein subunits linking GPCRs to JNK provided evidence that free βγ dimers (68) and, in some cellular systems, Gt12G7 (70) transfer signals from this class of receptors to JNK.

The pathway(s) connecting GPCRs to other, recently discovered members of the MAPK superfamily, such as ERK6, ERK5, and SAPK4, have not yet been defined. However, GPCRs have recently been shown to activate a novel pathway that involves the transcriptional regulation of the serum response factor by the small GTP-binding protein Rho (71), and a recent study suggests that both G12 and Gβγ might connect GPCRs to Rho and to serum response factor (72). Those molecules linking GPCRs and heterotrimeric G proteins to Rho remain undefined.

**Conclusion**

The emerging picture from recent reports is that in mammalian cells, βγ subunits of heterotrimeric G proteins communicate GPCRs with the MAPK and JNK pathways acting, respectively, on a Ras and Rac1/Cdc42-dependent biochemical route. These findings together strongly suggest that βγ complexes provide a molecular bridge between heterotrimeric G proteins and small GTP-binding proteins. This connection is strikingly similar to the pathway linking G protein-coupled pheromone receptors to MAPK-related enzymes in the budding yeast *Saccharomyces cerevisiae*. In
yeast, the G protein subunit can initiate activity of a MAPK cascade by binding an exchange factor for the small GTP-binding protein Cdc42, and then this GTP-binding protein physically interacts with the most upstream kinase, Ste20, causing its activation (73). An additional scaffolding protein, Ste5, binds yeast βγ and several components of this MAPK cascade. In mammalian cells a number of sequentially acting molecular switches are required instead to connect GPCRs to the ERK/MAPK pathway, including tyrosine kinases, lipid kinases, adapters, and protein scaffolds, to form a signaling network in this area is expected to help identify the nature of all bio-chemical routes, including those connecting heterotrimeric G proteins to the visual system of the fruit fly (63). Thus, it is conceivable that still unidentified scaffolding proteins might also participate in the mammalian pathway connecting heterotrimeric G proteins to MAPK cascades.

We can conclude that the molecular complexity of the signaling pathways connecting GPCRs to the nucleus has just begun to be appreciated. These pathways involve an unsuspected number of biochemical routes, including those connecting heterotrimeric G proteins to small GTP-binding proteins of the Ras and Rho family, their regulated kinases, and their nuclear targets (Fig. 2). Further work is needed to help identify the nature of all contributing molecular switches, as well as to elucidate fully their functional relationships. Emerging areas of interest also include exploring how all these signaling events that are initiated upon agonist binding to GPCRs, including second messenger generating systems, cytoskeletal changes, and physical interaction of heterotrimeric G protein subunits with molecules regulating kinase cascades, are integrated in space and time to elicit biologically relevant responses, including normal and aberrant cell growth.

REFERENCES


