Journal of Cellular Biochemistry Supplements 30/31:137–146 (1998)

G-Protein Regulatory Pathways: Rocketing into the Twenty-first Century

Cindy Knall[1]* **and Gary L. Johnson**[1,2]

[1]Department of Pediatrics, Program in Molecular Signal Transduction, Division of Basic Sciences, National Jewish Medical and Research Center, Denver, CO 80206
[2]Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80206

Abstract Complex cellular responses involve the integration of heterotrimeric G protein systems with protein kinase signal transduction pathways. Key in this integration is the control of small GTP-binding proteins including Ras and Rho family members. In this paper, we discuss the control of signal transduction pathways by G proteins and their integration with specific tyrosine kinases. The integration of G proteins, kinases, and small GTP-binding proteins in controlling cellular responses is illustrated through the newly defined $G\alpha_{12/13}$-regulated pathways. Furthermore, the polymorphonuclear leukocyte provides a primary cell system for analyzing the integration of G proteins, kinases, and small GTP-binding proteins in controlling cellular functions such as superoxide production, adherence, chemotaxis, and granule secretion. J. Cell. Biochem. Suppls. 30/31:137–146, 1998.

Key words: G proteins; signal transduction; protein tyrosine kinases; PMN

At times, it is hard to fathom the changes that have occurred in the field of G-protein systems over the last quarter of a century. Twenty-five years ago, adenyl cyclase [Birnbaumer, 1973] and the β-adrenergic receptor [Lefkowitz, 1974] were mainstream topics in pharmacology, but no receptor had been purified, and G proteins were yet to be discovered. As we approach the twenty-first century, 20 Gα, 6 Gβ, and 12 Gγ subunits have been identified, and more than 1,000 G-protein-coupled receptors are known. With the various genome projects nearing completion, additional G-protein subunits and receptors will almost certainly be identified.

In addition to their identification, great advances have been made in the structural biology of G proteins and their receptors [Hamm, 1998]. The structures of Gα and Gβγ have been solved in both the active and inactive states. Additionally, a low-resolution structure of rhodopsin, a G-protein-coupled receptor, exists. Likewise, a number of studies have defined contact points between G proteins and their receptors. The high-resolution structural determination of the G-protein-coupled receptor, and the identification of the structural relationship of the receptor with its G protein are most certainly close at hand.

Even though nearly two dozen Gα subunits are known, the number of direct effector molecules has remained small (Table I). Members of the $G\alpha_q$ family can regulate phospholipase Cβ (PLCβ), protein kinase C (PKC), and Bruton's tyrosine kinase (BTK). The $G\alpha_s$ family members positively regulate all isoforms of adenyl cyclase and a voltage-sensitive Ca^{2+} channel. $G\alpha_i$ family members negatively regulate some isoforms of adenyl cyclase, while positively regulating K^+ channels, Gα-interacting protein, phosphatidylinositol 3-kinase-γ (PI3Kγ) and cGMP phosphodiesterase. Interestingly, p115RhoGEF has just been identified as the first direct effector for the $G\alpha_{12/13}$ family [Hart et al., 1998].

In addition to the Gα effectors, the number of Gβγ effectors continues to grow (Table I). Interestingly, many of the classes of effectors are

Contract grant sponsor: National Institutes of Health; Contract grant number: HL09640; Contract grant number: GM30324; Contract grant number: DK37871; Contract grant sponsor: Helen Wohlberg and Herman Lambert Fellowship in Cancer Biology.

*Correspondence to: Cindy Knall, Department of Pediatrics, National Jewish Medical and Research Center, 1400 Jackson St., K1007, Denver, CO 80206.
E-mail: johnsonlab@njc.org

Received 7 October 1998; Accepted 8 October 1998

TABLE I. Heterotrimeric G-Protein Effectors

Subunit family	Effectors
$G\alpha_s$	All isotypes of adenyl cyclase
	Voltage-sensitive Ca^{2+} channel
$G\alpha_i$	Type 5 and 6 adenyl cyclase
	K^+ channel
	Gα-interacting protein
	PI3Kγ
	CGMP phosphodiesterase
$G\alpha_q$	PLCβ
	PKC
	BTK
$G\alpha_{12}$	P115RhoGEF
Gβγ	PLCβ
	PI3K
	Type 1, 2, 4, and 7 adenyl cyclase
	β-Adrenergic receptor kinase
	Phosducin
	K^+, Ca^{2+}, and Na^+ channels
	Src-family kinases
	BTK/Tec family kinases
	Rho family members
	Arf

shared between Gβγ and Gα subunits. Gβγ subunits regulate several isoforms of adenyl cyclase, both positively and negatively, as well as PLCβ, multiple isoforms of PI3K, β-adrenergic receptor kinase, phosducin, various ion channels, protein tyrosine kinases, and specific low-molecular-weight GTP binding proteins. Therefore, the Gβγ complex is as effective as the Gα subunit at transmitting a signal from the activated receptor to the interior of the cell, and in certain instances may actually be the primary transducer.

The research focus in the field has thus moved from questions of simple identification to questions of the mechanism of action of these effectors working in concert to regulate whole signaling pathways and cellular processes. Three areas of intense interest to our laboratory are the role of protein tyrosine kinases as G-protein effectors, the pathways regulated by $G\alpha_{12/13}$, and the use of primary polymorphonuclear leukocytes as a model for the regulation of cell functions by G-protein-coupled receptors.

PROTEIN TYROSINE KINASES: THE NEW G-PROTEIN EFFECTORS

Protein tyrosine kinases (PTK) were first implicated in G-protein-mediated signal transduction because lysophosphatidic acid (LPA)-stimulated responses were blocked by genistein, a PTK inhibitor [van Corven et al., 1993]. We now know that a variety of G-protein-coupled receptor agonists stimulate tyrosine phosphorylation. Furthermore, the evidence is now mounting that different classes of PTK interact to mediate this response (Table II). The identification of the specific kinases responsible for these phosphorylation events has remained controversial, however, and is an area of intense investigation.

Src and Src-Family Kinases

Just as in many other receptor systems, c-Src, and Src-family kinases have been implicated in G-protein-coupled receptor systems. Gq-coupled m1 muscarinic acetylcholine receptor

TABLE II. G-Protein-Regulated Protein Tyrosine Kinases

Kinase	Stimuli
c-Src and Src family	Angiotensin II
	Carbachol
	Endothelin-1
	fMLP
	IL-8
	LPA
	Thrombin
Pyk2	Angiotensin II
	Bradykinin
	Carbachol
	Mip1β
	LPA
	Rantes
	SDF-1
BTK/Tec family	$G\alpha_i$
	$G\alpha_q$
	Gβγ
Syk	Carbachol
	fMLP
	IL-8
	GROα
JAK	Angiotensin II
	α-Melanocyte-stimulating hormone
Receptor PTK	Angiotensin II
	α2A adrenergic receptor
	Bombesin
	Carbachol
	Endothelin-1
	Gβγ
	LPA
	Thrombin

*See text for discussion.

(m1AChR), transiently expressed in a B-cell lymphoma, required the activation of Lyn, a member of the Src-family, to activate extracellular signal-regulated kinase (ERK) [Wan et al., 1996]. In a similar manner, c-Src was required for angiotensin II, endothelin-1, and thrombin-stimulated vascular smooth muscle cell proliferation [Schieffer et al., 1997], and for LPA to transiently inhibit gap junction mediated cell-cell communication between Rat-1 fibroblasts [Postma et al., 1998]. In COS-7 cells, LPA [Luttrell et al., 1996], m1AChR, and m2AChR [Igishi and Gutkind, 1998] activated c-Src, which was required for the activation of ERK. Interestingly, c-Src activation could be mimicked by overexpression of Gβγ subunits, suggesting that the Gβγ complex, rather than Gα, mediates the activation of c-Src [Luttrell et al., 1996; Igishi and Gutkind, 1998]. These data suggest that c-Src and/or Src-family members play a key role in G-protein-mediated responses. However, c-Src may not be the whole story. Kranenburg et al. [1997] reported that through its Gi-coupled receptor, LPA stimulated ERK activation in Rat-1 fibroblasts and COS cells independent of c-Src. Similarly, Gi-coupled m2AChR did not require Lyn for ERK activation in a B-cell lymphoma [Wan et al., 1996]. Therefore, the role of c-Src and Src-family kinases remains controversial. The contradictory results may reflect differences in G protein-coupling and/or differences in the expression patterns of the various kinases that constitute the Src-family. More extensive analyses using a variety of cell types and receptor systems will need to be performed to reconcile the discrepancies in the data, as well as to establish the specific role of c-Src and Src-family kinases.

Pyk2

The PTK Pyk2 may hold the most promise as a common G-protein effector because of its wide distribution [Lev et al., 1995]. A variety of G-protein receptor agonists activate Pyk2 including bradykinin [Lev et al., 1995], angiotensin II, and LPA [Yu et al., 1996], Rantes and SDF-1α [Davis et al., 1997], MIP-1β [Ganju et al., 1998], and carbachol through either the nicotinic acetylcholine receptor [Lev et al., 1995] or the m1AChR [Felsch et al., 1998]. Pyk2 activation has been linked with c-Src in the Gq-coupled and Gi-coupled receptor-mediated activation of ERK in PC12 cells [Dikic et al., 1996], HEK 293 cells [Della Rocca et al., 1997], and osteoblasts [Jeschke et al., 1998]. Similarly, Pyk2 activation is required for MIP-1β activation of c-jun N-terminal kinase (JNK) and p38-mitogen-activated protein kinase (p38-MAPK) [Ganju et al., 1998]. These data suggest that Pyk2 has a common role as a G-protein effector for multiple G-protein-coupled receptors in a variety of cell types.

Btk/Tec Family Kinases

The Btk/Tec family [Rawlings and Witte, 1995], which has a wide distribution, also has been implicated in G-protein-regulated systems. Gβγ subunits bind to Btk via the pleckstrin homology domain in Btk [Tsukada et al., 1994]. However, the significance of this in terms of activation of Btk is unclear. More recently, it was demonstrated that $G\alpha_q$ could activate Btk directly both in vitro and in vivo, and that this activation was required for the activation of p38-MAPK by Gq-coupled receptors [Bence et al., 1997]. Although $G\alpha_q$ could activate Btk, $G\alpha_{i1}$, $G\alpha_o$ and $G\alpha_z$ could not, suggesting that activation of Btk may be Gα subunit specific. Of course, this does not rule out the possibility that other members of this family could be effectors for other Gα subunits. Interestingly, Gi-coupled receptor-mediated activation of ERK was blocked in B cells lacking Btk [Wan et al., 1997]. This finding suggests that the activation of PTK may be pathway specific or that Gβγ subunits associated with $G\alpha_i$ may mediate this activation. Also, like other nonreceptor cytoplasmic PTK, the Btk/Tec family members have tissue and cell type specific expression patterns that may influence their interaction with Gα, as well as Gβγ subunits [Rawlings and Witte, 1995]. Defining a common role for Btk/Tec family kinases as G protein effectors will require further investigation using multiple G protein-coupled receptors expressed on a variety of cell types.

Jak

Most recently, the Janus kinase (JAK) family [Ihle, 1995], which is required for cytokine-mediated responses, has been implicated in G-protein-regulated signal transduction. Inhibition of JAK2, blocked angiotensin II-stimulated vascular smooth muscle cell proliferation [Marrero et al., 1997]. Similarly, JAK2 was activated in B cells by α-melanocyte-stimulating hormone [Buggy, 1998]. The immediate downstream mediator of JAK2, signal transducers,

and activators of transcription protein (STAT1) [Ihle, 1996] was also activated in both systems. Whether other members of the JAK/STAT families can be activated by G-protein-coupled receptors remains to be tested. Like the other kinase families, it remains to be seen whether this represents a common theme among G-protein-coupled receptors.

Syk

In addition to the widely expressed cytoplasmic PTK, the cytoplasmic PTK Syk [van Oers and Weiss, 1995] has been implicated in cell type and lineage-specific G-protein-coupled receptor systems. In a B-cell lymphoma-deficient in Syk, both Gq-coupled m1AChR and Gi-coupled m2AChR could not activate ERK [Wan et al., 1996]. Furthermore, Syk is activated in polymorphonuclear leukocytes (PMN) by a number of G-protein-coupled receptor agonists [Asahi et al., 1995; Fernandez and Suchard, 1998]. Syk is only known to be expressed in cells of the hematopoietic lineage. Therefore, a role for Syk in G-protein systems is restricted to cells of this lineage.

Receptor PTK

Finally, an alternative to cytoplasmic PTK as effectors of G-protein-coupled receptor systems is the G-protein-mediated transactivation of a receptor PTK. G-protein-coupled receptors agonists angiotensin II [Linseman et al., 1995], thrombin [Rao et al., 1995], and endothelin-1, LPA, and thrombin [Daub et al., 1996] induced the transactivation of the platelet-derived growth factor receptor (PDGFR), the insulin-like growth factor-1 receptor (IGF-1R), and the epidermal growth factor receptor (EGFR), respectively. Additionally, α2A adrenergic receptor [Luttrell et al., 1997], m1AChR [Tsai et al., 1997], bombesin receptor, and m2AChR [Daub et al., 1997] also transactivated EGFR. This transactivation was stimulated not only by a variety of agonists, but also in a variety of cell types, including rat smooth muscle cells [Linseman et al., 1995; Rao et al., 1995], rat-1 fibroblasts [Daub et al., 1996], 293 cells [Tsai et al., 1997], and keratinocytes, astrocytes, and Cos-7 cells [Daub et al., 1997]. Therefore, this process may well be a common theme among G-protein-coupled receptor systems and cell types. Furthermore, the data suggest that this transactivation is required for G-protein-mediated activation of ERK [Daub et al., 1996, 1997; Tsai et al., 1997].

The mechanism by which G-protein-coupled receptors transactivate these receptor PTK is not completely understood. Overexpression of the Gβγ complex mimicked the transactivation of EGFR by Gi-coupled receptors [Luttrell et al., 1997]. Transactivation of other receptor PTK by this mechanism has not been demonstrated, nor has it been demonstrated for other classes of G-protein-coupled receptors. A common theme that is emerging suggests that activation of c-Src may, at least in part, account for the transactivation [Daub et al., 1997; Luttrell et al., 1997]. These results then continue the theme that different classes of PTK work together to activate G-protein-regulated pathways.

$G\alpha_{12/13}$: THE MOST RECENTLY DEFINED G-PROTEIN PATHWAY

The most recently identified family of Gα subunits is composed of $G\alpha_{12/13}$. This family is unique because until just months ago there were no known direct effectors. Nevertheless in recent years, several groups have demonstrated $G\alpha_{12/13}$-mediated regulation of specific cellular processes, including Na^+/H^+ exchangers, the cytoskeleton, transcription, and DNA synthesis, and vascular development (Fig. 1). Conse-

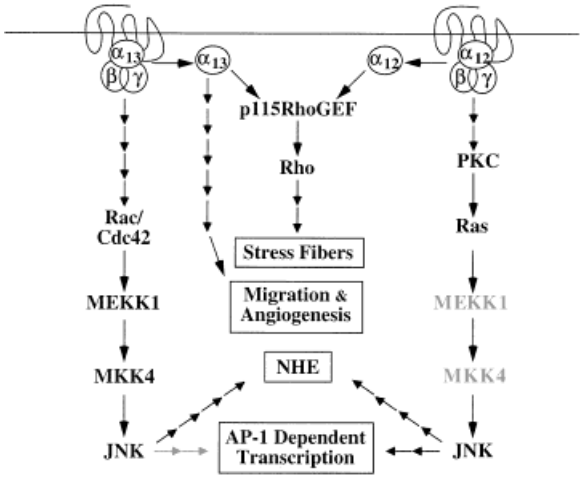

Fig. 1. $G\alpha_{12/13}$ regulation of specific effector pathways and cellular responses. *Black arrows* and text denote defined relationships, while *gray arrows* and text denote presumed relationships. $G\alpha_{12/13}$ use the same effector, p115RhoGEF, to regulate Rho and stress fiber formation. The direct effectors for the other pathways are unknown. $G\alpha_{12/13}$ use distinct low-molecular-weight GTP binding proteins to regulate JNK activation and AP-1-mediated transcription. Interestingly, only $G\alpha_{13}$ regulates angiogenesis in mice.

quently, these observations have established $G\alpha_{12/13}$ as the newest G-protein regulators of cellular processes.

Na^+/H^+ Exchanger

The first function defined for $G\alpha_{12/13}$ was the regulation of a Na^+/H^+ exchanger (NHE) [Dhanasekaran et al., 1994]. Subsequently, it was shown that $G\alpha_{13}$ activates all three NHE, while $G\alpha_{12}$ activates NHE2 and NHE3, but inhibits NHE1 [Lin et al., 1996]. Interestingly, $G\alpha_{12}$, but not $G\alpha_{13}$, regulation of NHE was shown to be dependent on PKC [Dhanasekaran et al., 1994], and Ras, but not Rac/Cdc42 or JNK [Wadsworth et al., 1997]. By contrast, $G\alpha_{13}$ was shown to use a Rac/Cdc42/MEKK1/JNK pathway to regulate NHE1 [Hooley et al., 1996; Wadsworth et al., 1997]. The direct effector(s) for $G\alpha_{12/13}$ regulation of NHE, however, remain to be identified.

Cytoskeleton

Buhl et al. [1995] demonstrated for the first time that $G\alpha_{12/13}$ regulate the cytoskeleton. This work demonstrated that $G\alpha_{12/13}$ themselves, but not $G\beta\gamma$, $G\alpha_{i2}$ or $G\alpha_q$, stimulated the Rho-dependent formation of stress fibers and focal adhesions in Swiss 3T3 cells. Recently, it was shown that $G\alpha_{13}$ was required for LPA-stimulated Rho-dependent stress fiber formation in these cells [Gohla et al., 1998]. Interestingly, both LPA and activated $G\alpha_{13}$-stimulated stress fiber formation were inhibited by dominate negative EGFR expression, suggesting that the EGFR pathway is required for $G\alpha_{13}$ regulation of this process [Gohla et al., 1998]. Additionally, an activator of Rho, p115RhoGEF, was recently identified as the first direct effector of $G\alpha_{12/13}$ [Hart et al., 1998] establishing the first complete $G\alpha_{12/13}$ pathway. It will be interesting to see whether other G12/13-coupled receptors possess a similar ability to regulate the cytoskeleton in other cell types.

Transcription and DNA Synthesis

In addition to the regulation of the Na^+/H^+ exchanger and the cytoskeleton, $G\alpha_{12}$ has been implicated in the regulation of AP-1 mediated transcription and DNA synthesis in astrocytes stimulated by thrombin [Aragay et al., 1995; Post et al., 1996]. $G\alpha_{12}$ required Ras, but not ERK [Post et al., 1996], to regulate AP-1-stimulated transcription [Aragay et al., 1995]. This is consistent with the observation that constitutive active forms of $G\alpha_{12/13}$ do not activate ERK, but do activate JNK, the upstream activator of c-jun a component of the AP-1 complex, in a Ras dependent manner [Prasad et al., 1995; Collins et al., 1996].

Vascular Development and Migration

The best evidence for the critical role of this G-protein family in cellular, as well as organismal biology comes from the results of the disruption of the $G\alpha_{13}$ gene in mice [Offermanns et al., 1997]. Using standard homologous recombination technology, mice heterozygous for the $G\alpha_{13}$ gene were generated and these mice were normal. Surprisingly, no homozygous mice were born. Upon histological analysis, it was determined that mice lacking $G\alpha_{13}$ failed to develop a vascular system at E8.5, even though $G\alpha_{12}$ expression was normal in these embryos and endothelial cells had differentiated. These results indicate that $G\alpha_{13}$ is required for angiogenesis and that $G\alpha_{12}$ cannot substitute for $G\alpha_{13}$. Angiogenesis is dependent on endothelial migration which may be defective in these mice since fibroblasts generated from these embryos do not migrate in response to G13-coupled receptor agonists such as thrombin.

PMN: A MODEL SYSTEM OF G-PROTEIN REGULATION

PMN serve as the first line of defense against bacterial infections, and as such they are the most abundant cell type in peripheral blood. Because of their repertoire of defense mechanisms, including the ability to rapidly migrate, generate reactive oxygen intermediates, release hydrolytic enzymes and engulf large particles, PMN exist in a quiescent, but rapidly inducible state in the circulation. Consequently, large numbers of resting PMN are easily isolated from healthy human donors. These PMN can be activated using any one of a number of soluble inflammatory mediators most of which bind to G-protein-coupled receptors. These properties have made PMN an ideal system for investigating G-protein-regulated signal transduction pathways, and their relationship to the induction and control of cell functions in primary cells (Fig. 2).

G Proteins and G-Protein-Coupled Receptors

PMN express a number of G proteins and G-protein-coupled receptors. PMN express the

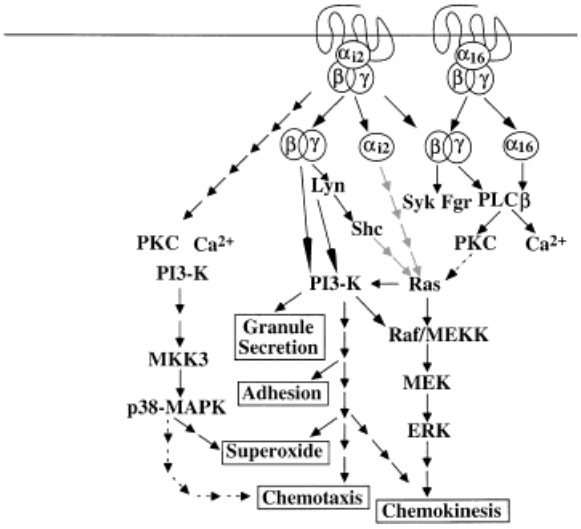

Fig. 2. Signal transduction pathways known to be activated in polymorphonuclear leukocytes (PMN) by G-protein-coupled receptors. *Black arrows* and text denote defined relationships, while *gray arrows* and text denote presumed relationships. *Dashed lines,* relationships defined for some but not all agonists. G-protein-coupled receptor agonists activate a number of signal transduction molecules in PMN. The relationship of direct effectors to the activation of these molecules is still being defined. The relationship of the various signal transduction molecules to the control of cellular functions is an area of vigorous investigation.

G proteins $G\alpha_s$, $G\alpha_{i2}$, $G\alpha_{i3}$, $G\alpha_q$, $G\alpha_{16}$, and $G\alpha_{12/13}$ [Spiegel, 1992]. Examples of G protein-coupled receptors expressed by PMN include receptors for leukotriene B4 (LTB4), platelet-activating factor (PAF), formyl-methionyl-leucyl-phenylalanine (fMLF/fMLP), complement component 5a (C5a), IL-8, and growth-regulated oncogene-α (GROα) [Murphy, 1994]. Therefore, PMN can be stimulated by a variety of factors, including nucleotides, lipid molecules, and proteins. The signal transduction pathways regulated by these stimuli will of course depend on the G proteins to which each receptor is coupled. Further investigation is required to identify all the combinations of receptors and G proteins in PMN.

G-protein-Regulated Pathways

Steady progress has been made in defining the pathways used to control the variety of PMN functions stimulated through G-protein-coupled receptors. A previous review by Bokoch [1995] covered a large body of work in this field. Therefore, we will focus on more recent observations in particular concerning the activation of kinases, both protein and phospholipid, and to highlight the areas that require further attention.

Tyrosine kinases. As noted previously [Bokoch, 1995], several groups demonstrated early on the inhibitory effect of PTK inhibitors on PMN function, as well as the tyrosine phosphorylation of several proteins in PMN after stimulation through G-protein-coupled receptors. More recently, the activation of Src-family kinases was shown to occur in PMN stimulated by various agonists. Lyn was activated in PMN stimulated by fMLP [Ptasznik et al., 1995], IL-8, and GROα [Gaudry et al., 1995]. However, Lyn may not be required by these agonists. FMLP can activate ERK in the absence of Lyn expression, indicating that this kinase is not required for the activation of ERK by G-protein-coupled receptors [Torres and Ye, 1996]. Furthermore, we were unable to detect Lyn activation in response to IL-8 or GROα (C.K., unpublished observation). Recently, we demonstrated the activation of Fgr in PMN stimulated by IL-8, but not GROα (C.K., unpublished observation). Additionally, we were unable to detect any activation of Hck in PMN stimulated by either IL8 or GROα (C.K., unpublished observation). Interestingly, non-adherent PMN from $fgr^{-/-}/hck^{-/-}$ mice function normally indicating that these kinases are not required in nonadherent PMN [Lowell et al., 1996]. Therefore, the specific role of Src-family PTK in G protein-regulated pathways in PMN remains unclear and may depend on the specific G protein coupling of a receptor.

In addition to the Src-family kinases, PMN express Syk. This kinase was activated in PMN stimulated by fMLP [Asahi et al., 1995; Fernandez and Suchard, 1998], IL-8, and GROα (C.K., unpublished observation). Although, Syk was essential for both Gq-coupled m1AChR and Gi-coupled m2AChR activation of ERK in a B-cell lymphoma [Wan et al., 1996], it remains to be determined whether activation of Syk is required by G-protein-coupled receptors in PMN.

Phosphatidylinositol 3-kinases. As in other cell types, PI3K has come to the forefront in the regulation of PMN functions stimulated through G-protein-coupled receptors. PMN are known to express three distinct PI3K isoforms, α [Arcaro and Wymann, 1993], γ [Stephens et al., 1994; Stoyanov et al., 1995], and δ [Vanhaesebroeck et al., 1997]. The activation of PI3Kα and δ is believed to be dependent on tyrosine phosphorylation events. The activa-

tion of PI3Kγ is dependent on the release of Gβγ subunits. In PMN, PI3K activity is stimulated by a number of agonists such as fMLP [Traynor-Kaplan et al., 1988] and IL-8 [Knall et al., 1996]. Whether all three isoforms are activated by each stimulus is unknown. Ptasznik et al. [1996] argued that Lyn-mediated activation of PI3Kα is the predominant source of phosphatidylinositol 3,4,5-trisphosphate in PMN. However, the activation of PI3Kα and PI3Kδ is most likely similar, as these isoforms share common regulatory subunits [Vanhaesebroeck et al., 1997]. Therefore, Ptasznik et al. may have been inhibiting PI3Kδ, as well as PI3Kα. Furthermore, all three isoforms are sensitive to the commonly used PI3K inhibitors wortmannin [Arcaro and Wymann, 1993] and Ly294002 [Vlahos et al., 1994]. These inhibitors block superoxide production [Baggiolini et al., 1987], granule secretion [Knall et al., 1996], adhesion [Knall et al., 1996; Capodici et al., 1998], chemotaxis, and chemokinesis [Knall et al., 1997] of PMN. Which specific isoforms of PI3K regulate these PMN functions remains to be determined. Nevertheless, the evidence strongly points to the fact that PI3K activity plays a central regulatory role in the induction of these functions by G-protein-coupled receptors on PMN.

Mitogen-activated protein kinases. During the past few years, much attention has been focused on the activation of the MAPK family by a variety of stimuli in a number of cell types. PMN are no exception. ERK and p38-MAPK are activated in PMN stimulated by G-protein-coupled receptor agonists such as fMLP [Torres et al., 1993; Krump et al., 1997; Nick et al., 1997], C5a [Buhl et al., 1994], IL-8 [Knall et al., 1996, 1997], and PAF [Nick et al., 1997]. Interestingly, there is no report of JNK activation in PMN stimulated by any soluble mediator. The reason for the lack of a JNK response in PMN is unknown.

PMN use the Ras/Raf/MEK pathway [Buhl et al., 1994; Worthen et al., 1994; Knall et al., 1996] and the Ras/MEKK1/MEK pathway [Avdi et al., 1996] to activate ERK. Additionally, PI3K activity is required for activation of ERK at the level of Raf [Knall et al., 1996] and MEKK1 [Avdi et al., 1996] in PMN. The pathway(s) leading from the G-protein-coupled receptor to the activation of Ras in PMN is less well defined. Ptasznik et al. [1995] suggested that Lyn/Shc complexes formed in PMN stimulated by fMLP could regulate the activation of Ras by activating Grb2/SOS, but this has not been proven. PKC appears to play a role in C5a [Buhl et al., 1994], but not fMLP [Worthen et al., 1994] or IL8 (C.K., unpublished observation) stimulation of ERK, whereas increases in intracellular calcium may have a role in fMLP activation of this pathway [Dusi et al., 1994]. Therefore, the identification of pathway components which link the G protein-coupled receptors to the activation of Ras and ERK activation in PMN await further investigation.

The pathway leading to p38-MAPK activation in PMN is even more poorly defined. Although MKK3, the direct activator of p38-MAPK, was activated by fMLP and PAF [Nick et al., 1997] little else is known about the components that lie upstream of MKK3. Inhibiting intracellular calcium increases, PKC or PI3K partially blocked p38-MAPK activation stimulated by fMLP [Krump et al., 1997], suggesting that these signal transduction molecules regulate p38-MAPK activation in PMN. It remains to be seen whether p38-MAPK pathway components defined in other cell types will apply to p38-MAPK activation in PMN.

Although the activation of ERK and p38-MAPK in PMN in response to a number of stimuli is well established, the role of these kinases in the G protein regulation of PMN functions is still evolving. PMN chemotaxis and primary granule secretion occur normally in response to IL8 [Knall et al., 1997] or GROα (C.K., unpublished observation) when the activation of either ERK or p38-MAPK is blocked. However, in the case of fMLP or PAF, PMN migration is partially inhibited, and superoxide production is completely inhibited if p38-MAPK activity is blocked [Nick et al., 1997]. Therefore, ERK and p38-MAPK may regulate some PMN functions in response to some but not all agonists. These differences may reflect differences in G-protein-coupling to specific receptors.

CONCLUSION

The human genome project has provided a growing database that suggests that G-protein-coupled receptors represent 1–5% of human genes. This prediction is based on the frequency of predicted G-protein-coupled receptors, characterized by their seven transmembrane receptor structure. Given the estimated 50,000–100,000 human genes, there are a predicted 500–5,000 human G-protein-coupled receptors. Subtracting the predicted number of olfaction

receptors, several hundred to thousands of G-protein-coupled receptors remain. The ligands for only 150 or so of these receptors are known. Thus, most of these receptors are "orphan" receptors. Undoubtedly, these "orphans" will be found to play critical regulatory roles in complex cellular processes. As discussed above, these responses will involve the integration of signaling by Gα and Gβγ effectors, tyrosine kinases, and small GTP binding proteins. Many unknowns exist, such as the reason so many RGS proteins are expressed and if they have effector functions in addition to GTPase-activating function. The interest in these questions is keen and answers are rapidly being obtained. The next few years will be extremely informative as we define the number of players from genome sequencing. The hard work will be defining the integration of G-protein signaling in the control of complex regulatory systems.

ACKNOWLEDGMENTS

This work was supported by grants HL09640 (C.K.), GM30324, and DK37871 (G.L.J.) from the National Institutes of Health, and by the Helen Wohlberg and Herman Lambert Fellowship in Cancer Biology (C.K.).

REFERENCES

Aragay AM, Collins LR, Post GR, Watson AJ, Feramisco JR, Brown JH, Simon MI (1995): G12 requirement for thrombin-stimulated gene expression and DNA synthesis in 1321N1 astrocytoma cells. J Biol Chem 270:20073–20077.

Arcaro A, Wymann MP (1993): Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: The role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses. Biochem J 296:297–301.

Asahi M, Tanaka Y, Qin S, Tsubokawa M, Sada K, Minami Y, Yamamura H (1995): Cyclic AMP-elevating agents negatively regulate the activation of p72syk in N-formylmethionyl-leucyl-phenylalanine receptor signaling. Biochem Biophys Res Commun 212:887–893.

Avdi NJ, Winston BW, Russel M, Young SK, Johnson GL, Worthen GS (1996): Activation of MEKK by formylmethionyl-leucyl-phenylalanine in human neutrophils. Mapping pathways for mitogen-activated protein kinase activation. J Biol Chem 271:33598–33606.

Baggiolini M, Dewald B, Schnyder J, Ruch W, Cooper PH, Payne TG (1987): Inhibition of the phagocytosis-induced respiratory burst by the fungal metabolite wortmannin and some analogues. Exp Cell Res 169:408–418.

Bence K, Ma W, Kozasa T, Huang XY (1997): Direct stimulation of Bruton's tyrosine kinase by G(q)-protein alpha-subunit. Nature 389:296–299.

Birnbaumer L (1973): Hormone-sensitive adenylyl cyclases. Useful models for studying hormone receptor functions in cell-free systems. Biochim Biophys Acta 300:129–158.

Bokoch GM (1995): Chemoattractant signaling and leukocyte activation. Blood 86:1649–1660.

Buggy JJ (1998): Binding of alpha-melanocyte-stimulating hormone to its G-protein-coupled receptor on B-lymphocytes activates the Jak/STAT pathway. Biochem J 331: 211–216.

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.

Buhl AM, Johnson NL, Dhanasekaran N, Johnson GL (1995): G alpha 12 and G alpha 13 stimulate Rho-dependent stress fiber formation and focal adhesion assembly. J Biol Chem 270:24631–24634.

Capodici C, Hanft S, Feoktistov M, Pillinger MH (1998): Phosphatidylinositol 3-kinase mediates chemoattractant-stimulated, CD11b/CD18-dependent cell-cell adhesion of human neutrophils: evidence for an ERK-independent pathway. J Immunol 160:1901–1909.

Collins LR, Minden A, Karin M, Brown JH (1996): Galpha12 stimulates c-Jun NH2-terminal kinase through the small G proteins Ras and Rac. J Biol Chem 271:17349–17353.

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.

Daub H, Wallasch C, Lankenau A, Herrlich A, Ullrich A (1997): Signal characteristics of G protein-transactivated EGF receptor. EMBO J 16:7032–7044.

Davis CB, Dikic I, Unutmaz D, Hill CM, Arthos J, Siani MA, Thompson DA, Schlessinger J, Littman DR (1997): Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4or CCR5. J Exp Med 186:1793–1798.

Della Rocca GJ, van Biesen T, Daaka Y, Luttrell DK, Luttrell LM, Lefkowitz RJ (1997): Ras-dependent mitogen-activated protein kinase activation by G protein-coupled receptors. Convergence of Gi- and Gq-mediated pathways on calcium/calmodulin, Pyk2, and Src kinase. J Biol Chem 272:19125–19132.

Dhanasekaran N, Prasad MV, Wadsworth SJ, Dermott JM, van Rossum G (1994): Protein kinase C-dependent and -independent activation of Na^+/H^+ exchanger by G alpha 12 class of G proteins. J Biol Chem 269:11802–11806.

Dikic I, Tokiwa G, Lev S, Courtneidge SA, Schlessinger J (1996): A role for Pyk2 and Src in linking G-protein-coupled receptors with MAP kinase activation. Nature 383:547–550.

Dusi S, Donini M, Rossi F (1994): Tyrosine phosphorylation and activation of NADPH oxidase in human neutrophils: A possible role for MAP kinases and for a 75 kDa protein. Biochem J 304:243–250.

Felsch JS, Cachero TG, Peralta EG (1998): Activation of protein tyrosine kinase PYK2 by the m1 muscarinic acetylcholine receptor. Proc Natl Acad Sci USA 95:5051–5056.

Fernandez R, Suchard SJ (1998): Syk activation is required for spreading and H2O2 release in adherent human neutrophils. J Immunol 160:5154–5162.

Ganju RK, Dutt P, Wu L, Newman W, Avraham H, Avraham S, Groopman JE (1998): Beta-chemokine receptor CCR5 signals via the novel tyrosine kinase RAFTK. Blood 91: 791–797.

Gaudry M, Gilbert C, Barabe F, Poubelle PE, Naccache PH (1995): Activation of Lyn is a common element of the stimulation of human neutrophils by soluble and particulate agonists. Blood 86:3567–3574.

Gohla A, Harhammer R, Schultz G (1998): The G-protein G13 but not G12 mediates signaling from lysophosphatidic acid receptor via epidermal growth factor receptor to Rho. J Biol Chem 273:4653–4659.

Hamm HE (1998): The many faces of G protein signaling. J Biol Chem 273:669–672.

Hart MJ, Jiang X, Kozasa T, Roscoe W, Singer WD, Gilman AG, Sternweis PC, Bollag G (1998): Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by Galpha13 [see comments]. Science 280:2112–2114.

Hooley R, Yu CY, Symons M, Barber DL (1996): G alpha 13 stimulates Na^+-H^+ exchange through distinct Cdc42-dependent and RhoA-dependent pathways. J Biol Chem 271:6152–6158.

Igishi T, Gutkind JS (1998): Tyrosine kinases of the Src family participate in signaling to MAP kinase from both Gq and Gi-coupled receptors. Biochem Biophys Res Commun 244:5–10.

Ihle JN (1995): The Janus protein tyrosine kinases in hematopoietic cytokine signaling. Semin Immunol 7:247–254.

Ihle JN (1996): STATs: Signal transducers and activators of transcription. Cell 84:331–334.

Jeschke M, Standke GJR, Scaronuscarona M (1998): Fluoroaluminate induces activation and association of Src and Pyk2 tyrosine kinases in osteoblastic MC3T3-E1 cells. J Biol Chem 273:11354–11361.

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.

Knall C, Worthen GS, Johnson GL (1997): Interleukin 8-stimulated phosphatidylinositol-3-kinase activity regulates the migration of human neutrophils independent of extracellular signal-regulated kinase and p38 mitogen-activated protein kinases. Proc Natl Acad Sci USA 94: 3052–3057.

Kranenburg O, Verlaan I, Hordijk PL, Moolenaar WH (1997): Gi-mediated activation of the Ras/MAP kinase pathway involves a 100 kDa tyrosine-phosphorylated Grb2 SH3 binding protein, but not Src nor Shc. EMBO J 16:3097–3105.

Krump E, Sanghera JS, Pelech SL, Furuya W, Grinstein S (1997): Chemotactic peptide N-formyl-met-leu-phe activation of p38 mitogen-activated protein kinase (MAPK) and MAPK-activated protein kinase-2 in human neutrophils. J Biol Chem 272:937–944.

Lefkowitz RJ (1974): Commentary. Molecular pharmacology of beta-adrenergic receptors—A status report. Biochem Pharmacol 23:2069–2076.

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 [see comments]. Nature 376:737–745.

Lin X, Voyno-Yasenetskaya TA, Hooley R, Lin CY, Orlowski J, Barber DL (1996): Galpha12 differentially regulates Na+-H+ exchanger isoforms. J Biol Chem 271:22604–22610.

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.

Lowell CA, Fumagalli L, Berton G (1996): Deficiency of Src family kinases p59/61hck and p58c-fgr results in defective adhesion-dependent neutrophil functions. J Cell Biol 133:895–910.

Luttrell LM, Della Rocca GJ, van Biesen T, Luttrell DK, Lefkowitz RJ (1997): Gbetagamma subunits mediate Src-dependent phosphorylation of the epidermal growth factor receptor. A scaffold for G protein-coupled receptor-mediated Ras activation. J Biol Chem 272:4637–4644.

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 Gbetagamma subunit-mediated activation of mitogen-activated protein kinases. J Biol Chem 271:19443–19450.

Marrero MB, Schieffer B, Li B, Sun J, Harp JB, Ling BN (1997): Role of Janus kinase/signal transducer and activator of transcription and mitogen-activated protein kinase cascades in angiotensin II- and platelet-derived growth factor-induced vascular smooth muscle cell proliferation. J Biol Chem 272:24684–24690.

Murphy PM (1994): The molecular biology of leukocyte chemoattractant receptors. Annu Rev Immunol 12:593–633.

Nick JA, Avdi NJ, Young SK, Knall C, Gerwins P, Johnson GL, Worthen GS (1997): Common and distinct intracellular signaling pathways in human neutrophils utilized by platelet activating factor and FMLP. J Clin Invest 99:975–986.

Offermanns S, Mancino V, Revel JP, Simon MI (1997): Vascular system defects and impaired cell chemokinesis as a result of Galpha13 deficiency. Science 275:533–536.

Post GR, Collins LR, Kennedy ED, Moskowitz SA, Aragay AM, Goldstein D, Brown JH (1996): Coupling of the thrombin receptor to G12 may account for selective effects of thrombin on gene expression and DNA synthesis in 1321N1 astrocytoma cells. Mol Biol Cell 7:1679–1690.

Postma FR, Hengeveld T, Alblas J, Giepmans BN, Zondag GC, Jalink K, Moolenaar WH (1998): Acute loss of cell-cell communication caused by G protein-coupled receptors: A critical role for c-Src. J Cell Biol 140:1199–1209.

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.

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.

Ptasznik A, Prossnitz ER, Yoshikawa D, Smrcka A, Traynor-Kaplan AE, Bokoch GM (1996): A tyrosine kinase signaling pathway accounts for the majority of phosphatidylinositol 3,4,5-trisphosphate formation in chemoattractant-stimulated human neutrophils. J Biol Chem 271:25204–252007.

Rao GN, Delafontaine P, Runge MS (1995): Thrombin stimulates phosphorylation of insulin-like growth factor-1 receptor, insulin receptor substrate-1, and phospholipase C-gamma 1 in rat aortic smooth muscle cells. J Biol Chem 270:27871–27875.

Rawlings DJ, Witte ON (1995): The Btk subfamily of cytoplasmic tyrosine kinases: Structure, regulation and function. Semin Immunol 7:237–246.

Schieffer B, Drexler H, Ling BN, Marrero MB (1997): G protein-coupled receptors control vascular smooth muscle cell proliferation via pp60c-src and p21ras. Am J Physiol 272:C2019–C2030.

Spiegel AM (1992): G proteins in cellular control. Curr Opin Cell Biol 4:203–211.

Stephens L, Smrcka A, Cooke FT, Jackson TR, Sternweis PC, Hawkins PT (1994): A novel phosphoinositide 3 kinase activity in myeloid-derived cells is activated by G protein beta gamma subunits. Cell 77:83–93.

Stoyanov B, Volinia S, Hanck T, Rubio T, Loubtchenkov M, Malek D, Stoyanova S, Vanhaesebroeck B, Dhand R, Nurnberg 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.

Torres M, Hall FL, O'Neill K (1993): Stimulation of human neutrophils with formyl-methionyl-leucyl-phenylalanine induces tyrosine phosphorylation and activation of two distinct mitogen-activated protein-kinases. J Immunol 150:1563–1577.

Torres M, Ye RD (1996): Activation of the mitogen-activated protein kinase pathway by fMet-leu-Phe in the absence of Lyn and tyrosine phosphorylation of SHC in transfected cells. J Biol Chem 271:13244–13249.

Traynor-Kaplan AE, Harris AL, Thompson BL, Taylor P, Sklar LA (1988): An inositol tetrakisphosphate-containing phospholipid in activated neutrophils. Nature 334: 353–356.

Tsai W, Morielli AD, Peralta EG (1997): The m1 muscarinic acetylcholine receptor transactivates the EGF receptor to modulate ion channel activity. EMBO J 16:4597–4605.

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.

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.

van Oers NS, Weiss A (1995): The Syk/ZAP-70 protein tyrosine kinase connection to antigen receptor signalling processes. Semin Immunol 7:227–236.

Vanhaesebroeck B, Welham MJ, Kotani K, Stein R, Warne PH, Zvelebil MJ, Higashi K, Volinia S, Downward J, Waterfield MD (1997): P110delta, a novel phosphoinositide 3-kinase in leukocytes. Proc Natl Acad Sci USA 94:4330–4335.

Vlahos CJ, Matter WF, Hui KY, Brown RF (1994): A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). J Biol Chem 269:5241–5248.

Wadsworth SJ, Gebauer G, van Rossum GD, Dhanasekaran N (1997): Ras-dependent signaling by the GTPase-deficient mutant of Galpha12. J Biol Chem 272:28829–28832.

Wan Y, Bence K, Hata A, Kurosaki T, Veillette A, Huang XY (1997): Genetic evidence for a tyrosine kinase cascade preceding the mitogen-activated protein kinase cascade in vertebrate G protein signaling. J Biol Chem 272:17209–17215.

Wan Y, Kurosaki T, Huang XY (1996): Tyrosine kinases in activation of the MAP kinase cascade by G-protein-coupled receptors. Nature 380:541–544.

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.

Yu H, Li X, Marchetto GS, Dy R, Hunter D, Calvo B, Dawson TL, Wilm M, Anderegg RJ, Graves LM, Earp HS (1996): Activation of a novel calcium-dependent protein-tyrosine kinase. Correlation with c-Jun N-terminal kinase but not mitogen-activated protein kinase activation. J Biol Chem 271:29993–29998.