



# Regulation of cell proliferation by G proteins

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**G Proteins provide signal transduction mechanisms to seven transmembrane receptors. Recent studies have indicated that the  $\alpha$ -subunits as well as the  $\beta\gamma$ -subunits of these proteins regulate several critical signaling pathways involved in cell proliferation, differentiation and apoptosis. Of the 17  $\alpha$ -subunits that have been cloned, at least ten of them have been shown to couple mitogenic signaling in fibroblast cells. Activating mutations in  $G\alpha_s$ ,  $G\alpha_{12}$ , and  $G\alpha_{12}$  have been correlated with different types of tumors. In addition, the ability of the  $\beta\gamma$ -subunits to activate mitogenic pathways in different cell-types has been defined. The present review briefly summarizes the diverse and novel signaling pathways regulated by the  $\alpha$ - as well as the  $\beta\gamma$ -subunits of G proteins in regulating cell proliferation.**

**Keywords:** G proteins; oncogene; signaling; ERK; JNK; Ras

## Introduction

Heterotrimeric guanine nucleotide binding proteins, commonly known as G proteins, form the super-family of signal transduction proteins that provide signal coupling mechanisms to seven transmembrane receptors. The requirement of GTP for the signal coupling of glucagon receptor to adenylyl cyclase (Rodbell *et al.*, 1971) and subsequent characterization of a GTP-binding protein identified the role of G proteins in diverse signal transduction processes (Ross and Gilman, 1977; Gilman, 1987; Hepler and Gilman, 1992). G proteins are peripherally associated with the cytoplasmic phase of the plasma membrane and are composed of monomers of  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits. Upon activation by an appropriate signal, the receptor interacts with the G protein and catalyzes the exchange of 'bound GDP' for GTP in the  $\alpha$ -subunit ( $G\alpha$ ). Subsequently, the GTP-bound  $\alpha$ -subunit and the  $\beta\gamma$ -subunits ( $G\beta\gamma$ ) dissociate from the receptor as well as from each other. The 'active'  $\alpha$ -subunit and the 'free'  $\beta\gamma$ -subunits initiate cellular responses by altering the activity of intracellular effector molecules. Meanwhile, the intrinsic GTPase activity of the  $G\alpha$  hydrolyzes the bound-GTP to GDP thus deactivating itself. The  $G\alpha$ -GDP re-associates with  $G\beta\gamma$ , possibly attenuating the  $\beta\gamma$ -effector interaction as well. The re-associated  $G\alpha\beta\gamma$ -GDP heterotrimer resets itself to interact with another molecule of receptor (Hepler and Gilman, 1992).

Although it has been known that G proteins are involved in signal transduction processes ranging from sensory perception to cell-growth regulation, their oncogenic properties have been realized only recently (Gilman, 1987; Johnson and Dhanasekaran, 1989; Dhanasekaran *et al.*, 1995, van Biesen *et al.*, 1996; Dhanasekaran and Vara Prasad, 1998). Studies from several laboratories have defined the novel mechanisms through which G proteins regulate cell growth, differentiation, and oncogenesis. The focus of this review is to present a brief summary of the different signaling pathways activated by G proteins in the regulation of cell proliferation and to discuss the possible interrelationships between these pathways.

## Diversity of G protein signaling

At present, the family of heterotrimeric G proteins consists of 17  $\alpha$ -subunits, five  $\beta$ -subunits, and 12  $\gamma$ -subunits. The  $\beta$ - and  $\gamma$ -subunits are tightly associated and can be separated only upon denaturation. Although both  $\alpha$ - and  $\beta\gamma$ -subunits are involved in cell signaling, and all the three of them show heterogeneity, the heterogeneity of the  $\alpha$ -subunit is used for the classification of G proteins. Based on the sequence similarity of the  $\alpha$ -subunits, G proteins are classified into four distinct classes – namely  $G_s$ ,  $G_i$ ,  $G_q$ , and  $G_{12}$  – by grouping the  $\alpha$ -subunits which show 50% or more identity into a class or subfamily (Strathman *et al.*, 1989; Strathman and Simon, 1991). Table 1 lists the members of the four different classes of  $G\alpha$ -subunits, their known receptors and effectors. Since this list grows rapidly now, only a representative number of receptors and effectors coupled to the respective G proteins are listed. While most of the  $\alpha$ -subunits are ubiquitously expressed (Strathman and Simon, 1991; Kaziro *et al.*, 1991), a few of them such as  $G\alpha_{t_1}$ ,  $G\alpha_{g_2}$ , and  $G\alpha_{15/16}$  show tissue-specific expression indicating their more specialized signaling role.  $G\alpha_{t_1}$ , expressed in retina and  $G\alpha_{g_2}$  expressed in gustatory epithelial tissue are involved in visual and taste signaling mechanisms respectively (Kaziro *et al.*, 1991, McLaughlin *et al.*, 1992). Although the determinant role of  $G\alpha_{15}$  and its human homologue  $\alpha_{16}$  in hematopoiesis are not known, the hematopoietic cell-specific expression of these  $\alpha$ -subunits points to the critical cell-specific signaling role played by them (Amatruda *et al.*, 1991; Wilkie *et al.*, 1991).

The  $\beta$ -subunits range in molecular weight from 34–36 kDa (Clapham and Neer, 1993). Five distinct  $\beta$ -subunits referred as  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ,  $\beta_4$  and  $\beta_5$  have been identified (Clapham and Neer, 1993; Watson *et al.*, 1994, 1996). While  $\beta_1$ ,  $\beta_2$ ,  $\beta_3$ ,  $\beta_4$  are ubiquitously expressed,  $\beta_5$  appears to have tissue-specific expres-

**Table 1** G proteins: their receptors and effectors

Class/ sub-family	G $\alpha$ -subunit	Regulation of cell growth	Receptors	Effectors
G <sub>s</sub>	G $\alpha_s$	+	$\beta$ 1,2-Adrenergic, Glucagon, Dopamine, Serotonin, $\alpha$ 2-Adenosine, ACTH, LH, FSH, MSH, GNRH, and TSH	Adenylyl Cyclase $\uparrow$ Ca <sup>++</sup> Channel $\uparrow$ Na <sup>+</sup> Channel $\uparrow$
	G $\alpha_{olf}$		Odorant receptors	Adenylyl Cyclase $\uparrow$
G <sub>i</sub>	G $\alpha_{i1}$	+	$\alpha$ 2-Adrenergic, thrombin,	Adenylyl Cyclase $\downarrow$
	G $\alpha_{i2}$	+	m2-, 4-Muscarinic	K <sup>+</sup> Channel $\uparrow$
	G $\alpha_{i3}$		acetylcholine, $\alpha$ 1- Adenosine, Vasopressin, Somatostatin	Ca <sup>++</sup> Channel $\downarrow$ Phospholipase C (?) $\uparrow$ Phospholipase A <sub>2</sub> (?) $\uparrow$
	G $\alpha_{oA,B}$	+	Muscarinic	K <sup>+</sup> Channel $\uparrow$ Ca <sup>++</sup> Channel $\downarrow$ Phospholipase C (?) $\uparrow$
	G $\alpha_{t1,2}$	+	Opsins	cGMP- Phosphodiesterase $\uparrow$
	G $\alpha_g$		Taste receptors	cGMP- Phosphodiesterase $\uparrow$
	G $\alpha_z$		Dopaminergic, 5-HT <sub>1A</sub> Serotonin	Phospholipase C- $\beta$ (?) Adenylyl cyclase (?)
G <sub>q</sub>	G $\alpha_q$	+	$\alpha$ 1-Adrenergic, LPA,	Phospholipase C- $\beta$ $\uparrow$
	G $\alpha_{11}$	+	Chemokine, Bradykinin,	
	G $\alpha_{16}$	+	m1-, m-3, m-5-Muscarinic	
	G $\alpha_{14}$		acetylcholine, Thrombin, LPA, Vasopressin,	
	G $\alpha_{15}$		Thromboxane, Chemokine Histaminergic	
G <sub>12</sub>	G $\alpha_{12}$	+	Thrombin, Thromboxane, LPA, Neurokinin-1	GEFs, GDIs, GAPs (?)
	G $\alpha_{13}$	+	Thrombin, Thromboxane, Bradykinin, LPA, Neurokinin-1, Angiotensin II-AT <sub>1</sub>	GEFs, GDIs, GAPs (?)

sions present only in the retina, neuronal tissue, and platelets. Although it has been demonstrated that the  $\beta$ -subunits can interact with distinct effector molecules independent of the  $\alpha$ -subunits, it is worth noting that G $\alpha$ -subunits have a seminal role even in these ' $\beta$ -subunit regulated' pathways. The 'turning on' and 'off' of these signaling pathways are still dependent on the GDP/GTP cycle and the GTPase activity of  $\alpha$ -subunits. In addition, the receptor interaction to specific G proteins primarily relies on the configuration of the  $\alpha$ -subunit of the heterotrimer. Nevertheless, the signaling role of  $\beta$ -subunit suggests the possibility that a single signaling event at the receptor level can activate two different signal transducers consisting of the  $\alpha$ - and  $\beta\gamma$ -subunits. G $\alpha$  and  $\beta\gamma$ , in turn, can elicit either a single potent response or two distinct responses. The  $\gamma$ -subunits, that range in molecular weights from 8–10 kDa have tissue specific expression profiles. Recent biochemical as well as crystallographic analysis of the G protein heterotrimer indicates that the  $\gamma$ -subunit physically interacts with both the  $\alpha$ - and the  $\beta$ -subunit (Rahmatullah *et al.*, 1996; Wall *et al.*, 1995; Lambright *et al.*, 1996). However, it is more tightly associated with the  $\beta$ -subunit and thus behaves as an integral component of the  $\beta$ -subunit. The carboxyl-termini of  $\gamma$ -subunits are prenylated and proposed to have a role in the membrane association of the heterotrimer (Clapham and Neer, 1993). Although, no signaling role has been assigned to the  $\gamma$ -subunit, its close association with the  $\beta$ -subunit, its greater diversity in relation to

the  $\beta$ -subunits, its tissue specific expression patterns, and its specificity in forming complex with different  $\beta$ -subunits suggest that the  $\gamma$ -subunit may have a determinant role in  $\beta\gamma$ -signaling.

Despite the presence of such heterogeneity among the G protein subunits, a certain degree of promiscuity is seen in receptor interaction to specific G proteins as well as G protein interaction with specific effector molecules. It is likely that the observed promiscuity may be due to our inability to define the specific  $\alpha$ -,  $\beta$ - and  $\gamma$ -configuration in each of these receptor-G protein or G-protein-effector interactions. In this context, it is worth noting that a total of 800 different  $\alpha\beta\gamma$ -configurations are theoretically possible and it is likely that some of these configurations define the specificity of G protein interactions. The findings that the G $\beta\gamma$ -subunit configuration can confer specificity to the signaling pathways regulated by G $\alpha_o$ , also supports this view (Kleuss *et al.*, 1991; Kleuss *et al.*, 1992).

### G proteins in the regulation of cell growth and differentiation

The observation that tropic hormones activate cell proliferation and differentiation through the cyclic AMP (cAMP)-dependent pathway suggested the possibility that G<sub>s</sub> proteins may be involved in the regulation of cell growth (Dumont *et al.*, 1989). Identification of the GTPase-inhibitory, activating

mutations of  $G\alpha_s$  and  $G\alpha_i$  in a subset of endocrine tumors established the oncogenic potential of the  $\alpha$ -subunits. Subsequently, these observations led to the investigation of growth altering properties of other G proteins (Gupta *et al.*, 1992a; Pouyssegur and Seuwen, 1992; Dhanasekaran *et al.*, 1995; Dhanasekaran and Vara Prasad, 1998). Growth-promoting activities of different  $G\alpha$ -subunits were investigated by expressing the GTPase-deficient mutants of different  $G\alpha$ -subunits in various cell-lines. A great tool in identifying the role of G proteins in growth-regulation is the fibroblast transformation assay which is widely used to monitor the growth promoting and transforming ability of diverse proteins (Copeland *et al.*, 1979). The fibroblast cell lines such as Swiss 3T3, NIH3T3 and Rat 1a cells are in a sensitive state in which the activation of growth-regulating cytoplasmic signaling pathways promotes cellular transformation. The major limitation of fibroblast transformation assays is that they depend on a single mesenchymally derived cell which is not sensitive to transformation by all genes (Hunter, 1990). In spite of such a limitation, the  $G\alpha$ -transformed fibroblasts have revealed the signaling components involved in the activation of cell proliferation (Dhanasekaran and Vara Prasad, 1998).

Similar to the use of fibroblast transformation assays to define the mitogenic pathway, different cell lines such as F9 teratocarcinoma, PC 12 pheochromocytoma and 3T3-L1 fibroblast cells have been used to identify the role of the G protein subunits in cell differentiation. The F9 teratocarcinoma cells has been used to investigate the role of  $G\alpha_{12}$  in the differentiation of these cells into primitive endoderm (Watkins *et al.*, 1992). Expressing anti-sense RNA for  $G\alpha_{12}$  that reduces the endogenous  $\alpha_{12}$  levels, it has been shown that the ablation of  $G\alpha_{12}$  induced the differentiation of F9 cells into a primitive endoderm. A similar strategy has defined the role of  $G\alpha_{12}$  and  $G\alpha_{13}$  in retinoic acid induced differentiation of these cells (Jho and Malbon, 1997; Jho *et al.*, 1997). Likewise, studies using  $G\alpha$ -antisense oligonucleotide in 3T3-L1 fibroblasts indicated that  $G\alpha_s$  transmits a differentiation-inhibition signal (Wang *et al.*, 1992). The role of  $G\alpha_{16}$  and  $G\alpha_q$  in inducing PC12 cell differentiation has been also defined using the GTPase deficient mutants of these  $\alpha$ -subunits (Heasley *et al.*, 1996). Differential expression of distinct  $\alpha$ -subunits has been documented in several differentiating systems. An interesting correlation with regard to  $G\alpha_{16}$  and  $G\alpha_{12}$  expression is seen during DMSO-induced neutrophil differentiation of the human myeloid cell line, HL 60. In these cells, upon DMSO-induced differentiation, the expression of  $G\alpha_{16}$  is reduced by 90% whereas the expression of  $G\alpha_{12}$  increased by 160% (Amatruda *et al.*, 1991; Wilkie *et al.*, 1991). These findings are interesting in the light of the fact that  $G\alpha_{16}$  expression is restricted specifically to hematopoietic cells. However, whether the differential expression of these  $\alpha$ -subunits is the cause or effect of differentiated phenotype has not been resolved.

As an alternative to these fibroblast-based growth assays, microinjection of different cells with antibodies raised against specific  $\alpha$ -subunit has been used to identify the growth-promoting role of the respective  $\alpha$ -subunit. Using the  $\alpha_{12}$ -antibodies, it has been shown that the microinjection of  $\alpha_{12}$  antibodies inhibited the

serum-induced DNA synthesis in Balb/c3T3 cells. (LaMorte *et al.*, 1992). Further analyses have shown that  $\alpha_q$ -antibodies inhibited both thrombin and bradykinin stimulated DNA synthesis whereas  $\alpha_{12}$ -antibodies inhibited only the thrombin-stimulated DNA synthesis. These results indicate that activation of mitogenic pathway by thrombin receptor depends upon both  $G\alpha_q$  and  $G\alpha_{12}$  whereas bradykinin depends only on  $G\alpha_q$  (LaMorte *et al.*, 1993). Using this approach, the role of  $G\alpha_{12}$  in thrombin-stimulated mitogenic pathway of 1321N1 astrocytoma cells has been confirmed (Aragay *et al.*, 1995).

Studies using the activated mutants of different  $\alpha$ -subunits in diverse cell-types have identified novel mechanisms through which these  $\alpha$ -subunits regulate cell growth and differentiation. Of the 17  $\alpha$ -subunits that have been cloned to date, ten of them,  $G\alpha_s$ ,  $G\alpha_{11}$ ,  $G\alpha_{12}$ ,  $G\alpha_o$ ,  $G\alpha_q$ ,  $G\alpha_{11}$ ,  $G\alpha_{16}$ ,  $G\alpha_z$ ,  $G\alpha_{12}$ , and  $G\alpha_{13}$  have been shown to be involved in the regulation of cell growth. Although these studies have unequivocally indicated the role of  $\beta\gamma$ -subunits in cell signaling, overexpression of  $\beta\gamma$ -subunits has never been shown to activate either cell proliferation or differentiation. In contrast, the ability of  $\alpha$ -subunits to activate cell proliferation and subsequent oncogenic transformation of fibroblast cells supports the emerging view that the  $\alpha$ -subunits form a new class of oncogenes.

### $G\alpha_s$ and the *gsp* oncogene

The signaling pathway mediated cAMP has been found to be mitogenic in hormone-dependent endocrine tissues such as adrenal cortex, thyroid, ovary, pituitary, and testes (Dumont *et al.*, 1989; Vallar, 1996). These cells are endowed with seven transmembrane receptors that are coupled to the adenylyl cyclase pathway through  $G\alpha_s$ . Tropic hormones such as growth hormone releasing hormone (GHRH) and thyroid stimulating hormone (TSH) stimulate cell proliferation in pituitary and thyroid tissues respectively through their respective  $G_s$ -coupled receptors. Therefore, the observation that the  $G\alpha_s$ -mediated signaling pathway is constitutively activated in a subset of human pituitary tumors (Vallar *et al.*, 1987) led to an intense search for the activating mutations of  $G\alpha_s$  in endocrine tumors. Point mutations in the  $\alpha_s$  subunit involving the replacement of either arginine 201 (R201) or glutamine 227 (Q227) were identified as the activating mutations involved in pituitary GH secreting tumors and thyroid hyperfunctioning adenomas (Landis *et al.*, 1989; Lyons *et al.*, 1990). Accordingly, the mutationally activated form of  $G\alpha_s$  is known as the *gsp* oncogene (derived from *Gs* protein).

To date, *gsp* mutations have been detected in pituitary tumors, thyroid adenomas and thyroid carcinomas. In these tumors, the activating mutations result in the constitutive activation of adenylyl cyclase-cAMP-cAMP-dependent protein kinase (PKA) signaling pathway which leads to abnormal cell proliferation (Vallar, 1996). The role of the *gsp* mutations in thyroid and pituitary neoplasia was investigated using thyroid-derived FRTL-5 or pituitary-derived GH3 cells (Muca and Vallar, 1994; Zieger *et al.*, 1996; Ham *et al.*, 1997). Expression of *gsp* has been observed to stimulate the adenylyl cyclase activity followed by an increased rate

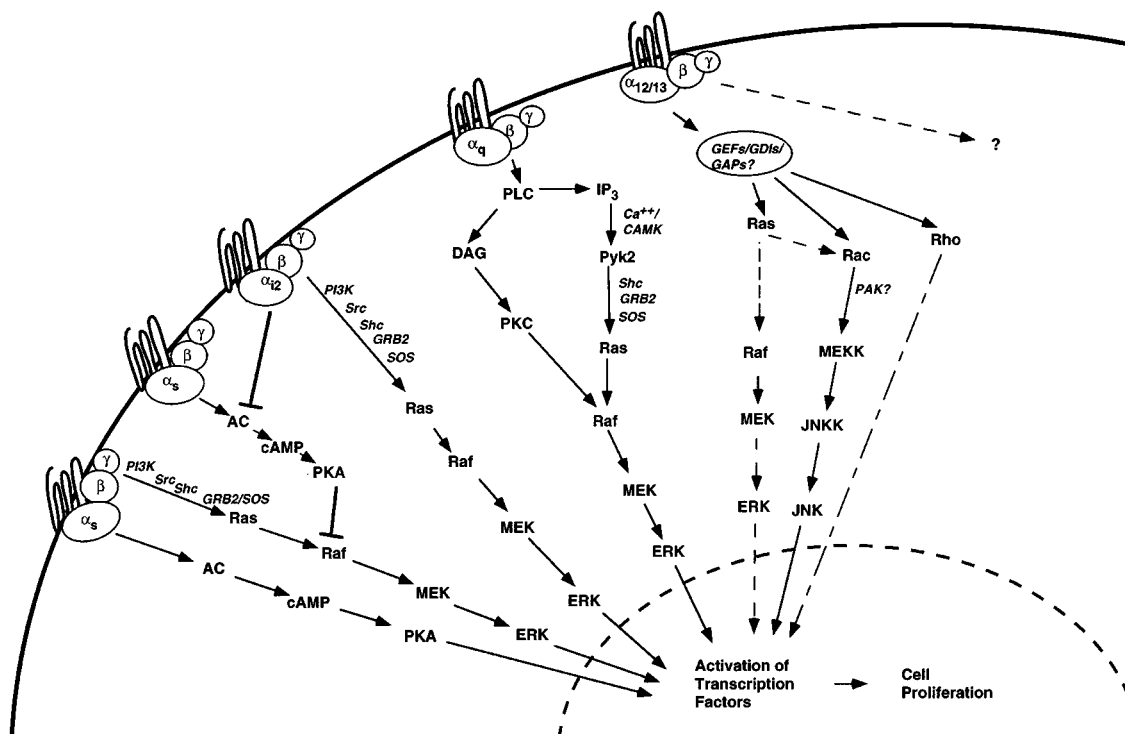
of DNA synthesis independent of the mitogenic hormone, TSH. GH3 cells expressing *gsp* also showed a similar increase in cAMP levels along with the activation of cell proliferation (Ham *et al.*, 1997). It has been also shown that the persistent activation of adenylyl cyclase by expressing the A1-fragment of the cholera toxin led to an increased proliferation of FRTL-5 cells (Zieger *et al.*, 1996). In addition, the finding that the injection of these cells into nude mice resulted in the formation of tumors clearly indicated the role of cAMP in TSH-independent cell proliferation and neoplastic transformation of thyroid tissue (Muca and Vallar, 1994; Zieger *et al.*, 1996). The growth promoting activity of *gsp* was also assessed using Swiss 3T3 cells (Zachary *et al.*, 1990). Expression of the *gsp* oncogene resulted in an increase in the rate of DNA synthesis and cell proliferation that could be correlated with elevated levels of cAMP. Although the expression of *gsp* potentiated the mitogenic pathway of Swiss 3T3 cells, it did not lead to the transformation of these cells (Zachary *et al.*, 1990).

The growth promoting activity of *gsp* is mainly due to the constitutive activation of the adenylyl cyclase (AC) pathway. AC mediated increase in cAMP levels leads to the persistent activation of PKA resulting in the phosphorylation and subsequent activation of cAMP responsive element-binding protein (CREBP). CREBP binds to cyclic AMP responsive elements (CREs) to transactivate the transcription of specific primary response genes that initiate cell proliferative machinery. In the case of pituitary somatotrophs (in

which *gsp* has been identified as an oncogene), CREBP binds to the CRE located in the promoter region of the transcription factor known as growth hormone factor 1 (GHF1) and activates its transcription. Subsequently, GHF1 transcriptionally activates the expression of other growth-promoting genes. However, it should be noted here that  $G_s$  stimulates proliferation only in the cells – such as those present in endocrine tissues – that are positively responsive to cAMP-PKA signaling pathway for their cell growth. By contrast, in other cells,  $G_{\alpha_s}$  appears to have a growth inhibitory effect through its negative regulation of Ras-Raf signaling pathway (Burgering *et al.*, 1993; Graves *et al.*, 1993; Cook and McCormick, 1993; Wu *et al.*, 1993; Severson *et al.*, 1993). In these cell types, PKA activated by  $G_{\alpha_s}$  through cAMP directly phosphorylates Raf at Ser 43 and/or Ser 671 (Wu *et al.*, 1993; Mischak *et al.*, 1996) thus inhibiting Raf and its downstream MEK-ERK cascade. Thus, *gsp* can have two opposing effects depending on the cells in which they are expressed (Figure 1).

### $G_{\alpha_i}$ and the *gip2* oncogene

Based on its sequence homology with  $G_{\alpha_s}$ , the analogous activating mutations of  $G_{\alpha_{i2}}$  are R179 and Q205. Mutations in these residues have been observed in different forms of tumors. While R179-mutation of  $G_{\alpha_{i2}}$  was identified in ovarian sex cord stromal tumors and adrenal cortex tumors (Lyons *et al.*, 1990), Q205-



**Figure 1** Schematic model for the regulation of cell proliferation by G proteins. Stimulation of a receptor coupled to a specific G protein generates both  $\alpha$ -specific and  $\beta\gamma$ -specific signals as illustrated whereas the activated (GTPase-deficient)  $\alpha$ -subunit stimulates only the  $\alpha$ -specific events (see text for details). AC, adenylyl cyclase; PKA, cyclic AMP-dependent protein kinase; PLC, phospholipase-C; DAG, diacylglycerol; IP<sub>3</sub>, inositol 1,4,5 trisphosphate; Pyk2, proline-rich tyrosine kinase-2; PKC, protein kinase C; PI3K, Phosphatidylinositol 3-kinase; GEF, guanine nucleotide exchange factor; GDI, guanine nucleotide dissociation inhibitor; GAP, GTPase-activating protein; PAK, p21-activated kinase; ERK, extracellular-signal regulated kinase; MEK, mitogen-activated extracellular-signal regulated kinase kinase; MEKK, MEK kinase; JNK, Jun N-terminal kinase; JNKK, Jun N-terminal kinase kinase

mutation has been detected in non-functioning pituitary tumors (Tordjman *et al.*, 1993). These activated mutant forms of  $G\alpha_{i2}$  are denoted as *gip2* oncogenes (for *Gi* protein-2). Unlike *gsp*, the over-expression of *gip2* resulted in the oncogenic transformation of Rat1a cells (Pace *et al.*, 1991; Gupta *et al.*, 1992a,b,c). Surprisingly, *gip2* failed to transform other fibroblast cell lines such as NIH3T3 cells. Nevertheless, it has been shown that the *gip2*-transformed Rat1a cells can form colonies on soft agar and the injection of these cells has been shown to form tumors in athymic nude mice (Pace *et al.*, 1991; Gupta *et al.*, 1992a). Consistent with these findings, inactivating *gip2* protooncogene function has been shown to inhibit cell growth and tumor formation (Hermouet *et al.*, 1996a,b). Injection of murine melanoma cells expressing the dominant negative mutant of  $G\alpha_{i2}$  ( $G\alpha_iG204A$ ) into nude mice greatly reduced the tumorigenic potential of the melanoma cells (Hermouet *et al.*, 1996b). When K1735-CL19 cells expressing  $G\alpha_{i2}G204A$  were injected into athymic nude mice, the tumor formation was much delayed compared to the animals injected with either control cells or cells expressing *gip2*. Furthermore, the tumors formed in these animals were of reduced in size. It has also been observed that the expression of  $G\alpha_{i2}G204A$  in these melanoma cells inhibited their growth by 50%. Taken together, these results suggest that the *gip2* oncogene, as well as its protooncogene  $G\alpha_{i2}$ , are involved in the onset and the propagation of many different forms of tumors.

The mechanism through which *gip2* activates cell proliferation and oncogenesis is not fully understood. However, the finding that the expression of *gip2* constitutively activates MEK-ERK signaling in Rat1a cells indicated that this could be the major mechanism through which *gip2* regulates cell growth (Gupta *et al.*, 1992b). Interestingly it has been observed that *gip2*-mediated activation of MEK-ERK pathway is independent of functional Ras (Winitz *et al.*, 1993). Based on these observations, it has been proposed that the Ras-independent activation of MEK-ERK signaling pathway by *gip2* is due to the inhibitory effect of *gip2* on the levels of intracellular cAMP (van Biesen *et al.*, 1996). It has been known that the expression of *gip2* leads to a decrease the cAMP levels and the resultant PKA activity in Rat1a cells (Gupta *et al.*, 1992a,b; Lowndes *et al.*, 1991). Since PKA is known to inhibit Raf through phosphorylation, a reduction in PKA activity can relieve its inhibitory effect of PKA and subsequently on MEK-ERK signaling pathway (van Biesen *et al.*, 1996; Dhanasekaran and Vara Prasad, 1998). Thus, through its downregulation of AC-cAMP-PKA pathway, *gip2* can upregulate MEK-ERK signaling pathway and promote cell proliferation (Figure 1). Such a view would be consistent with the observation that the activation of ERK by *gip2* is independent of Ras (Winitz *et al.*, 1993). The activation of MEK-ERK signaling pathway couples *gip2* signaling to the transactivation of TCFs in the nucleus.

Based on the observation that the *gip2* mutations activate cell proliferation, the growth regulating properties of the other members of  $G\alpha_i$  family was investigated. The respective  $\alpha$ -subunits were expressed in different fibroblasts to test their ability to transform the fibroblast cell lines. These studies have indicated that the GTPase-deficient mutant of  $G\alpha_{i1}$  is very similar

to to the *gip2* oncogene in activating cell proliferation whereas the analogous activated mutant of  $G\alpha_{i3}$  is not (Hermouet *et al.*, 1993).  $G\alpha_z$  and  $G\alpha_o$ , both of which belong to the  $G_i$  family of G proteins, have also been shown to activate mitogenic pathways in different cell-types. The expression of an activated mutant of  $G\alpha_z$  ( $G\alpha_zQ205L$ ) has been shown to transform Swiss 3T3 cells (Wong *et al.*, 1995). Although the specific mechanism by which  $G\alpha_zQ205L$  activates cell proliferation has not been identified yet, considering the ability of  $G\alpha_z$  in inhibiting the adenylyl cyclase activity, it is likely that the signaling pathway activated by  $G\alpha_z$  is analogous to the ones regulated by  $G\alpha_{i2}$ . Expression of an activated mutant of  $G\alpha_o$  ( $G\alpha_oQ205L$ ) has been shown to transform NIH3T3 cells (Kroll *et al.*, 1992). Although the mechanism by which  $G\alpha_oQ205L$  regulates cell proliferation is not fully understood, protein kinase C (PKC)-dependent activation of ERK-pathway has been implicated (van Biesen *et al.*, 1996).

### $G\alpha_q$ , the conditional oncogene

Although the mutations in  $G\alpha_q$  has not been observed in any tumors, the growth promoting activity of  $G\alpha_q$  has been investigated using the activating mutation of  $G\alpha_q$  ( $G\alpha_qQ209L$ ). While the expression of  $G\alpha_qQ209L$  has been shown to transform NIH3T3 cells,  $G\alpha_qQ209L$ -induced foci formation in NIH3T3 cells was conditional upon priming the transfected cells with dexamethasone (De Vivo *et al.*, 1992). Kalinec *et al.*, (1992) also observed  $\alpha_qQ209L$ -mediated transformation of NIH3T3 cells. While it has been demonstrated that the injection of the transformed NIH3T3 cells into athymic nude mice induced tumors by 1 week, it has also been noticed that the expression of  $G\alpha_qQ209L$  in NIH3T3 cells caused more cell death than transformed phenotype. Expression of  $G\alpha_qQ209L$  proved to be cytotoxic to 80% of the transfectants (Kalinec *et al.*, 1992). Based on these findings it was proposed that the low levels of expression of  $G\alpha_qQ209L$  transforms the fibroblasts while higher levels of expression leads to their death. Hence the growth promoting activity of  $G\alpha_q$  appears to be conditional upon the physiological status of the cell as well as the expression levels of the activated  $G\alpha_q$ . The observation that the expression of  $G\alpha_qQ209L$  inhibits cell growth (Wu *et al.*, 1992; Qian *et al.*, 1993), along with the findings that the persistent activation of phospholipase C (PLC) by  $G\alpha_{i6}Q212L$  inhibits PDGF- and serum-stimulated growth responses in Swiss 3T3 cells by 50–80%, also supports this view (Qian *et al.*, 1994).

Since the activated mutants of  $G\alpha_q$  were known to activate PI-PLC $\beta$  (Wu *et al.*, 1992; Conklin *et al.*, 1992), the role of PLC in  $G\alpha_q$ -mediated cell proliferation has been investigated. It has been observed that the microinjection of PLC triggers transformation in NIH3T3 cells (Smith *et al.*, 1989). In addition, it has been noticed that the expression of m1, m2, m5 muscarinic-(Gutkind *et al.*, 1991),  $\alpha_{1b}$  adrenergic- or 5HT $_{1c}$ -receptors (Allen *et al.*, 1991) that are known to be coupled to PLC pathways leads to NIH3T3 cell transformation. These observations suggest that the activation of PLC is involved in the mitogenic pathway regulated by  $G\alpha_q$ . The activation of PLC leads to cleavage of phosphatidylinositols generating inositol



1,4,5-trisphosphate (IP<sub>3</sub>) and diacylglycerol (DAG). The diacylglycerols generated from these pathways activate PKC and PKC in turn can stimulate ERK through Raf (Zou *et al.*, 1996; Marais *et al.*, 1998). Thus, in cell types such as CHO, it has been observed that G $\alpha_q$  can activate an ERK-mediated proliferative pathway through a PKC-dependent but Ras-independent mechanism (Figure 1). However, in other cell types such as the rat vascular smooth muscle and NIH3T3 cells, G $\alpha_q$  appear to activate ERK through a novel pathway involving proline-rich tyrosine kinase-2 (Pyk2) and Ras (Lev *et al.*, 1995; Bourne, 1995). This pathway appears to be activated by the IP<sub>3</sub> generated by the activation of PLC. IP<sub>3</sub> increases the cellular levels of Ca<sup>2+</sup> levels by stimulating the intra- as well as the extracellular mobilization of Ca<sup>2+</sup>. The IP<sub>3</sub>-induced increase in Ca<sup>2+</sup> levels, presumably through a Ca<sup>2+</sup>/calmodulin-dependent kinase, stimulate the activity of Pyk2 to activate Shc through tyrosine phosphorylation. The resultant Shc-GRB2-SOS complex stimulates Ras which leads to the activation of ERK (Lev *et al.*, 1995; Bourne, 1995). Thus, G $\alpha_q$  can regulate growth through either a Ras-dependent or Ras-independent but PKC-dependent ERK pathway (Figure 1). Either of these pathways can couple G $\alpha_q$  signaling to the nuclear events through the activation of TCFs or TRE-specific transcription factors.

#### G $\alpha_{12/13}$ : the *gcp* oncogenes?

The observation that *cta*, a *Drosophila* gene closely related to G $\alpha_{12}$  and G $\alpha_{13}$ , is involved in *Drosophila* embryogenesis, gave an early indication that the G<sub>12</sub> class may be involved in cell growth and development (Parks and Wieschaus, 1991). A direct role for G $\alpha_{12}$  in the regulation of cell proliferation rather came from an unrelated study focussed on characterizing tumor-specific oncogenes. In an attempt to identify the causative oncogene of soft tissue sarcoma, Aaronson and his co-workers used an expression cloning method in which the fibroblast transformation ability of the putative oncogene was used as a reporter-assay (Chan *et al.*, 1993). Pooled cDNA libraries from the human sinovial tumor-derived cell line, A2095 and Ewin's sarcoma cell line, RD-ES-1 were screened for the presence of the causative oncogene. This led to the identification of G $\alpha_{12}$  as the transforming oncogene present in the tumor derived cDNA library.

NIH3T3 cells transformed by G $\alpha_{12}$  were characterized by their decreased doubling time, loss of saturation density of growth, and ability to form colonies on soft-agar in addition to forming tumors in athymic nude mice. Further characterization of the transforming property of G $\alpha_{12}$  indicated that there was no mutational change in the G $\alpha_{12}$  cloned from this tumor cell-line and the expression of wild-type G $\alpha_{12}$  itself was sufficient to alter the growth properties of NIH3T3 cells (Chan *et al.*, 1993). Interestingly, serum starvation inhibited the transforming ability of wild-type G $\alpha_{12}$  suggesting serum/agonist-dependent nature of this transformation. However, the GTPase deficient, activated mutant of G $\alpha_{12}$ , G $\alpha_{12}$ Q229L, can abrogate the need for the serum/agonist-dependency for transformation (Xu *et al.*, 1993). Subsequently, the serum-independent, potent transforming activities of the QL-

mutants of G $\alpha_{12}$  and G $\alpha_{13}$  have been demonstrated by several laboratories (Jiang *et al.*, 1993, Xu *et al.*, 1994; Vara Prasad *et al.*, 1994; Voyna-Yasenetskaya *et al.*, 1994). In this context, it is significant to note that the activated mutants of G $\alpha_{12}$  and G $\alpha_{13}$  are the most potent transforming  $\alpha$ -subunits that have been tested so far. Based on these observations, Gutkind and his colleagues have designated the G12 class of  $\alpha$ -subunits as the *gcp* family of oncogenes since human G $\alpha_{12}$  was cloned as a transforming oncogene from Ewin's sarcoma expression library (Xu *et al.*, 1994).

Despite their strong transforming activity (the strongest among all the  $\alpha$ -subunits), the role of G $\alpha_{12}$  and G $\alpha_{13}$  in naturally occurring tumors is yet to be established. Nonetheless G $\alpha_{12}$ QL- and G $\alpha_{13}$ QL-transformed fibroblasts have begun to reveal the components of the signaling network involved in the regulation of cell proliferation. Although the effector molecules that physically interact with G $\alpha_{12}$  and G $\alpha_{13}$  remain to be identified, recent studies have defined several novel mechanisms through which these  $\alpha$ -subunits can activate cell proliferation. NIH3T3 cells transformed with G $\alpha_{12}$ QL or G $\alpha_{13}$ QL indicated that conventional secondary messenger pathways involving cAMP, Ca<sup>2+</sup>, or IP<sub>3</sub> were not altered in these cells (Chan *et al.*, 1993; Xu *et al.*, 1993; Jiang *et al.*, 1993, Xu *et al.*, 1994; Vara Prasad *et al.*, 1994; Voyna-Yasenetskaya *et al.*, 1994). However, specific sets of primary response genes appear to be activated in G $\alpha_{12}$ QL- and G $\alpha_{13}$ QL-transformed NIH3T3 cells (Fromm *et al.*, 1997; Vara Prasad *et al.*, 1994). G $\alpha_{12}$ QL has been shown to activate SRFs through a Rho-dependent pathway (Fromm *et al.*, 1997). G $\alpha_{13}$ QL has been shown to activate the transcription of Egr-1 a primary response gene implicated in cell proliferation as well as differentiation (Vara Prasad *et al.*, 1994). While the ERK-MAPK pathway is not significantly altered in these cells, JNK, a kinase distantly related to ERK-MAPK is constitutively activated in G $\alpha_{12}$ QL- and G $\alpha_{13}$ QL-transformed and -transfected cell lines (Vara Prasad *et al.*, 1995; Collins *et al.*, 1996; Voyna-Yasenetskaya *et al.*, 1996). This is one of the very few instances where the constitutive activation of JNK has an anti-apoptotic effect. G $\alpha_{12}$  and G $\alpha_{13}$  are also involved in transmitting additional growth-promoting signals (Dhanasekaran and Dermott, 1996). These include the Ras/Rac-dependent transient activation of ERK (Mitsui *et al.*, 1997) and Rho-dependent activation of focal adhesion complex formation (Buhl *et al.*, 1996; Hooley *et al.*, 1996). The observations that cell transformation by G $\alpha_{12}$  is dependent on both the functional Ras, Raf, Rho, and Rac supports the view that multiple signaling inputs are involved in G $\alpha_{12}$ -mediated cell proliferation (Mitsui *et al.*, 1997, Zhang *et al.*, 1996; Tolkacheva *et al.*, 1997). It is worth noting that through their activation of Ras, Rac, Rho and CDC42, G $\alpha_{12/13}$  also stimulate other downstream events regulated by these small GTPases. It has been observed that G $\alpha_{12}$  can stimulate the activities of Phosphatidyl inositol 5-phosphate kinase, a Rho-dependent kinase and probably Phosphatidyl inositol 4-phosphate kinase but not Phosphatidyl inositol 3-kinase in NIH3T3 cells (Gebauer and Dhanasekaran, unpublished observations). Furthermore, it has been shown that G $\alpha_{13}$ QL stimulate PLD through a Rho-dependent pathway (Plonk *et al.*, 1998). In addition, it has been shown

that  $G\alpha_{12}$  can activate PKC-dependent signaling pathways in other cell types (Dhanasekaran *et al.*, 1994; Wadsworth *et al.*, 1997). These additional reinforcements of cell-survival signals should be contributing to the accelerated cell growth seen in cells expressing  $G\alpha_{12}QL$  and  $G\alpha_{13}QL$ .

How are the multiple signaling inputs generated from  $G\alpha_{12/13}$  integrated into a cell proliferation signal? It appears that the signals from  $G\alpha_{12/13}$  are translated into growth-promoting signals by the small GTPases Ras, Rac, and Rho. It has been reported that Ras, Rho, Rac, and CDC42 play an essential role in cell cycle progression from G1 to S phase (Aktas *et al.*, 1997; Takuwa and Takuwa, 1997; Olson *et al.*, 1995; Hirai *et al.*, 1997). An additional role for Rac in progression from G2 to M phase has been also identified (Moore *et al.*, 1997). Based on these observations, it is likely that  $G\alpha_{12/13}$  activation of Ras-, Rac-, and Rho-regulated signaling mechanisms accelerate G1/S and G2/M phase cell cycle progression. The observation that  $G\alpha_{12}$ -induced cell proliferation depends on both Ras-regulated ERK and Ras/Rac-regulated JNK to progress through G1 to S phase provide strong support to this view (Mitsui *et al.*, 1997). Moreover, it is likely  $G\alpha_{12/13}$ -activated Rho plays additional roles. Rho is involved in the regulation of cytoskeletal rearrangements associated with cell division and proliferation (Ridley and Hall, 1992; Takai *et al.*, 1995). Furthermore, Rho has been known to activate specific SRFs through a hitherto unidentified mechanism (Hill *et al.*, 1995). Thus activation of Rho, in addition to Ras and Rac may greatly facilitate cell growth in cells expressing  $G\alpha_{12/13}$ . In this context, it is significant to note that  $G\alpha_{12}$ -mediated transformation of NIH3T3 cell line is dependent on Rho and Rho-mediated activation of SRFs (Fromm *et al.*, 1997). Thus,  $G\alpha_{12/13}$  coordinate several critical signaling events through its interactions with the Ras- and Rho-family of GTPases. These include the regulation of different kinase modules as well as the activation of several transcription factors such as SRFs, TCFs, Jun and ATF2.

The temporal sequence of these signaling events can be analysed using the serum/LPA-inducible transformation of NIH3T3 cells expressing wild type  $G\alpha_{12}$ . Using this model system, it has been observed that in addition to the activation of different mitogenic pathways,  $G\alpha_{12}$  mediates the inhibition of an apoptotic pathway involving p38MAPK (Dermott and Dhanasekaran, Unpublished observations). In the light of the recent observation that p38MAPK is the primary signaling module involved in the apoptotic pathways activated by serum-deprivation (Kummer *et al.*, 1997), this finding, for the first time demonstrates the ability of  $G\alpha_{12}$  to inhibit an apoptotic pathway to promote cell growth. Presumably, the combination of multiple proliferative signals together with a strong anti-apoptotic signal leads to the oncogenic transformation of cells expressing these  $\alpha$ -subunits. Perhaps, the ability to activate of such multiple growth promoting signaling-inputs confers the potent oncogenic property unique to  $G\alpha_{12/13}$ .

The mechanisms through which  $G\alpha_{12}$  and  $G\alpha_{13}$  transmit their signals to the respective low molecular weight GTPases are presently not known. It is likely that they stimulate specific guanine nucleotide ex-

change factors (GEFs), compete with guanine nucleotide dissociation inhibitors (GDIs), or inhibit specific GAPs (Dhanasekaran and Dermott, 1996). A role for tyrosine phosphorylation of Shc in the signal coupling between  $G\alpha_{12}$  and Ras has been proposed in thrombin-stimulated  $G\alpha_{12}$ -mediated mitogenic pathway of astrocytoma cells (Collins *et al.*, 1997). A recent observation that LPA receptors can activate Tiam-1, an exchange factor for Rac through a PKC-dependent mechanism (Fleming *et al.*, 1997), suggests an interesting possibility that  $G\alpha_{12}$ , which activates PKC-dependent pathway in some cell types, can stimulate Rac and possibly Rac-mediated JNK activity through PKC and Tiam-1. The observation that the activation of JNK by  $G\alpha_{12}QL$  is PKC-dependent in HeLa cells fits well with this hypothesis (Tsim and Dhanasekaran, unpublished observations). Further analysis of the mitogenic pathways activated by these  $\alpha$ -subunits and identifying the mechanism(s) through which  $G\alpha_{12/13}$  activate different small GTPase and kinases may finally define the mitogenic pathways regulated by these  $G\alpha$ -subunits.

Although both  $G\alpha_{12}$  and  $G\alpha_{13}$  activate cell proliferation and induce oncogenic transformation in different fibroblast cell lines,  $G\alpha_{12}$  appears to be more potent than  $G\alpha_{13}$  in these pathways (Jiang *et al.*, 1993; Vara Prasad *et al.*, 1994). This is significant in light of the recent observation that the fibroblasts derived from  $G\alpha_{13}^{-/-}$  mouse embryos in which both the alleles of wild-type  $G\alpha_{12}$  were present, did not show any decrease in ligand-induced DNA synthesis or cell proliferation (Offermanns *et al.*, 1997). Furthermore, in some cell types, the asynchronous activation of  $G\alpha_{13}$  activates an apoptotic pathway (Althoefer *et al.*, 1997). Thus, it is likely that  $G\alpha_{12}$  is more involved in regulation of cell proliferation than  $G\alpha_{13}$ . Our recent finding that  $G\alpha_{12}$  more avidly interacts with Ras supports such a hypothesis (Wadsworth *et al.*, 1997). Considering the potent oncogenic property of  $G\alpha_{12}$ , it is likely that activating mutations in  $G\alpha_{12}$  should be highly oncogenic leading to human tumors. However, the role of the activating mutations of  $G\alpha_{12}$  in naturally occurring human tumors remains to be established.

### The $\beta\gamma$ -subunits

The unique structure of  $\beta\gamma$ -subunits with seven WD-40 repeats with the resultant seven propeller motif, points to an active signaling role for  $\beta\gamma$ -subunits (Wall *et al.*, 1995; Lambright *et al.*, 1996; Sondek *et al.*, 1996). All of the signaling pathways regulated by the  $\alpha$ -subunits can be equally or more potently activated by the  $\beta\gamma$ -subunits (Clapham and Neer, 1993; Gutkind, 1998). In spite of its ability to potentially activate diverse signaling pathways – the pathways used by the  $G\alpha$ -subunits to activate cell proliferation – the expression of  $G\beta\gamma$  fails to activate cell proliferation. Although the overexpression of  $\beta\gamma$ -subunit has not been shown to stimulate the rate of DNA synthesis or cell proliferation,  $\beta\gamma$ -subunits play a major role in mediating the seven transmembrane receptor-activation of ERK and JNK pathways (Crespo *et al.*, 1994; Coso *et al.*, 1996). The mechanism by which  $\beta\gamma$ -subunit activate the Ras-dependent ERK pathway has been the focus of many laboratories. The ability of  $\beta\gamma$ -subunits to activate PLC and hence PKC



or  $\text{Ca}^{2+}$ -dependent pathways suggest that  $\beta\gamma$  can activate the ERK pathway through more than one mechanism (van Biesen *et al.*, 1996; Dhanasekaran and Vara Prasad, 1998; Gutkind, 1998).

Lefkowitz and co-workers have characterized the ability of different configurations of  $\beta\gamma$ -subunits to activate ERK in COS-7 cells (Hawes *et al.*, 1995). Results from these studies indicated that the expression of the  $G\beta1\gamma1$ ,  $G\beta1\gamma2$ ,  $G\beta1\gamma3$ , and  $G\beta2\gamma2$ -configurations increased the basal levels of ERK by 6–10-folds while the configurations involving  $G\beta3$  or  $G\beta4$  failed to activate ERK. Similarly, only the  $G\beta1\gamma1$ ,  $G\beta1\gamma2$ ,  $G\beta1\gamma3$ , and  $G\beta2\gamma2$  configurations stimulated 3–4-fold increase in PI hydrolysis suggesting their activation of PI-PLC pathways (Hawes *et al.*, 1995). Coexpression of a dominant negative mutant of Ras along with the  $G\beta\gamma$  completely inhibited the activation of ERK without having any effect on PLC activity. Since  $G\beta\gamma$  can physically interact and activate PLC it is likely that the  $\text{IP}_3$ -derived from the PLC pathway can increase intracellular  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$ /CAMK can activate Pyk2 kinases. Pyk2 in turn, can phosphorylate Shc, leading to the activation of GRB2 and SOS. The resultant activation of Ras can lead to the activation of ERK through Raf-MEK pathway (Rocca *et al.*, 1997). This is consistent with the observation that the  $\beta\gamma$ -activated ERK is not sensitive to PKC-inhibitors but sensitive to Ras, Raf and tyrosine kinase inhibitors (Hawes *et al.*, 1995).  $G\beta\gamma$ -activation of PLC can also lead to an increase in DAGs and subsequently to the activation of PKC. Therefore, at least in some cell types,  $G\beta\gamma$  should be able to activate ERK-pathway through a PKC-dependent but Ras-independent pathway similar to that of  $G\alpha_q$ . The role of  $G\beta5$  in activating mitogenic signaling is not known. Although the observation that all of the configurations involving  $G\beta1$  and  $G\beta2$  raises the question of specificity in  $\beta\gamma$ -signaling (Hawes *et al.*, 1995), perhaps, the  $\gamma$ -subunits that were not tested in this study may have more determinant role in conferring specificity. Recent observations that mutations of specific amino acid residues in the  $\beta$ -subunit can selectively inhibit its interactions with different effectors, suggesting the  $\beta\gamma$ -effector specificity at the molecular level (Yan and Gautam, 1997).

Interestingly, the  $\beta\gamma$ -subunit-activation of Ras and the subsequent activation of ERK prove to be dependent upon the non-receptor tyrosine kinase activity of Src as well as PI3K (Luttrell *et al.*, 1996; Hawes *et al.*, 1996). Expression of a truncated inhibitory mutant of Src has effectively been shown to block the  $\beta\gamma$ -induced stimulation of Ras-mediated ERK activity. Further analyses have shown that the coupling between  $\beta\gamma$ -subunit and Src is provided by a wortmannin-sensitive phosphatidyl 3-kinase (PI3K). In cells overexpressing  $\beta\gamma$ -subunits, wortmannin treatment blocks the  $\beta\gamma$ -induced Src-phosphorylation as well as the ERK activation (Hawes *et al.*, 1996). The molecular basis for the activation of Src by PI3K is speculated to be phosphatidylinositol-3,4,5-trisphosphate, one of the products of PI3K (Hawes *et al.*, 1996). Thus the emerging view of  $\beta\gamma$ -subunit regulation of ERK activity involves PI3K, Src and Ras in a linear pathway (Rocca *et al.*, 1997). The role of Src in activating Ras through Shc-GRB2-SOS pathway has been already established (van Biesen *et al.*, 1996; Gutkind, 1998). The recruitment of different

signaling proteins such as Src, Pyk2 and PI3K by  $\beta\gamma$ -subunits appears to be cell type and/or receptor dependent.

### G protein interactions with other signaling pathways

It is being realized that the critical signaling pathways involved in cell proliferation, differentiation and apoptosis are stringently regulated. Often multiple signaling inputs are required to initiate and sustain the signals involved in cell proliferation. A case to the point is the activation of multiple signaling pathways by  $G_{12}$  and  $G_{13}$  (Dhanasekaran and Dermott, 1996). In some cases, multiple signaling inputs can be generated by two different G proteins and their subunits converging towards the activation of a single mitogenic response. Experimentally, this has been shown in HEK 293 cells in which the coexpression and stimulation of  $G_i$ -coupled  $\alpha2A$ -adrenergic receptor and the  $G_{q/11}$  coupled  $\alpha1B$  adrenergic receptors, converge towards the activation of ERK pathway through different routes - one through  $G\beta\gamma$ - and the other through  $G\alpha_q$ -subunits respectively (Rocca *et al.*, 1997). It is very much likely that such positive cooperation between different G proteins exists *in vivo*. In addition, there seems to be dynamic interactions between G protein signaling pathways and other signaling cascades in the regulation of cell proliferation. Such interactions between different signaling cascades are more apparent in the mitogenic pathways regulated by tyrosine kinases and G proteins. For example, epidermal growth factor is a receptor tyrosine kinase that activates PLC $\gamma$  and Ras-Raf-ERK pathways in diverse cell types through the activation of both tyrosine and ser/thr kinases (Chech *et al.*, 1988; Buday and Downward, 1993). However in hepatocytes, EGF mediated stimulation of inositol triphosphate and DAG accumulation is dependent the presence of  $G\alpha_i$  since this pathway can be inhibited by treating the cells with pertussis toxin (Yang *et al.*, 1993). Similarly, a critical role for  $G\alpha_{12}$  in insulin signaling pathway has been reported (Moxham and Malbon, 1996; Chen *et al.*, 1997). Using inducible expression of antisense RNA to  $G\alpha_{12}$ , it has been shown that the ablation of  $G\alpha_{12}$  leads to physiological conditions similar to those seen in noninsulin-dependent diabetes mellitus (Moxham and Malbon, 1996) defining a critical role for  $G\alpha_{12}$  in insulin-receptor signaling pathway. Interestingly, tissue-specific expression of activated  $G\alpha_{12}$  ( $G\alpha_{12}Q205L$ ) mimicked the actions of insulin, thus further supporting a 'permissive' role for  $G\alpha_{12}$  in insulin-receptor signaling pathway (Chen *et al.*, 1997). Recently it has been shown that the stimulation of receptors for endothelin, thrombin, and LPA transactivate EGFR and this transactivation is necessary for the activation of ERK activation, *fos* gene expression, and DNA synthesis suggesting the critical role played by EGFR in G protein mediated signaling (Daub *et al.*, 1996). Such interactions between different  $G\alpha$ -subunits and other signaling cascades such as those between  $G\alpha_{11}$  and TGF $\beta$ R (Kataoka *et al.*, 1993),  $G\alpha_i$  and bFGFR (Sa and Fox, 1994),  $G\alpha_q$  and PDGFR (De Vivo and Iyengar, 1994),  $G\alpha_{12}$  and CSF-1R (Corre and Hermouet, 1995),  $G\alpha_{13}$  and IGF-1R (Liu *et al.*, 1997) and  $G\alpha_{13}$  and EGFR (Gohla, 1998) have been

identified. However, the molecular mechanisms underlying these interactions remain to be resolved.

The role of Src in  $G\alpha_q$  and  $\beta\gamma$ -mediated stimulation of ERK-module has been discussed above. In addition, it has been observed that v-Src transformed Rat-1 fibroblasts show an enhanced  $G_q$  and  $G_{11}$  response to endothelins (Liu *et al.*, 1996). An analysis of  $G\alpha_q$  and  $G\alpha_{11}$  in these cells indicated that they are hyperphosphorylated at the tyrosine residues and their ability to activate PLC has been greatly increased. Taken together with the role of Src in providing signal coupling between Pyk2 and Shc in  $G\alpha_q$  as well as  $\beta\gamma$  signaling pathways (van Biesen *et al.*, 1996; Gutkind, 1998), it appears that there is dynamic positive interaction between Src and  $G\alpha_{q/11}$  (Liu *et al.*, 1996). It has been proposed that the tyrosine-phosphorylation of  $G\alpha_{q/11}$  plays a role in their interactions with their receptors (Umemori *et al.*, 1997). In this context, it is noteworthy that  $G\alpha_s$  as well as  $G\alpha_i$  can also be phosphorylated *in vitro* by Src (Hausdorff *et al.*, 1992). In these studies, it has been observed that the immunoprecipitated Src preferentially phosphorylates the purified  $G\alpha_s$  and  $G\alpha_i$  in an immunocomplex kinase assay. Although the tyrosine-phosphorylation of  $G\alpha$ -GDP slightly enhanced its affinity for GTP $\gamma$ S in these studies, the significance of these phosphorylations yet to be determined. In contrast, the phosphorylation of  $G\alpha_z$  and  $G\alpha_{12}$  by PKC have been shown to reduce the affinity of these  $\alpha$ -subunits to  $\beta\gamma$  (Fields and Casey, 1995; Kozasa and Gilman, 1996). Interestingly,  $G\alpha_{12}$  and  $G\alpha_{13}$  have been shown to be rapidly phosphorylated in response to thrombin- or thromboxane A2 in a PKC dependent manner (Offermanns *et al.*, 1996). Although these studies propose a regulatory role for PKC in G protein signaling, the functional significance of these phosphorylations remains to be established.

G proteins have been also implicated in the tyrosine-phosphorylation and subsequent activation of the STAT-family of transcription factors (Bhat *et al.*, 1994; Marrero *et al.*, 1997). It has been observed that the stimulation of the AT<sub>1</sub>-Angiotensin II receptor that primarily couples through  $G_q$  results in the phosphorylation of STAT-1 and STAT-3 through a JAK2-mediated mechanism. In this context, it is worth noting that recent studies have indicated that Src family of kinases and not the Janus kinases (JAKs) activate STATs (Chaturvedi *et al.*, 1998). Considering the critical role of Src in transmitting the signals from both  $G\alpha_q$  and  $\beta\gamma$  to Ras pathway, it is conceivable that AT<sub>1</sub>-Angiotensin II receptor-stimulated  $G\alpha_q$  and  $G\beta\gamma$  utilize Src to activate the STATs. This tantalizing link remains to be established. Similarly, the functional significance of the ability of  $G\alpha_q$  to stimulate the novel Bruton's tyrosine kinase remains to be established (Bence *et al.*, 1997). Nevertheless it is interesting to note that the cells deficient in BTK show a blunted p38MAPK-response to  $G\alpha_q$ -coupled receptors (Bence *et al.*, 1997).

In contrast to the positive interactions between G proteins and other signaling molecules, there are some instances in which these interactions led to the negative-regulation of specific mitogenic pathways. A case to the point is the ability of cAMP from  $G_s$  signaling pathway to inhibit ERK-signaling pathway (Burgering *et al.*, 1993; Graves *et al.*, 1993; Cook and McCormick, 1993; Wu *et al.*, 1993; Sevetson *et al.*,

1993). Recently it has been shown that during genotoxic stress, p53, the well characterized tumor suppressor, downregulates the activity of  $G\alpha_q$  and  $G\alpha_{11}$  through  $G\alpha_{q/11}$ -specific RGS protein (Buckbinder *et al.*, 1997), a negative regulator of G protein activity (Berman and Gilman, 1998). Taken together, it appears that the parallel signals from different pathways modulate G protein signaling pathways. Either by recruiting multiple growth promoting signals or by actively canceling out the inhibitory signals, G proteins coordinate different signaling pathways so as to commit the cells to proliferation.

### Concluding remarks

G protein signaling involves two specific signaling components, namely the  $\alpha$ - and the  $\beta\gamma$ -subunits. It should be noted here that the *gsp*, *gip2*, and the signaling pathways activated by the QL mutants of  $G\alpha_q$  or  $G\alpha_{12/13}$  are  $\alpha$ -dependent pathways. Since the overexpression of the activated mutants of  $G\alpha$  does not involve a compensatory increase in the  $\beta\gamma$ -subunits, the signaling pathways stimulated by GTPase-deficient  $\alpha$ -subunits are independent of the  $\beta\gamma$ -subunits. Although the overexpression  $G\beta\gamma$  leads to the activation of ERK, JNK, and PLC pathways,  $G\beta\gamma$  fails to stimulate cell proliferation oncogenic transformation. Thus, it appears as though  $G\alpha$  generates a unique signaling input to activate cell proliferation. Nevertheless, it should be noted that  $G\beta\gamma$ -signaling plays a definitive role in the normal physiological activation of G protein coupled receptors by their effectors. Perhaps, during such receptor-activated mitogenic pathways, in the absence of the activating mutations in the  $G\alpha$ -subunits, dual signaling inputs from both the subunits may be required to increase the duration and the strength of specific mitogenic signal. Accordingly, the stimulation of  $G_s$  coupled receptor in GH3 or thyroid cells can lead to two mitogenic signaling inputs: 1, AC-cAMP-PKA signaling from  $G\alpha_s$  and 2, Ras-Raf-MEK-ERK signaling from  $G\beta\gamma$ . Even in cell types in which  $G\alpha_s$  is growth inhibitory, the  $\beta\gamma$ -mediated ERK-signaling can bypass the inhibitory signal of  $G\alpha_s$  (Crespo *et al.*, 1995).

In the case of  $G\alpha_i\beta\gamma$  heterotrimer, upon receptor stimulation both  $G\alpha_i$ -GTP and  $G\beta\gamma$ - can stimulate ERK pathway through two different mechanisms. While  $G\alpha_i$  can stimulate ERK through Ras-independent pathway,  $G\beta\gamma$  stimulates ERK through Ras. Similarly, in the case of  $G\alpha_q\beta\gamma$  heterotrimer, both  $G\alpha_q$  and  $G\beta\gamma$  can activate PLC, thus strengthening the PLC-mediated mitogenic responses. Theoretically,  $G\beta\gamma$  released from  $G\alpha_{12}\beta\gamma$  and  $G\alpha_{13}\beta\gamma$  heterotrimers can activate both ERK and JNK through Ras and Rac respectively. However, the finding that the stimulation of wild-type  $G\alpha_{12}$  leads to the activation of JNK with little or no activation of ERK suggests that  $G\alpha_{12}$  somehow channelizes the  $G\beta\gamma$ -subunits to specific effector molecules.

Teleologically, the ability of  $\alpha$ - and the  $\beta\gamma$ -subunits of the heterotrimer to regulate similar responses may provide the optimal threshold levels of signaling strength required to initiate the mitogenic response. In contrast, the activated mutants of  $\alpha$ -subunits, through constitutively and persistently activating the

specific responses, may readily reach this threshold independent of the  $\beta\gamma$ -subunit. Moreover, diverse signaling pathways cooperate with G protein signaling in converging towards the activation of specific kinases and transcription factors to initiate cell proliferation. Such dynamic cooperative signaling between G proteins and other signaling pathways finally define the specific signaling network involved in cell proliferation. Further analyses of G protein signaling in relation to other signaling mechanisms should define the dynamic interactions that occur *in vivo* between different signaling pathways. Now that the critical players involved in these the signaling pathways have

been identified, further studies should clarify the mechanism by which they interact and integrate to form a signaling network involved in the regulation of cell proliferation.

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