REGULATION OF TRANSCRIPTION FACTORS BY HETEROTRIMERIC G PROTEINS

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Abstract

Lessons from viral hijacks of cells and cancer biology suggest that the activation of G protein-coupled receptors (GPCRs) often results in the modulation of various transcription factors and cofactors. Since drugs acting on GPCRs represent a significant portion of therapeutic agents currently in use, it is important to understand the actions of GPCRs on gene expression. GPCRs and their associated heterotrimeric G proteins are known to regulate gene transcription through complex signaling networks. The G protein-mediated signaling cascades have been extensively studied and accumulating evidence indicates that the four subfamilies of G proteins may utilize both common and unique pathways for transcriptional regulation. This review aims to provide a contemporary account of our understanding on the regulation of transcription factors by GPCRs, with a special emphasis on specific regulations of transcription factors such as STAT3 and NF- κ B by individual G protein subfamilies. Functional impacts of the signal integration between different pathways and the contributions by other GPCR-interacting molecules will also be briefly discussed.

Key Words

GPCR, heterotrimeric G proteins, transcriptional regulation, signal integration

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Introduction

Heterotrimeric G proteins mediate diverse signaling events triggered by the activation of canonical seven-transmembrane G protein-coupled receptors (GPCRs) as well as by a growing repertoire of "atypical GPCRs" such as multimeric growth factor receptors. Sustained activation of the $G\alpha$ subunits of heterotrimeric G proteins (e.g., by mutations that inhibit their GTPase activity or by constitutively active GPCRs) can transform fibroblasts and result in carcinogenesis. Cancer cells can play a similar trick by expressing GPCRs and/or their agonists ectopically to create a positive feedback signal that promotes growth. Some G protein-mediated proliferation and transforming signals are so effective and convenient such that cytomegaloviruses and herpesviruses express their own copies of decoy GPCRs during infection to subvert the host cells. These viral GPCRs are typically constitutively active and stimulate the G protein signals in a promiscuous manner, and in some cases the hijacked host cells eventually become cancerous.

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Numerous reports have substantiated the long-term functional impacts of G protein signals on cell proliferation, differentiation and many other cellular processes. Transient boosts of second messengers by the rapid activation/deactivation signaling cycle of G proteins are probably insufficient to drive these complex cellular processes. In fact, transient activation of G proteins can greatly perturb intracellular signaling events and propagate changes into the nucleus to modulate the actions of a great variety of transcription factors. Manifestation of complex cross-talks between different G protein subfamilies as well as with other signaling molecules such as receptor tyrosine kinases may configure the eventual transcriptional events.

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Amino acid sequence homology of the $G\alpha$ subunits and their functional similarities were initially employed to define the four subfamilies of heterotrimeric G proteins. However, the signaling characteristics of each subfamily often converge at some common downstream

targets such as the mitogen-activated protein kinases (MAPKs) that represent major regulatory inputs for transcriptional control. Besides, the functional roles of the $G\beta\gamma$ complex in transcriptional regulations are becoming increasingly eminent. This review attempts to provide an updated account on the regulations of various transcription factors by the four subfamilies of $G\alpha$ subunits as well as their associated $G\beta\gamma$ complex, with an emphasis on the regulations of STATs, NF- κ B and those targets specialized to individual subfamilies of G proteins. Representatives of GPCRs triggering the corresponding G protein-mediated regulations are provided. Functional linkages with other closely interacting molecules and different aspects of signal integration will also be briefly discussed.

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G_s Subfamily

The principal downstream signal for members of the G_s subfamily – $G\alpha_s$ and $G\alpha_{olf}$ – is the stimulation of adenylyl cyclases (ACs) that produce cAMP. Subsequent activation of cAMP-activated protein kinase (PKA) leads to the phosphorylation of cAMP-responsive element (CRE) binding protein (CREB) at Ser¹³³ in the nucleus, which in turn binds to p300/CREB-binding protein (CBP) and regulates the transcription of a great variety of genes [1]. Such G_s -mediated CREB regulation has been demonstrated in different native cellular contexts. Early studies in Aplysia neurons have already shown that continuous serotonin administration increases the cAMP levels and CREB activation, thereby defining a critical role of CREB in memory function [2]. Follicle-stimulating hormone can stimulate the production of cAMP and activate CREB in Sertoli cells [3], and the positive autoregulation of CREB [4] is essential for spermatogenesis. Application of β -adrenergic agonist isoproterenol initiates CREB phosphorylation in primary chick muscle cultures [5]. While an increase of cAMP provokes the PKA-mediated CREB phosphorylation, it also prevents protein phosphatase-1-mediated CREB dephosphorylation through the PKA-mediated

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phosphorylation of dopamine- and cAMP-regulated 32 kDa phosphoprotein (DARPP-32) [6] (Fig. (1), pathway I). As CREB is one of the major signal integrators, activation of G_s-coupled receptors could affect the overall duration of CREB activation and hence the profile of gene transcription [7]. For example, sustained CREB activation is required for c-fos expression in the developing striatum, and the two-way positive regulation by PKA is important in such a process [8].

PKA-induced association of CREB and p300/CBP modulates diverse transcriptional events through several different mechanisms [9]. p300/CBP interacts with various components of the basic transcriptional machinery including RNA polymerase II, TATA-binding protein and TF_{II}B. The acetyltransferase activity of p300/CBP covalently modifies histone proteins and allows chromatin restructuring, as well as other important non-histone transcription factors like p53, Kruppel-like factor, GATA-1, T cell factor and HMG in a differential manner [10]. The role of p300/CBP in G_s-regulated transcriptional activity is exemplified by the study of androgen receptor (AR) in prostate cancer cells [11] (Fig. (1), pathway II). Isoproterenol and expression of constitutively active Gα_s cause PKA-dependent nuclear translocation and activation of AR, albeit AR is not a direct PKA substrate. It is plausible that G_s activation facilitates the nuclear transfer of AR through the PKA-mediated modulation of nuclear transfer machinery and direct acetylation of AR by p300/CBP within the nucleus [12]. A PKA-activated transcription coactivator GT198 identified in certain cancerous tissues may also enhance AR activation [13].

An increase of intracellular cAMP levels can also stimulate the cAMP-sensitive guanine-nucleotide-exchange factors Epac1 and Epac2 [14, 15] that were initially believed to promote the activation of monomeric GTPases Rap1 and Rap2, respectively. In fact, Epac proteins can also activate Ras directly through a region different from the Rap-interacting domain, and stimulate Rac indirectly through the interactions with RacGEFs like Vav2 and

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Tiam1 [16]. Modulation of the various categories of monomeric GTPases by Epac isoforms can subsequently affect the regulation of MAPK pathways and hence the downstream transcription factors (Fig. (1), pathway III).

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An example of the transcriptional consequence of cAMP-modulated Epac activation is in cardiac hypertrophy [17, 18], in which Epac1 forms a multi-protein complex with PKA, ERK5, phosphodiesterase 4D3, and the muscle-specific A-kinase anchoring protein mAKAP [17] (Fig. (1), pathway IV). The complex facilitates the Epac1-mediated ERK5 inhibition, leading to the modulation of a variety of transcription factors such as NF- κ B [19], c-Jun [20], MEK2C [21], c-fos and Fra-1 [22]. mAKAP also brings calcineurin A β and ryanodine receptor RyR2 to the regulation by PKA. Activation of the G_s-coupled β_2 -adrenergic receptor mediates a hypertrophic response in which PKA directly phosphorylates and inactivates RyR2 and contributes to the full activation of NF-ATc through calcineurin [18].

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The well-known cytokine signaling partners – Janus kinases (JAKs) and signal transducers and activators of transcription (STATs) – have recently been identified as novel downstream effectors of different heterotrimeric G proteins, and different G proteins appear to regulate STAT phosphorylation in a slightly different manner. STAT3 becomes phosphorylated at both Tyr⁷⁰⁵ and Ser⁷²⁷ when HEK 293 cells are stimulated with isoproterenol or upon expression of a constitutively active mutant of G α_s [23]. This regulation is cAMP-dependent, as the adenylyl cyclase activator forskolin mimicked the response. PKA, JAK2/3, Rac1, c-Jun N-terminal kinase (JNK), and phosphatidylinositol 3-kinase (PI3K) are all required for the G_s-induced STAT3 phosphorylation at both sites, but only Ser⁷²⁷ but not Tyr⁷⁰⁵ is dependent on c-Src, Ras, Raf-1, and MEK1/2. Compared with the signaling intermediate profiles of other G α -mediated STAT3 activity, PKA, JNK, and PI3K are distinctively engaged in G_s-induced STAT3 phosphorylation [23].

G_i Subfamily

This subfamily consists of the largest number of G α subunits and most of them, except G α_z , can be functionally uncoupled from GPCRs by pertussis toxin (PTX)-catalyzed ADP-ribosylation. The prototypical G α_i subunits have three subtypes (G α_{i1-3}) initially identified to inhibit AC activity. G α_z and G α_o (G α_{oA-B}) share significant sequence homologies and functional similarities with the G α_i subunits. Rod and cone transducins, and gustducin are functionally distinct from other subfamily members by regulating cGMP- responsive phosphodiesterases in very specialized tissues.

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Although ample evidence have revealed the ability of G_i to activate all three classes of MAPKs, specific regulations limited to particular subsets of the signaling cascades are usually observed in different cellular contexts. Formyl peptide receptor like-1 receptor (FPRL-1) mediates ERK, JNK and p38 activation in a PTX-sensitive manner when expressed in CHO cells, but p38 is not activated in U87 astrocytoma cells [24]. Src is required for the activation of both ERK and JNK, but only JNK needed PLC β involvement. The signaling selectivity is further revealed in the gene expression of glial fibrillary acidic protein (GFAP) and interleukin (IL)-1 α ; the former is dependent on both ERK and JNK activation, but the latter is only affected by JNK. An interesting observation in this study pertains to the inability of the formyl peptide receptor to induce GFAP expression [24], thus implying a restriction of the signaling specificity of different G_i-coupled receptors within the same cellular context. Compartmentalization of signaling molecules in subcellular structures like lipid rafts may increase specificity by confining the signaling events. Activation of NF- κ B by FPRL-1 in U87 cells is shown to be mediated through G α_{i2} , Src and ERK. All these components are localized in lipid rafts and the phosphorylation of IKK is presumably regulated by this signaling complex [25].

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The four members of G protein-coupled opioid receptors are well known for their differential analgesic/hyperalgesic effects and importance in drug tolerance and withdrawal. (reviewed in [26]). Though all members are G_i-coupled (but differentially coupled to G_{14/16}), subtle differences between their regulations of transcriptional events may help to explain their distinctive functional roles (Fig. (2), pathway I). In human neuroblastoma SH-SY5Y cells, G_i-coupled μ-opioid receptor (MOR) mediates IKK and p65 phosphorylations ~~that~~ in turn stimulate NF-κB in a PTX-sensitive manner. ERK, PI3K and c-Src, as well as JNK and CaMKII, but not PLCβ and PKC, are involved [27]. ~~Actually~~, all four opioid receptors can stimulate JNK and regulate c-Jun activity [28-30], but different signaling components are involved. ~~MOR is the only one that requires PI3K for JNK activation [30].~~ Acute versus chronic morphine administration can affect the outcome of NF-κB activity. Acute MOR stimulation by morphine increases NF-κB activity and induces expression of opioid receptors and peptides, as well as nitric oxide synthase. The latter together with the superactivation of cAMP production under chronic morphine treatment suppress NF-κB activity ([31] and references therein).

~~A number of well-known growth factor receptor tyrosine kinases have recently been demonstrated to make use of heterotrimeric G proteins to transduce signals.~~ The pro-survival effect of nerve growth factor (NGF) but not EGF receptor for neurons is indeed sensitive to PTX treatment in terms of the regulation of Akt (Fig (2), pathway II). Subtype specificity of G protein coupling has been demonstrated, wherein Gα_{i1}, Gα_{i3}, Gα_{oA} and Gα_{oB} but not Gα_{i2} are involved. The NGF-induced G protein-mediated Akt activation can lead to the phosphorylation of Bad and IKK, as well as NF-κB transcriptional activity [32]. Another important downstream effector of Akt in this context is tuberin. NGF promotes Akt-mediated tuberin phosphorylation and degradation, which relieves the suppression of Rheb and allows its activation of mTOR/raptor. mTOR phosphorylates p70 S6 kinase and 4E-BP1 and leads

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to an increase of S6-mediated ribosome biogenesis and eIF4E-triggered translation process [33]. The NGF-mediated tuberlin degradation observed in PC12 cells and mouse cortical neuron cultures is partially sensitive to PTX treatment, overexpression of RGS proteins and G $\beta\gamma$ scavenger, indicating the requirement of G $_i$ activation [34]. Further evidence of the G $_i$ involvement in tuberlin degradation has been demonstrated by the activation of endogenously expressed α_2 -adrenergic, muscarinic acetylcholine M4 receptor and κ -opioid receptor in PC12 cells, δ -opioid receptor in NG108-15 cells and MOR in SH-SY5Y cells [34-36].

G α_o is abundant in neurons and is concentrated in growth cones, suggesting its essential functions in neurite outgrowth. G α_o is among the first few G α subunits identified to activate STAT3 through Src kinase [37]. Follow-up studies showed that the G $_o$ -dependent regulation of STAT3 is an essential pathway for cannabinoid CB $_1$ receptor-mediated neurite outgrowth in Neuro2A cells [38] (Fig (2), pathway III). Both tyrosine and serine/threonine phosphorylation of STAT3 are enhanced by CB $_1$ receptor, where the former modification is mediated by Src directly, and the latter is probably through Rac/JNK activation under the influence of Src. It turns out that the G α_o -mediated Src/STAT3 activation is dependent on the G α_o regulation of Rap1 monomeric G protein [39]. G α_o binds and reduces the stability of Rap1GAPII by promotes degradation, Rap1 activity is thus enhanced [39, 40] and stimulates Ral which in turn activates Src [38]. As shown in the same study, all three classes of MAPKs are activated by CB $_1$ receptor in neurons, but the Ras/B-Raf/ERK pathway appeared to be dispensable for neurite outgrowth. Analogous STAT3 regulation by serotonin 5-HT $_{1A}$ receptor in neurons has also been identified in a similar context [41].

Growing evidences suggest the crucial roles of G $_i$ subfamily members in the control of developmental processes, that involve the regulations of a distinctive profile of development-related transcription factors (Fig. (2), right panel). Wnt/Frizzled (Fz) signals regulate diverse cellular and developmental events and it has been recently shown that Fz

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proteins (the receptors of Wnts) can make use of various G_i subfamily members to transduce intracellular signals and act cooperatively with the canonical Wnt/Fz/Dishevelled pathways

[42]. Initial evidence hinted at a role for PTX-sensitive G proteins in mediating Fz2 (but not Fz1) activation of PLC β , PKC and its plasma membrane translocation in F9 teratocarcinoma

[43]. The PTX-sensitive candidates are subsequently identified as $G\alpha_o$ and $G\alpha_{i2}$ [44], thus extending the functional importance of transducin family from vision biology to developmental control. Activation of $G\alpha_{i2}$ by Fz2 facilitates the binding of $G\alpha_{i2}$ to cGMP-

selective phosphodiesterase 6 (PDE6) γ subunit and promotes cGMP degradation. The reduction of cGMP suppresses the cGMP-activated protein kinase (PKG)-mediated β -catenin phosphorylation and destabilization, and hence facilitates the β -catenin-enhanced Lef/Tcf transcriptional activity (Fig. (2), pathway IV). $G\beta\gamma$ complex released from G_o or G_{i2} upon Fz activation subsequently stimulates PLC β , and PKC, Ca^{2+} /calmodulin-sensitive protein kinase II (CaMKII) and calcineurin. Calcineurin directly activates the transcriptional activity of NF-AT (Fig. (2), pathway V).

Smoothed (Smo) protein is another, distantly related GPCR important in embryonic developmental patterning and tissue homeostasis that also make use of G_i subfamily members.

Unlike other typical GPCRs, Smo is a constitutively active GPCR whose activity is constantly suppressed by the coexistence of another transmembrane protein Patched1 (Ptch1). The binding of cholesterol-linked Sonic hedgehog N-terminal fragment (N-Shh) to Ptch1 de-represses Smo and subsequently activates Gli transcription factors (reviewed in [45]).

Reconstitutions of Smo with various G proteins by transfecting Sf9 insect cells indicated that only G_i subfamily members ($G_z \gg G_{i1} > G_{i2} \approx G_{i3} > G_o$), but not any member of the other three subfamilies, can be activated by Smo [46] (Fig. (2), pathway VI). Expression of an oncogenic Smo mutant or Shh in mammalian fibroblasts triggers Gli-mediated transcriptional events in a PTX-sensitive manner and the responses are blocked by various Shh antagonists.

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G_i appears to play dual roles in transducing N-Shh/Smo signals to regulate Gli activity.

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Gα₁₂QL could only partially rescue the Gli activation under PTX treatment, and the C-

terminal tail of Smo is dispensable for G protein coupling but essential for Gli activation [46].

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The Gβγ released from G_i may help to recruit G protein-coupled receptor kinase 2 (GRK2)

and allow β-arrestin2 to interact with the C-terminal tail of Smo [47], to mediate Gα-

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independent signals for Gli activation [48]. The direct effect of Gα_i subunits is believed to be

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the reduction of basal intracellular cAMP level that constantly suppresses Gli activity through

PKA [49], but the possible involvements of PI3K, MEK1 and PKC could not be excluded [50].

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Gα_z and the three Gα_i subtypes but not Gα_o can directly bind to Eya2 [52, 53], an important transcription cofactor in vertebrate muscle development [54]. The activated Gα subunits retain Eya2 on the plasma membrane and abolish its nuclear translocation with Six1/4 to suspend the corresponding transcription events, such as MEF2C expression (Fig. (2), pathway VII). Reciprocally, the ability of Gα subunits to inhibit AC is suppressed due to the association with Eya2 [52, 53]. More recent evidence suggests a role of Gα_z in muscle development. Gα_z has been identified in C2C12 myotubes and rat skeletal muscle at early postnatal stages. Unexpectedly, expression of activated Gα_z causes attenuation of myogenic differentiation of C2C12 cells [55]. Preliminary data indicates that myogenin expression is suppressed by Gα_z, and the effect can be reversed by siRNA against Gα_z or expressing a Gα_z mutant devoid of lipid modification. Expressions of other myogenic transcription factors like MyoD and MEF2 are also repressed (H. Mei, M. K. C. Ho and Y. H. Wong, unpublished data). Another possible route for Gα_z to suppress myogenesis is the regulation of Rap1 through the binding of Rap1GAP [56], since activation of Rap1 can inhibit myogenesis [57].

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G_q Subfamily

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The $G\alpha$ subunits of G_q subfamily link GPCRs to the activation of $PLC\beta$ isoforms that hydrolyzes membrane phospholipids phosphatidylinositol biphosphate, PIP_2 to release inositol trisphosphate (IP_3) and diacylglycerol (DAG) – two important second messengers causing Ca^{2+} mobilization and protein kinase C activation (Fig. (3), pathway I). The activated molecules each participates in multiple signaling networks and link $G\alpha_q$ family members to a host of different cellular events. Bradykinin (BK) receptor induces NF- κ B activation through both $G\alpha_q$ and $G\beta_1\gamma_2$ in BK receptor-transfected HeLa cells. PI3K and Akt are involved in this regulation, as the expressions of the functionally deficient mutants of PI3K or Akt greatly reduce BK-induced NF- κ B activity in the transfected cells [58]. Human cytomegalovirus intrusion also employs a similar mechanism to induce NF- κ B activation by encoding a type 1 CC chemokine receptor homolog US28 that constitutively activates both $G\alpha_{q/11}$ and $G\beta\gamma$ subunits [59, 60].

Growing evidence indicates that $G\alpha_q$, $G\alpha_{11}$, $G\alpha_{14}$ and $G\alpha_{16}$ are functionally diverse with regard to their differences in receptor coupling and the gene expression profiles that they regulate (Fig. (3)). Particular $G\alpha_q$ family members have recently been demonstrated to be involved in T cell receptor (TCR) signaling-related transcription activation [61-63] (Fig. (3), pathway II). These $G\alpha$ proteins are linked to TCR signaling upon extensive crosslinking of CD3. Instead of the traditional Lck-dependent signaling pathway, bacteria superantigens utilize an alternative $G\alpha_{11}$ -dependent, $PLC\beta$ -mediated pathway to activate transcription factors that ultimately induce the production of IL-2. In Lck-deficient T cells, bacteria superantigens initiates TCR-dependent activation of raft-enriched $G\alpha_{11}$ protein that in turn stimulates $PLC\beta$ and PKC . Consequently, ERK1/2 are activated and caused the nucleus translation of NF-AT and NF- κ B.

An intriguing role of G_q in transcriptional regulation is through the nuclear translocation of tubby-like protein 3 (TULP3) [64], a member of tubby protein family related

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Deleted: Human cytomegalovirus encodes a CCR1 chemokine receptor homolog US28 that binds RANTES, MCP-1, MIP-1 α , and fractalkine. In COS-7 cells expressing US28, $PLC\beta$ is activated via the constitutive activation of $G_{q/11}$, and both $G_{q/11}$ and $G\beta\gamma$ subunits trigger signaling pathways that converge at NF- κ B activation. Collectively, these data indicate that through expression of US28, cytomegalovirus may utilize the G proteins of the infected cell to alter cellular responses normally stimulated by chemokines

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to the adult-onset obesity as well as other developmental processes such as neural tube formation and photoreceptor cell integrity [64, 65]. Tubby proteins bind to the plasma membrane through interaction with PIP₂, the PLC substrate. Activation of G_q stimulates PLCβ and hydrolyzes PIP₂ that allows tubby proteins to translocate into the nucleus (Fig. (3), pathway III). The molecular consequence of tubby protein translocation is unclear. A more recent study indicates that tubby protein stimulates GTP hydrolysis of the small G protein Rab-7 through the RabGAP RBG-3, implying functional roles not only in the regulation of fat storage but also in the degradation of sensory molecule in neurons [66].

In addition to its expression in spleen, lung, kidney, pancreas, and testis, Gα₁₄ is primarily expressed in hematopoietic cells including bone marrow stromal cells, certain early myeloid cells, and progenitor B cells [67]. Although Gα₁₄ is less intensively studied as compared to Gα_q and Gα₁₁, several pieces of evidence suggest that activation of G₁₄-coupled receptors can lead to the induction of transcription factors such as NF-κB and STAT3 through a complex network consisting of multiple signaling intermediates (Fig. (3), pathway IV). In addition to Gα_{i/o}, type 2 somatostatin receptor (SSTR2) can also couple to Gα₁₄ and Gα₁₆ [68, 69]. In a pancreatic tumor cell line AR4J that endogenously expresses SSTR2 and Gα₁₄, activation of SSTR2 leads to the stimulation of IKK. Further investigation demonstrated that in HEK293 cells transiently expressing SSTR2 and Gα₁₄, NF-κB-driven luciferase activity can be promoted upon activation of SSTR2. Both Gα₁₄ and Gβγ contribute to the activation of three signaling cascades, including PLCβ/PKC/CaMKII, Ras/Raf-1/MEK/ERK and c-Src, and converge at the stimulation of IKK, a prerequisite of NF-κB activation [70]. In HEK293 cells, coexpression of Gα₁₄ and G_i-coupled δ-opioid receptor (DOR) result in DPDPE-induced activation of STAT3. Similar to the induction of NF-κB by Gα₁₄, multiple signaling intermediates are required for STAT3 regulation and they include PLC-β/PKC/CaMKII, c-Src/JAK2 and Ras (or Rac1)/Raf-1/ERK. However, PI3K and the two other MAPK

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Gα₁₄ is rather promiscuous with regard to its interaction with a variety of G_i- and G_s-coupled receptors. It can efficiently link G_i-coupled δ-opioid receptor (DOR) to activate PLCβ in COS-7 cells [69].

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subfamilies, JNK and p38 are not involved [71]. Due to the fact that the constitutively activated STAT3 leads to cellular transformation, it is possible that activation of GPCRs that couple to $G\alpha_{14}$ may cause neoplastic transformation of cells.

The functional uniqueness of $G\alpha_{16}$ in the G_q subfamily is firstly reflected in its receptor coupling promiscuity and highly restricted tissue distribution. Early studies show that $G\alpha_{16}$ can couple the $G_{i/o}$ -linked C5a chemotactic receptor to intracellular Ca^{2+} mobilization. Various heterologous expression studies have proved that $G\alpha_{16}$ indiscriminately interacts with a wide variety of G_s and $G_{i/o}$ -linked receptors [72, 73]. With its restrictive expression in the hematopoietic lineages, it is believed that $G\alpha_{16}$ may play a fundamental role in hematopoietic cell differentiation and function.

Although the underlying mechanisms as to how $G\alpha_{16}$ is involved in the regulation such as lineage exclusion and maturation remain elusive, there is evidence suggesting that $G\alpha_{16}$ mediates the activation of several important transcription factors such as NF- κ B and STAT3. It has been established previously that $G\alpha_{16}$ is able to couple G_i -linked receptors such as formyl peptide, C5a, C3a, and CCR8 receptors to NF- κ B activation [74], but the molecular details have yet to be delineated. In Reh cells, adenosine A_1 receptor (A_1R) can couple to both G_i [75] and G_{16} [68, 73], and its activation results in PTX-insensitive phosphorylation of IKK, an upstream modulator of NF- κ B activation. A_1R -mediated IKK phosphorylation and NF- κ B-driven luciferase activity can also be demonstrated in CHO cells expressing $G\alpha_{16}$. Further examination of $G\alpha_{16}$ -dependent NF- κ B activation revealed the participation of c-Src and several signaling intermediates along the ERK cascade, including Ras, Raf-1 and MEK1/2 [76].

Similarities exist in the signaling cascades involved in the activation of NF- κ B and STAT3. Activation of STAT3 by constitutively active $G\alpha_{16}$ ($G\alpha_{16}QL$) in transfected HEK293 cells requires PLC β /PKC/CaMKII and Ras/Raf-1/ERK. The non-receptor tyrosine kinases, c-

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Src, JAK2 and JAK3 are also involved as demonstrated by the use of specific inhibitors and dominant-negative mutants [77, 78]. Likewise, $G_{\alpha_{16}}$ QL stimulates STAT1 and c-fos transcriptional activities via PLC β , c-Src/JAK and ERK pathways [79]. In human erythroleukemia (HEL) cells, stimulation of prostacyclin receptor (hIPR) by its specific agonist cicaprost induces STAT1 and STAT3 phosphorylation in a PTX-insensitive manner [80]. It is speculated that hIPR may utilize $G_{\alpha_{16}}$ and $G_{\alpha_{14}}$ endogenously expressed in HEL cells to regulate STAT3. ERK and JNK, but not p38 MAPKs are phosphorylated in response to cicaprost and the physical association between STAT3, ERK and JNK can be enhanced by cicaprost. The regulation of STAT3 by hIPR may explain the effect of prostacyclin on Bcl-2 gene expression and in hematopoiesis [79].

The regulations of Ras-ERK pathway by G_q subfamily members are further complicated by the involvement of tetratricopeptide repeat 1 (TPR1). TPR1 serves as a direct linkage between $G_{\alpha_{16}}$ or G_{α_q} and Ras (Fig. (3), pathway V). Activated $G_{\alpha_{16}}$ preferentially binds to TPR1 and the interaction stabilizes the accumulation of activated Ras [81]. The coexistence of TPR1 and $G_{\alpha_{16}}$ in various hematopoietic cells as shown in the same study suggests that this interaction could have crucial roles of $G_{\alpha_{16}}$ functions in a tissue-specific manner. Preliminary evidence shows that $G_{\alpha_{14}}$ is also a binding partner of TPR1 (D. H. T. Kwan and Y. H. Wong, unpublished data). Together with the canonical PLC-related pathways, G_q subfamily members can generate a robust activation of Ras-ERK signal.

G₁₂ Subfamily

$G_{\alpha_{12}}$ and $G_{\alpha_{13}}$ constitute the fourth subfamily of G_{α} subunits. Their biological importance and signaling mechanisms have been intensely studied. The two G_{α} subunits are now known to induce malignant transformation of fibroblasts [82-84], and regulate cell growth [85-87], cell migration [88-90], and differentiation [91, 92]. To understand the

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signaling cascades underlying the biological responses of $G\alpha_{12}$ and $G\alpha_{13}$, attempts have been made to identify their downstream effectors or and functional regulators [83]. The interactions between $G\alpha_{12}$ and $G\alpha_{13}$ and their binding partners lead to a series of signaling events involving protein kinase cascades and trigger various transcriptional activities.

Rho GTPases, belonging to the Ras superfamily of small monomeric G proteins, have been identified as the downstream signaling mediators of $G\alpha_{12}$ subfamily (Fig. (4), pathways I and II). Rho GTPases are important for the regulation of actin cytoskeleton rearrangement, microtubule dynamics as well as transcription factor activity [82]. The abilities of $G\alpha_{12/13}$ to activate Rho GTPases through direct interactions of $G\alpha_{12/13}$ with guanine nucleotide exchange factors (RhoGEFs) have been reported [93-95]. Rho GTPases can regulate the expression of c-fos, in which the serum response element (SRE) on the c-fos promoter is essential for the regulation [84]. Over-expression of GTPase-deficient mutant for $G\alpha_{12/13}$ ($G\alpha_{12}QL$ or $G\alpha_{13}QL$) robustly stimulates transcriptional activity of SRE [96, 97], as well as the expression of early growth response 1 (Egr-1) and c-fos through Rho GTPase activation [87]. G_{12} -coupled receptors such as those for sphingosine 1-phosphate (S1P) [85], thromboxane A_2 [97], lysophosphatidic acid (LPA) [97], have been shown to activate Rho and SRE activity. Interestingly, some GPCRs such as the 5-hydroxytryptamine (5-HT) 4A receptor can specifically activate $G\alpha_{13}$ but not $G\alpha_{12}$ and result in agonist-induced SRE activation [86]. Yet, another subtype of 5-HT receptor, 5-HT₇ receptor, regulates SRE-mediated gene transcription through $G\alpha_{12}$ -dependent activation of RhoA and Cdc42 [98].

JNK has been reported as the major form of MAPKs regulated by $G\alpha_{12/13}$. Overexpression of $G\alpha_{12}QL$ or $G\alpha_{13}QL$ results in a robust stimulation of JNK [99-102] in diverse cell types. $G\alpha_{12}$ -induced JNK activation leads to an increase in c-Jun transcriptional activity [99] and promotes cell proliferation [88]. G_{12} -coupled receptors such as LPA [103, 104], thrombin [105], angiotensin II [106], and S1P [107], can indeed activate JNK. In

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1321N1 astrocytoma cells, the thrombin receptor requires $G\alpha_{12}$ to induce AP-1

transcriptional activity [108, 109]. Stimulation of G_{13} -coupled endothelin B receptor induces preproET-1 (ppET-1) gene transcription via JNK [110]. Recently, it has been discovered that

G_{12} -coupled S1P receptors specifically modulates NF- κ B-driven COX-2 induction through JNK-dependent ubiquitination and degradation of I κ B α [107] (Fig. (4), pathway III).

$G\alpha_{12/13}$ -mediated JNK activation stimulates not only c-Jun transcription factor, but also nuclear translocation and activation of NF-AT [111, 112]. In cardiac fibroblasts,

angiotensin II-induced NF-AT activation has been shown to be dependent on the activation of $G\alpha_{12/13}$ proteins, Rac, NADPH oxidase, JNK and $G\alpha_{12/13}$ -driven ROS production [111].

$G\alpha_{12/13}$ appears to be an important component in regulating endothelin-1-driven myofibroblast formation and collagen synthesis through activation of NF-AT [112].

Regulation of other transcription factors mediated by $G\alpha_{12/13}$ subfamily has also been reported. $G\alpha_{13}$ QL stimulates NF- κ B-dependent reporter gene in HeLa cells [113]. A recent

report shows that overexpression of $G\alpha_{12}$ QL induces STAT3 phosphorylation as well as DNA binding ability of STAT3 in diverse cell types [114].

$G\alpha_{12/13}$ proteins have been found to be a signaling element of cadherin complex [115].

More importantly, binding of activated $G\alpha_{12/13}$ to the cytoplasmic region of cadherins leads to the dissociation of the transcriptional activator β -catenin from cadherins and thereby

relocalizes β -catenin to the cytoplasm and nucleus [115] (Fig. (4), pathway IV). Expression of $G\alpha_{12}$ QL or $G\alpha_{13}$ QL up-regulates β -catenin-mediated Tcf/Lef-dependent transcriptional

activation in SW480 cells [115]. It is also found that tyrosine phosphorylation of β -catenin, and the activations of Src and Rho can be observed in $G\alpha_{12}$ QL-expressing Madin-Darby

canine kidney cells [116]. Tyrosine phosphorylation of β -catenin leads to dissociation of cadherin-catenin complex at the plasma membrane and in turn promotes β -catenin-mediated

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transcription [117]. A recent report showed an interaction between $G\alpha_{12}$ and catenin p120, p120^{ctn}, a cadherin-associated protein which binds to the juxtamembrane region of the cytoplasmic domain of E-cadherin [118] (Fig. (4), pathway V). Interestingly, a couple of zinc-finger transcription factors, such as Glis2 [119], Kaiso [120] and Nanos1 [121], have been identified as p120^{ctn}-specific binding partners [122]. These transcription factors are related to the disruption of cell-cell adhesion and increase of cellular mobility and invasiveness in tumor cells, recapitulating $G\alpha_{12/13}$ -induced malignant transformation. Together with the regulation of Rho-mediated cytoskeleton rearrangements, $G\alpha_{12/13}$ appear to play central roles in orchestrating different signaling pathways for driving cell movements.

Gβγ Complex

The roles of Gβγ complex in transcriptional regulation are perhaps far more intriguing than their $G\alpha$ counterparts. Common effectors like PLCβ and AC isoforms are differentially regulated by Gβγ complex, which adds another level of regulatory signals and thus modulates the related downstream transcriptional events (see next section for more details). Recently, accumulating evidences demonstrate the critical roles of Gβγ in the regulation of a distinct set of transcription factors (Figure 5). Using $G\gamma_5$ as a bait in yeast two-hybrid screening, adipocyte enhancer binding protein 1 (AEBP1) is found to interact directly with Gβγ₅ [123]. Gβγ₇ is ineffective in the AEBP1 interaction. Gβγ₅ attenuates the repressive activity of AEBP1 to adipogenesis-related gene transcription in 3T3-L1 cells (Fig. (5), pathway I). It should be noted that $G\gamma_5$ expression is reduced selectively in the mitotic clonal expansion phase early at the onset of adipogenesis and then resumes upon the entry of terminal differentiation, inferring a crucial temporal role of Gβγ₅ in adipocyte differentiation. AEBP1 is a known downstream effector of ERK [124], and recent evidence suggests that NF-κB is

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upregulated by AEBP1 through sequestering IκBα and promoting its degradation [125]. The functional impacts of Gβγ in the regulation of AEBP1 and the related signaling events are far more complex than one would expect.

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Another identified nuclear binding partner of Gβγ complex is glucocorticoid receptor (GR) [126]. The WD-40 motif of Gβ may be essential for the GR interaction, because a structural analog RACK1 (receptor for activated C-kinase 1) also binds GR. Activation of G_i-coupled somatostatin receptor in GH3 cells induces nuclear translocation of Gβγ and accumulation at GR element, and the transcriptional activity of GR is inhibited by the direct binding of Gβγ toward the AF-2 domain of GR (Fig. (5), pathway II). The inhibitory effect of Gβγ on GR is attenuated by forced nuclear export [126] but enhanced by blockade of the prenylation of the Gγ subunit [127], suggesting that the functional blockade of GR by Gβγ occurs within the nucleus.

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A third category of transcriptional modulators identified as Gβγ-interacting partners in the yeast two-hybrid assay are two class II histone deacetylases HDAC4 and HDAC5. Activation of α₂-adrenergic receptor enhances the Gβγ-HDAC5 interaction, whereas overexpression of Gα_o is suppressive [128]. The transcriptional repression property of HDAC5 toward the transcription factor MEF2C is relieved by the Gβγ interaction [128] (Fig. (5), pathway III). Interestingly, Gβγ has an additional enhancing effect on the MEF2C transcriptional activity. MEF2C is known to interact with the N-terminal region of HDAC5, where Gβγ also binds in addition to the C-terminus of HDAC5. Gβγ actually facilitates the HDAC5-MEF2C coupling by direct interaction with MEF2C [129], perhaps serving as a scaffold to allow MEF2C immediate access to particular gene promoters upon inactivation of HDAC5.

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Signal Integration

In a specific cell type, the temporal and spatial presentations of particular signaling pathways collectively determine the biological outcomes. Signal integration between different $G\alpha$ subfamilies has been demonstrated both in transfected and endogenous cell systems, where they act cooperatively to regulate multiple transcriptional events. A number of examples are described below.

While the regulations of cAMP production by $G\alpha_s$ and $G\alpha_i$ apparently oppose each other, the two streams of signals can converge and act synergistically at different levels. The type 2 subfamily of ACs (subtypes 2, 4 and 7) are insensitive to $G\alpha_i$ inactivation. However, synergistic activation of AC2 by the costimulation of G_s - and G_i -coupled receptors has been documented in many studies [130-134]. In fact, the $G\beta\gamma$ complex released from the activation of G_i stimulates AC2 coincidently with $G\alpha_s$ activation. The compositions of AC subtypes in a particular cell type could therefore determine the characteristics of ligand-induced cAMP production and hence the downstream regulation of transcriptional activity.

Both $G\alpha_s$ and $G\alpha_{i2}$ show transforming capabilities when overexpressed in different types of fibroblasts [135, 136]. The phenomena definitely can not be fully explained by their opposite regulation of cAMP production. One of the most intriguing findings is the stimulation of Src family tyrosine kinases (Src and Hck) by the direct interactions with either $G\alpha_s$ or $G\alpha_i$, whereas both of them opened up the catalytic domain of Src in a similar fashion [137]. Since uncontrolled upregulation of Src family tyrosine kinases will generally lead to cancerous effects, such coincidence may explain the fact that mutations of both $G\alpha_s$ and $G\alpha_{i2}$ could be found in various genetic disorders and cancers [138].

In human SK-N-MC neuroepithelioma cells, simultaneous stimulation of endogenously expressed G_i -coupled neuropeptide Y_1 and G_q -coupled muscarinic acetylcholine M_1 receptors results in synergistic activation of ERK. The enhanced ERK

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activation is associated with an increase in CREB phosphorylation. Further investigation indicated that Ca^{2+} and Src-family tyrosine kinases provided the two primary signals for this synergistic activity [139]. In COS-7 cells cotransfected with G_s -coupled dopamine D_1 receptor (D_1R) and G_q -coupled gastrin-releasing peptide-prefering bombesin receptor (GRPR), despite the fact that D_1R or GRPR agonist alone is able to trigger the JNK kinase activity, activation of G_s -coupled D_1R exhibits a significant inhibitory effect on this kinase activity triggered by GRPR stimulation. This result suggests that “fine tuning” of JNK activation is accomplished by receptor cross-talk [140]. Signal integration also occurs during the activation of other MAPKs. In transfected PC12 cells, simultaneous occupancy of G_q -coupled 5-HT_{2A} receptor and G_s -coupled 5-HT_{7A} receptor enhances the activation of Akt and ERK [141].

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Most GPCRs can couple to several G proteins within a single cell. Although bradykinin B_2 receptor (B_2R) is often described as a $G_{q/11}$ -coupled receptor, it is also able to utilize G_{α_i} upon agonist stimulation. In B_2R -expressing HEK293 cells, bradykinin-induced ERK2 and transcriptional activity of Elk-1 are dependent on G_{α_q} -mediated PKC and on G_{α_i} -induced Ras activation. Furthermore, constitutively active mutants of $G_{\alpha_{q/11}}$ or G_{α_i} alone poorly stimulate ERK2, whereas a combination of both led to a synergistic effect. These results indicate that the cooperation between the signaling pathways elicited by dually coupled GPCRs are required for the efficient stimulation of the ERK cascade [142].

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GPCR-mediated transcription control could further be affected by auxiliary proteins that modulate GPCR signals. The actions of regulators of G protein signaling (RGS) can either enhance (as in ion channel regulations) or reduce (such as enzymatic actions of AC or PLC) the duration of G protein-mediated downstream signals. RGS proteins with multiple functional domains can also serve as molecular scaffolds to support interactions between trimeric and monomeric G proteins. The functional importance of RGS proteins has been

extensively discussed [143, 144]. In addition to G protein-mediated signals, GPCRs make use of other means to transduce G protein-independent signals. Besides receptor internalization, β -arrestins play important roles in the regulation of MAPK cascades as well as transcription factors such as p53, NF- κ B and CREB [145]. A number of GPCRs bearing PDZ-binding motifs at the C-terminal tails bind to PDZ domain-containing signaling molecules such as PSD-95, shank and PDZ-RGS3 and provide yet another level of regulatory mechanisms [146]. Homer proteins bind to group I metabotropic glutamate receptors and regulate MAPK cascades, which is believed to be crucial for modulating synaptic plasticity [147]. These examples are only a small number of ample evidence of the GPCR-mediated regulation of transcriptional events in a multi-faceted manner.

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Conclusions

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Numerous studies of the transcriptional controls through the GPCR-G protein axis accumulated for decades enrich the knowledge on the possible physiological impacts of the expression of a particular type of GPCR, and facilitate the exploration of the possible pathological consequences due to ectopic GPCR or G protein expression in the affected tissues. Ligand-bound GPCR activates a specific group of G proteins and trigger various acute signaling cascades as well as long-term complex regulations towards many types of transcription factors. The plethora of overlapping downstream signaling of the four G protein subfamilies are not perplexing observations but implicates the fine controls of the common transcriptional events in particular cellular contexts. Different scenarios of G protein-mediated transcriptional regulations have been discussed in the context of particular cell types or tissues, but it does not exclude the possibility of combinatorial effects of the discussed pathways to interact with each other. Knowledge of colocalization and segregation of particular subsets of G protein-related signaling molecules is not in the scope of our

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discussion, and that would be one of the major concerns for ones who would like to compare and contrast the signaling uniqueness of individual GPCRs. Nevertheless, continuous investigations of G protein-mediated regulatory mechanisms towards novel transcription factors will unravel new ideas on the important roles of GPCR and G proteins in developmental control, cancer progression and long-term modulations of cellular physiology.

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Table 1. Regulations of transcriptional activity by G protein subfamilies

G Subfamily	Regulated Transcription Factors	Signaling Intermediates	Cell / Tissue Type	Possible Function	References
G_s	CREB	AC, PKA, DARPP-32, PP1	Neuron	Learning and memory	[6-8]
	NF-ATc	mAKAP, calcineurin A β , RyR2	Cardiomyocytes	Cardiac hypertrophy and cell transformation	[18]
	NF- κ B c-Jun MEF2C c-fos / Fra-1	Epac1, PKA, ERK5, PDE4D3, mAKAP	Cardiomyocytes	Cardiac hypertrophy and cellular transformation	[17, 19-22]
	STAT3	PKA, JAK2/3, Rac1, JNK, PI3K, Src, Ras, Raf-1, MEK1/2	Various fibroblasts	Cellular transformation	[23]
G_i	Gli1-3	AC, PKA, GSK3	Mouse embryonic fibroblast	Smoothed-mediated developmental signaling	[46, 49]
	Lef / Tcf	PDE6, PKG, β -catenin	F9 teratocarcinoma	Wnt / Fz2-mediated developmental signaling	[42-44]
	NF- κ B	Src, ERK, IKK, PI3K, JNK, CaMKII	U87 astrocytoma; SH-SY5Y neuroblastoma	Cytokine release; acute and chronic morphine actions	[24, 25, 27-31]

Deleted: Table 1. Regulations of transcriptional activity by G protein subfamilies¶
G α Subfamily

	Six1/4	Eya2	Myoblast	Suppress myogenic transcription	[52, 53]
	STAT3	Rap1GAPII, Rap1, Ral, Src	Neuron, Neuro2A cells	Neurite outgrowth	[37-40]
G_q	NF-AT	PLC β , PKC, ERK1/2	T cells	T cell receptor signaling	[61-63]
	NF- κ B	PI3K, Akt, PLC β , PKC, ERK1/2, c-Src, JAK2/3	Immune cells, AR4J pancreatic carcinoma, Reh cells	Chemoattractant actions; inflammatory responses	[70, 74, 76]
	STAT1/3, c-fos	PLC β , TPR1, PKC, CaMKII, Ras, Raf-1, MEK, ERK1/2, c-Src, JAK2/3	HEL erythroleukemia		[71, 77-80]
	TULP3	PLC β	Adipocytes; neurons	Fat storage; neuronal development	[64-66]
G₁₂	AP-1, c-Jun, NF- κ B	p115RhoGEF, Ras, Rac, JNK, p38	Fibroblasts, 1321N1 astrocytoma	Cell proliferation and migration	[88, 90, 99-106]
	c-fos, Egr-1, SRF	RhoGEF, Rho	NIH3T3 cells	Cytoskeletal rearrangement	[84, 87, 96, 97]
	Glis2, Kaiso, Nano1	E-cadherin, p120 ^{ctn}	SW480 and DLD1 colorectal carcinoma, MDCK and HCT116	Cellular adhesion; tumor metastasis	[117, 119-122]

			cells		
	Lcf / Tef	Cadherins, β -catenin	SW480 cells, MDCK cells	Cellular adhesion; tumor metastasis	[115-117]
	NF-AT	Rac, NADPH oxidase, JNK	Myofibroblasts, HeLa cells	Cellular transformation	[111, 112]
	STAT3	PDGF α , PI3K, JAK, Src	Various fibroblasts	Transactivation of PDGF α	[113, 114]
G$\beta\gamma$	AEBP1	G γ_5 (direct binding)	3T3-L1 cells	Relieves adipogenic transcription events	[123]
	c-Jun	Src, PLC β , JNK	U87 astrocytoma	Cytokine expression	[24]
	GR	G $\beta_2\gamma_2$ (direct binding)	GH3 cells, HCT116 cells	Suppress GRE-mediated gene transcription	[126, 127]
	NF-AT	PLC β , PKC, CaMKII, calcineurin	F9 teratocarcinoma	Fz2-mediated developmental signaling	[43, 44]
	MEF2C	HDAC5	HEK293 cells	Facilitate MEF2C-mediated transcription	[128, 129]

Figure Legends

Fig. (1) Transcriptional regulatory pathways mediated by G_s . Four different routes of G_s -mediated signals that regulate different transcriptional activities are depicted schematically. (I) Increase of cAMP production activates CREB through PKA-mediated phosphorylation events. (II) PKA regulates the nuclear entry of androgen receptor (AR). (III) cAMP-activated Epac isoforms link to the activation of various small GTPases and regulates transcription factors like STAT3 through MAPKs. (IV) A signalosome containing Epac and PKA mediates a variety of transcriptional activities. The pathways shown may not be necessary to coexist in a single cell. Details of interactions are discussed in the text.

Fig. (2) Transcriptional regulatory pathways mediated by G_i subfamily members in two different cellular contexts. Inside a neuronal environment, (I) GPCRs like opioid receptors activate various MAPKs and trigger transcriptional activities of STAT3, NF- κ B and c-Jun. (II) Non-7-transmembrane growth factor receptors like TrkA (NGF receptor) make use of G_i subfamily members for MAPK regulations and Akt-mediated survival signals. (III) Rap1GAPs bridge G_i subfamily members to small GTPase Rap-mediated STAT3 activation. G proteins take parts in different developmental signals, including (IV) Wnt-Fz2 mediated Lef/Tcf activation through $G\alpha_{i2}$; (V) Fz2 triggers $G\beta\gamma$ release from G_o for activation of NF-AT through PLC β ; (VI) Smo couples to G_i and G_z and regulates Gli transcription factors; and (VII) Eya2 is sequestered by $G\alpha_i$ and $G\alpha_z$ and prevented from association with Six1 for transcription.

Fig. (3) Transcriptional regulatory pathways mediated by G_q subfamily members. (I) $PLC\beta$ is the common downstream effectors for G_q subfamily members. Productions of IP_3 and DAG mediates great varieties of signaling networks including MAPK cascades. (II) T cell receptor/CD3 complex also make use of G_q subfamily members for signaling. (III) G_q -mediated PIP_2 hydrolysis releases TULP3 from plasma membrane to nucleus for transcriptional regulations in neurons and adipocytes. (IV) G_{14} and G_{16} are more promiscuous than G_q and G_{11} in terms of their receptor coupling specificity, and thus transmit more diverse GPCR signals to $PLC\beta$ activation. (V) TPR1 links $G\alpha_q$ and $G\alpha_{16}$ to promote Ras-mediated downstream signals.

Fig. (4) Transcriptional regulatory pathways mediated by $G_{12/13}$. Both members couple to particular sets of GEFs and activate different small GTPases ((I) for G_{12} and (II) for G_{13}). Besides the converged roles on the regulation of Rho-mediated cytoskeletal rearrangements, the coupled small GTPases also activates different transcriptional events through MAPKs. (III) $G_{12/13}$ proteins also make use of other signaling intermediates such as ASK1, Pyk2 and SNAP for regulating STAT3, c-Jun and NF- κ B. (IV) $G\alpha_{12/13}$ seize cadherins from catenins and release them for nuclear entries and transcriptional regulations.

Fig. (5) Direct regulations of transcription events by $G\beta\gamma$ complex. (I) $G\gamma_5$ -containing $G\beta\gamma$ complex removes AEBP1 from nuclear entry for adipogenic transcription. (II) $G\beta\gamma$ serves as scaffold to modulate the HDAC5-MEF2C interaction and the corresponding transcriptional activity. (III) $G\beta\gamma$ colocalizes with glucocorticoid receptor (GR) on the GR elements of gene promoters and blocks its transcriptional activity.