REVIEW

# The puzzling uniqueness of the heterotrimeric G15 protein and its potential beyond hematopoiesis

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## Abstract

Heterotrimeric G proteins transduce the signals of the largest family of membrane receptors (G protein-coupled receptors, GPCRs) hence triggering the activation of a wide variety of physiological responses. G15 is a G protein characterized by a number of functional peculiarities that make its signaling exceptional: 1) it can couple a variety of Gs-, Gi/o-, and Gq-linked receptors to phospholipase C activation; 2) relatively to other G proteins, it is poorly affected by β-arrestin-dependent desensitization, the general mechanism that regulates GPCR function and 3) at the protein level, its expression is only detected in highly specific cell types (hematopoietic and epithelial cells). G15 α-subunit displays unique structural and biochemical properties, and is phylogenetically the most recent and divergent component of the Gαq/11 subfamily. All these aspects shed a mysterious light on G15 biological role, which remains substantially elusive. Thus, far, G15 signaling has been analyzed in the context of hematopoiesis. Here, we highlight observations supporting the view that G15 functions may extend further beyond the immune system. In addition, we describe puzzling aspects of G15 signaling that offer a novel perspective in the understanding of its physiological role.

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## Introduction

The α-subunit of the heterotrimeric G15 protein (Gα15) is the most divergent member of the Gαq family sharing 57% amino acid sequence identity with Gαq (for a sequence alignment see Hubbard & Hepler (2006)). Gα15 was originally cloned in mouse (Wilkie *et al.* 1991). The human isoform was named Gα16 assuming it represented a novel subtype (Amatruda *et al.* 1991), but later on it was recognized as the poorly conserved ortholog of Gα15 sharing only 85% sequence identity.

G15 is best known for its ability to create a functional link between hundreds of different G protein-coupled receptors (GPCRs) and the β isoform of phospholipase C (PLCβ; Milligan *et al.* 1996). For this reason, G15 has often served as a versatile readout, particularly, in the preliminary characterization of orphan GPCRs when the lack of an agonist precludes any functional characterization of the downstream signaling pathway (and vice versa). Powerful cellular platforms for the screening of specific ligands have been created by co-expressing G15 together with orphan GPCRs. Furthermore, thanks to G15, it was confirmed that unconventional GPCRs like OA1 (Innamorati *et al.* 2006) and smoothened (Masdeu *et al.* 2006) are indeed G protein-coupled.

Despite the restricted expression profile (Table 1) and the great success as a pharmacological tool, a number of gene knockout studies (in animal or cellular models) revealed relatively little about G15 physiological activity (see below). Part of the mystery surrounding the role of this G protein may derive from assumptions drawn perhaps too prematurely after the cloning, such as an exclusive link with hematopoiesis.


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**Table 1** List of organs and cells from various organisms showing Gα15 expression according to literature data

| Organ, cells | Species | References | EST (m) | EST (H) |
|---|---|---|---|---|
| Immune system | | | | |
| Tonsil: B and T cells | H | Grant *et al.* (1997) | | − |
| Thymus (decreasing in adult) | m | Wilkie *et al.* (1991) | ++ | ++++ |
| Lymph nodes | m | Davignon *et al.* (2000) | − | − |
| Blood | | | − | +++ |
| Activated peripheral blood cells | H | Lippert *et al.* (1997) | | |
| Lymphocytes | | | | |
| Pre-B cells | H | Mapara *et al.* (1995) and Grant *et al.* (1997) | | |
| HSC | H | Lippert *et al.* (1997) | | |
| γδ | H | Lippert *et al.* (1997) | | |
| Activated T cells | H | Lippert *et al.* (1997) | | |
| Megakaryocytes | H | den Dekker *et al.* (2001*b*) | | |
| Platelets | H | Giesberts *et al.* (1997) and Tenailleau *et al.* (1997) | | |
| Neutrophils | m | Davignon *et al.* (2000) | | |
| Monocytes | H | Tenailleau *et al.* (1997) and Su *et al.* (2009) | | |
| Bone marrow: HSC and erythroid cells | H | Tenailleau *et al.* (1997) and Pfeilstocker *et al.* (2000) | ++++ | ++ |
| Epithelia | | | | |
| Hair follicle | b | Wilkie *et al.* (1991) | | |
| Skin: keratinocytes | b | Rock *et al.* (1997) | +++ | + |
| Tongue: taste bud | r | Kusakabe *et al.* (1998) | − | +++ |
| Thymus: epithelial cells | H | Fig. 2, this review | | |
| Other organs | | | | |
| Brain (traces) | m | Wilkie *et al.* (1991) | + | + |
| Heart (traces) | m | Wilkie *et al.* (1991) | +++ | − |
| Lungs (traces) | m | Wilkie *et al.* (1991) | − | ++ |
| Kidney (traces) | m | Wilkie *et al.* (1991) | − | + |

H, human; b, baboon; m, mouse; r, rat.
"Traces" reported in parenthesis means that mRNA expression is low and the protein is undetectable by immunoblotting. Other tissues where reported as substantially negative: yalk (m), uterus (m), testis (m), liver (m, H) (Fig. 2) (Wilkie *et al.* (1991)). In the last two columns the approximate expression patterns inferred from EST sources as reported by UniGene (NCBI). Reference numbers are Hs.73797 and Mm.1546 for human and mouse respectively. Symbols are present when data are available and refer to the number of transcripts per million of ESTs (−, absent; +, 1–9 copies; ++, 10–29 copies; +++, 30–99 copies; ++++, more than 100 copies).

## The evasive nature of Gα15 expression

### Gα15 distribution profile

#### *Hematopoiesis*

In the original characterization, the murine Gα15 was found to be selectively expressed by hematopoietic cells and therefore in the bone marrow, thymus (where it declines in the adult), spleen, and embryonic liver (Amatruda *et al.* 1991, Wilkie *et al.* 1991). Consequently, a wealth of attention was focused on early maturation stages of hematopoiesis (Hubbard & Hepler 2006, Su *et al.* 2009) such as cluster of differentiation 34 (CD34) positive hematopoietic stem cells (HSCs; Tenailleau *et al.* 1997, Pfeilstocker *et al.* 2000), erythroid precursors (Ghose *et al.* 1999), megakaryocytic (den Dekker *et al.* 2001*a*,*b*), and B cells progenitors (Mapara *et al.* 1995). The expression of Gα15 is subsequently lost upon cell maturation. This was reproduced *in vitro* using inducible cellular models, such as HL60 or WB4 cells (Amatruda *et al.* 1991, Grant *et al.* 1997, Tenailleau *et al.* 1997), where the protein expression progressively declines upon acquisition of a neutrophil-like phenotype. On the other hand, Gα15 expression may be transiently restored by committed hematopoietic cells upon specific stimulation, as shown by activating quiescent T cells with Leuco A (Lippert *et al.* 1997).

Based on data collected in four different cell lines, Wilkie *et al.* (1991) concluded that Gα15 is absent in stromal cells lines. In good agreement, we found only minimal mRNA traces, and no corresponding protein signal in mesenchymal stem cells from bone marrow or thymic stromal cells, either freshly isolated or cultured, were found (Fig. 1A and B). It appears therefore that, besides HSCs, other stem cells including more immature stages like embryonic stem cells and yolk do not express Gα15 (Wilkie *et al.* 1991). In summary, G15 expression cannot be generalized to all stem cells, but overlaps with the CD34 marker for stem and progenitor cell population (Pfeilstöcker *et al.* 1998, 2000).



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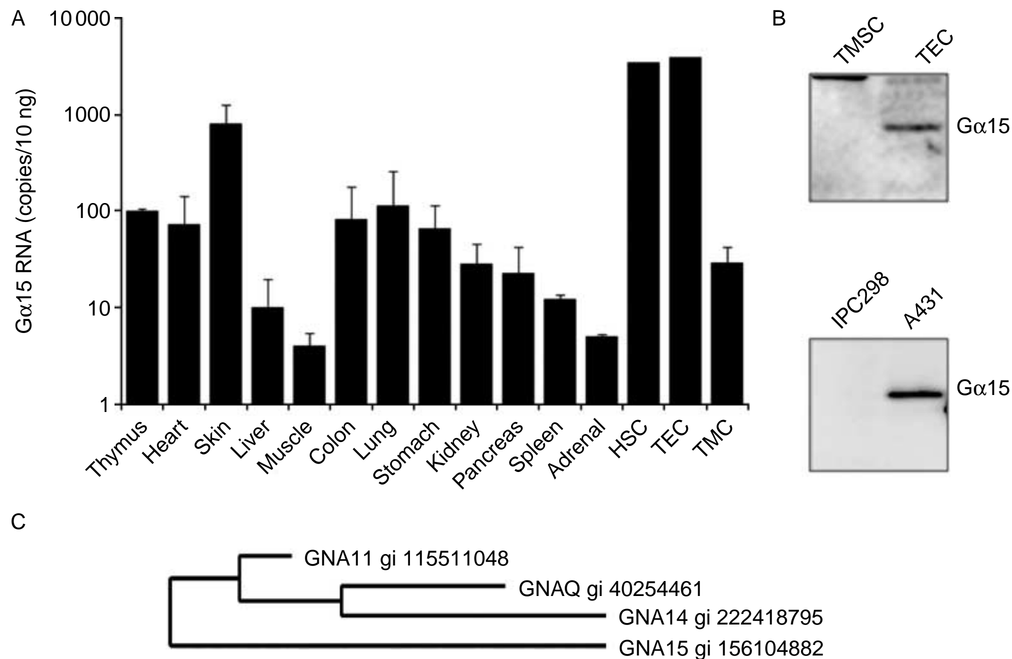


**Figure 1** Gα15 expression in human tissues and cells as assessed by quantitative RT-PCR (A) or immunoblotting (B). HSC is for hematopoietic stem cells, TEC for thymic epithelial cells, TMSC for mesenchymal stem cells. (A) GNA15 (Gα15 gene) expression analysis was performed by quantitative RT-PCR using the TaqMan assay Hs_00157720_m1 (Applied Biosystems). As an endogenous reference, glyceraldehyde-3-phosphate dehydrogenase transcript level was measured in parallel. GNA15 copy number (means of three measures ± S.D.) was assessed by the standard curve method, according to Lai *et al.* (2003). (B) Immunoblotting detected a positive signal in TEC, consistent with the robust Gα15 mRNA presence, while TMSC resulted negative. Gα15 was also present in the epithelioid cell line A413 as opposite to the melanoma cell line IPC298. (C) Phylogenetic tree of Gαq/11 family members obtained at http://www.phylogeny.fr, according to Dereeper *et al.* (2008). GNA15 appears as the most distant member within the Gαq family.

### *Epithelia*

Despite broadly described as hematopoietic specific (Amatruda *et al.* 1991, Hubbard & Hepler 2006, Su *et al.* 2009), G15 expression was occasionally reported in tissues that are not part of the immune system, particularly in a variety of epithelia. While analyzing baboon skin by *in situ* hybridization, Rock *et al.* (1997) reported the presence of Gα15 in hair follicular epithelium. In the hair follicle bulge of murine skin (Shi *et al.* 2003) reside CD34-positive cells that may serve as a reservoir for Langerhans cells as well as for other immune cell precursors (Gilliam *et al.* 1998, Kumamoto *et al.* 2003). The existence of a population of slowly cycling immature cells originated from hematopoietic precursors could thus explain the presence of Gα15 signal. On the other hand, CD34 was also specifically associated with keratinocyte stem cells characterized by high *in vitro* clonogenic potential (Trempus *et al.* 2003), and Gα15 mRNA was found in cultured human keratinocytes from neonatal foreskin (that originate from ectoderm rather than mesoderm) but not in fibroblasts, melanocytes, or endothelial cells (Rock *et al.* 1997). Consistent with this finding, we report robust expression in the skin and in the epidermoid A431 cell line (Fig. 1). Furthermore, northern blot analysis detected Gα15 mRNA in rat tongue epithelia (Kusakabe *et al.* 1998).

Since mature cutaneous epithelium is maintained by an unknown number of progenitor populations (Yan & Owens 2008), it would be interesting to define in deeper details the cellular localization of Gα15 in these tissues.

Consistent with an expression profile extended to epithelial cells, transcriptional levels comparable to HSCs are reached in cells of an internal epithelium, namely thymic epithelial cells (TECs, Fig. 1). By contrast, mesenchymal stem cells derived from the same organ resulted negative.

HSCs, TECs, and progenitor cells in epidermis share functional and phenotypic characteristics. For instance, epidermal keratinocytes can recruit hematopoietic precursors and support the development of a thymic microenvironment (Clark *et al.* 2005). Thus, one could wonder whether a common functional signature links these cell types to the expression of Gα15. A very intriguing possibility is that Gα15 is expressed at intermediate stages of maturation, when cells are in the process of leaving quiescence to generate lineages that can be stimulated to rapid proliferation. If demonstrated, this aspect could be relevant in pathological processes. Although there is no direct



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indication pointing to an involvement of G15 in tumor cell growth (Heasley *et al.* 1996*b*), Gα15 was coimmunoprecipitated to the M1 muscarinic receptor in prostate adenoma (Ruggieri *et al.* 1995, Luthin *et al.* 1997). Normal tissue was not analyzed, but we found the immunoblot of healthy prostate negative for Gα15 expression thus suggesting that its appearance may relate to initial phases of the transformation process.

#### *Or elsewhere?*

By performing Southern blotting analysis of PCR products, Wilkie *et al.* (1991) showed a weak Gα15 expression in several organs. We confirmed by quantitative PCR (Fig. 1A) that very low, albeit significant, levels of Gα15 are present in heart, kidney, and almost all tissues analyzed. Though abundance was much reduced compared with HSCs or TECs, a small number of Gα15 mRNA copies are therefore present in most tissues, nonetheless the translational level remains below the detection limit of the antibodies available for immunoblotting (estimated as <1 ng in HeLa cells (Krumins & Gilman 2006)). An antibody suitable for immunohistochemistry would allow analyzing different epithelia present in various organs and pinpoint positive cell lineages. Unfortunately, a similar tool has not yet been described in the literature, possibly because of the limited choice of epitopes presenting specific immunogenicity without cross-reaction with other members of the Gq/11 protein subfamily members.

Under these circumstances, there is a strong possibility that small subpopulations of cells derived from specialized epithelia may contribute the low signal detected in most human organs.

### Two Gα15 isoforms?

Another puzzling aspect about Gα15 expression is that, in addition to the most commonly observed 43 kDa form, a 46 kDa form was repeatedly described (Lippert *et al.* 1997, Tenailleau *et al.* 1997, den Dekker *et al.* 2001*a*,*b*). During megakaryocytic maturation (den Dekker *et al.* 2001*a*,*b*) and T lymphocyte activation, the transient appearance of the heavier band anticipates the similarly transient expression of the 43 kDa form (Lippert *et al.* 1997). The downmodulation of both forms by five specific shRNA sequences (G Innamorati 2009, unpublished observation) proves that both represent Gα15 rather than a cross-reacting α-subunit, or any other protein sharing a common epitope.

For other G protein α-subunits, alternative splicing causes the appearance of similar doublets in acrylamide gels (Robishaw *et al.* 1986, Tsukamoto *et al.* 1991). However, in the case of Gα15, neither protein kinase C (PKC) phosphorylation (Gu *et al.* 2003) nor cysteine palmitoylation at position 9 and 10 of the amino-terminus (Pedone & Hepler 2007) significantly affected the migration of the recombinant protein. The main gene transcript (NM_002068) of human *GNA15* (the gene encoding for Gα15) consists of seven exons. An alternative splice variant (AK300481) was found in NCBI database by SpliceMiner software (Kahn *et al.* 2007), however, this mRNA variant contains the first two and a larger third exon that are not compatible with the 46 kDa protein.

Further investigation is needed to unravel the molecular details that differentiate these two species and to verify if they fulfill specific roles.

## The evasive nature of G15 coupling

G15 coupling to GPCRs appears as characterized by low selectivity but high efficiency.

### G15 promiscuity and its physiological coupling

Many authors documented the peculiar promiscuity of G15 by showing functional interactions with a wide variety of different receptors in a large number of transfected cell lines (Offermanns & Simon 1995, Zhu & Birnbaumer 1996). Such versatility would predict that dozens of different GPCRs expressed by any given cell (Hakak *et al.* 2003) may act as physiological upstream activators of G15. In addition to various chemokine receptors, HSCs express other GPCRs such as the β2AR (Muthu *et al.* 2007), opioid receptors (Rozenfeld-Granot *et al.* 2002) and smoothened receptors (Masdeu *et al.* 2006). Needless to say, these receptors were proved to be good couplers of G15 in recombinant systems (Offermanns & Simon 1995, Wu *et al.* 1995, Zhu & Birnbaumer 1996, Lee *et al.* 1998, Gutierrez-Frias *et al.* 2004).

However, promiscuity remains to be demonstrated under naive conditions. The identification of specific receptor–G15 interactions represents a very challenging task as PLC is also activated by the ubiquitous Gq/11 or by other G proteins via release of βγ-subunit, or by indirect activation through other intermediate effectors (see for instance the activation of PLCε by Gs via PKA (Schmidt *et al.* 2001)). The lack of pharmacological inhibitors specific for Gα15 further complicates the analysis.

Thus, it is not surprising that only very few examples describe GPCR signaling through naive G15. Knocking out Gα15 expression in transgenic mice reduced the coupling of C5a to calcium release in macrophages while leaving intact the coupling to other GPCRs (Davignon *et al.* 2000), including P2Y2 receptor stimulated by UTP (Davignon *et al.* 2000). Surprisingly, in erythroleukemia cells, silencing Gα15 led to reduced mobilization of intracellular $Ca^{2+}$ upon stimulation of



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the same P2Y2 purinoceptor (Baltensperger & Porzig 1997). These results may be reconciled considering that cell-specific components contribute to define the specificity of receptor–G protein interaction (Ostrom & Insel 2004), and hence specialized cells may dictate specific coupling profiles.

Olfactory G proteins and transducins are exclusively expressed in sensory neurons to mediate the signaling of dedicated GPCRs (olfactory receptors and opsins respectively). Likewise, the selective expression of Gα15 in HSCs suggested that it may serve as a specific effector of GPCRs involved in immunity (Amatruda *et al.* 1993). In this context, sphingosine 1-phosphate receptor 4 (S1PR4) is a good candidate as a physiological activator of G15. The lymphoid tissue-specific S1PR4 is part of a family of receptors responding to lysophospholipids or lysosphingolipids (Rivera *et al.* 2008). It has been reported that the genes encoding for S1PR4 and GNA15 are located in tandem, likely under the control of the same promoter (Contos *et al.* 2002). Consistently, according to microarray data, both the genes are simultaneously expressed in mouse fetal liver cells, and are silenced during erythroid differentiation (A Ronchi, personal communication, 2007). An interaction between these two proteins could explain why, in the presence of S1P containing serum (Yatomi *et al.* 2001), the inhibition or downregulation of Gα15 affects erythroid cells growth and differentiation (Ghose *et al.* 1999). Nonetheless, when tested with GTP photoaffinity label in CHO cells (Graler *et al.* 2003), S1PR4 was shown to be coupled to Gi and G12/13 but not to Gq/11 and G15.

Another good candidate as a specific G15 activator would be CXCR4, the receptor responsible for retaining HSC in the bone marrow (Levesque & Winkler 2008). Bafflingly, several chemokine receptors including CXCR4 (Wu *et al.* 1995, Arai & Charo 1996) in addition to CCR5 (Tian *et al.* 2008), CCR7 (Tian *et al.* 2008), and CCR1 (Arai & Charo 1996, Kuang *et al.* 1996) are among the very few GPCRs that refrain to couple with G15 upon exogenous expression. Again, the cellular context could make a difference since silencing Gα15 mRNA in monocytic THP1 cells partially reduced chemotactic ability in response to CCR1 (Tian *et al.* 2008). Another exception to G15 promiscuity is CCR2A (Kuang *et al.* 1996, Tian *et al.* 2008), but not its splice variant CCR2B that only differs in the carboxyl-terminus. Yet, also CCR2A coupled to G15 when ectopically expressed in HEK-293 cells (Arai & Charo 1996) instead of COS-7 cells. If the cellular context influences the specificity of the interaction, many molecular mechanisms could be involved. Although the GPCR carboxyl-terminus is not generally considered to directly determine G protein specificity (G15 included), it could act indirectly by bridging GPCRs to preassembled signaling complexes. Likewise, post-translational modifications targeting the G protein to specific plasma membrane microdomains could physically restrict G15 interactions with GPCRs partitioned within these discrete areas. Another largely underestimated cause for coupling discrimination is the identity of the β- and γ-subunits forming the heterotrimeric complex. Five β- and twelve γ-subunits assemble in multiple combinations with the α-subunit. If the composition of the heterotrimer narrows the number of upstream GPCR partners (Robillard *et al.* 2000), the cellular repertoire of β- and γ-subunits (Kleuss *et al.* 1993) could be crucial to modulate specificity. In a similar manner, the formation of receptor heterodimers could provide an additional mechanism for discrimination (Maggio *et al.* 2007).

Discrepancies observed in different cellular systems are not unraveled by transgenic animal models that failed to provide a clear indication about which GPCRs are upstream G15. Gα15 knockout mice display normal maturation of all cell lineages and mount a normal response to the immune challenges (Davignon *et al.* 2000). Unfortunately, very limited data are available in non-hematopoietic tissues. For instance, the increase of cGMP occurring upon activation of the muscarinic M3 receptor in membrane fractions of tracheal smooth muscle was inactivated by a Gα15-specific antibody (Bruges *et al.* 2007). Expanding research focus beyond immunity could prove critical. In addition, newly emerged data suggest that G15 action may become particularly relevant under exceptional conditions, i.e. in the case of prolonged stimulation (see below).

## Differential sensitivity of G15 to receptor desensitization

There is an additional feature that makes G15 different from other G proteins, i.e. its relatively enduring activity under conditions in which the coupling efficiency of other G proteins is reduced by GPCR desensitization. GPCR desensitization is a general regulatory mechanism operated by cytosolic adaptor proteins, named β-arrestins, that rapidly translocate to a hundred different GPCRs after agonist stimulation (DeWire *et al.* 2007). The term 'arrestin' derives from the proteins' ability to dampen receptor signaling by steric hindrance of G protein coupling. Moreover, β-arrestins promote receptor endocytosis (internalization) by recruiting endosomal adaptor proteins. The interaction with β-arrestin is stabilized by receptor phosphorylation. Seven isoforms of GPCR kinases (GRKs) are responsible for phosphorylating multiple sites of the receptor carboxyl-terminus in response to ligand binding.

In respect to other G proteins, G15 signaling is poorly affected when desensitization is either induced by repeated GPCR activation or it is emphasized by β-arrestin overexpression (Innamorati *et al.* 2009).



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G15 is the only Gq/11 family member that does not interact with GRK2, whereas for other G proteins the interaction prompts GRK translocation from the cytosol to the plasma membrane. As a consequence, GRK2 is not recruited to GPCR upstream of G15 (Day *et al.* 2003). In addition to a reduced receptor phosphorylation, the missing interaction with GRK2 could have additional consequences since the negative modulation that GRK2 exerts on G protein signaling goes beyond its kinase activity. In fact, a kinase-dead GRK2 mutant was reported to equally modulate the activities of Gq, G11, and G14 while leaving the activity of G15 unaffected. GRKs contain regulator of G protein signaling (RGS) domains believed to accelerate G protein inactivation by stabilizing the transition state of Gα-catalyzed GTP hydrolysis. It is thus tempting to explain G15 enduring signaling with a prolonged permanence in the GTP bound state. However, the RGS domain of GRK2 was described as particularly weak (Carman *et al.* 1999), and other GRKs and RGS containing proteins (such as RGS2), known to interact with G15 (Day *et al.* 2003), could easily compensate. Further investigation is required to explain the mechanistic base of G15 refractoriness to β-arrestin-dependent desensitization that nevertheless implies the permanence of Gα15 in the complex assembled around the desensitized GPCR. This was shown with a V2 vasopressin receptor mutant constitutively stabilized in a desensitized state by a mutation in the conserved DRY sequence (R137H; Barak *et al.* 2001). The R137H-V2R coimmunoprecipitated with Gα15, but not with Gαq or Gαs, as if Gα15 possesses a better capability to remain in direct contact with activated receptors, possibly by competing with β-arrestin (Innamorati *et al.* 2009).

The desensitization process modulates GPCR function by adjusting receptor efficiency to the intensity and persistence of the stimulation. In immune cells GRKs and arrestins are dynamically regulated (Vroon *et al.* 2006) thus the specificity of GPCR response can drastically vary depending on the cell activation state. In the case of stimuli, particularly intense (as possibly during commitment to high proliferation states), G15 could represent a key element that, by better resisting to arrestin-dependent desensitization, takes over when other G proteins become ineffective. In this model, G15 would determine a qualitative evolution of the signal with the final result of reprogramming the cell.

### G15 sensitivity

The interactions occurring between GPCR and G15 may result in more stablity than the interaction with other G proteins. A steady interaction of wild-type P2Y2 receptor with Gα15 was assessed by FRET even in the absence of ligand (Kotevic *et al.* 2005). The presence of G15 biased the pharmacological profile of the κ opioid receptor (Su *et al.* 2009), again suggesting the existence of preformed receptor–G protein complexes. A similar interaction with G15 may even interfere with the activation of other G protein subtypes as shown for pUS28 (Moepps *et al.* 2008), a viral GPCR characterized by elevated ligand-independent constitutive activity and by increased phosphorylation (Minisini *et al.* 2003). This effect was unmasked because Gq/11 promotes serum response factor (SRF)-dependent transcriptional activity much more effectively than G15 or G14, and the overexpression of G15 reduced SRF effect by directly competing with Gq/11 for the chemokine-activated pUS28 (Moepps *et al.* 2008).

Exogenous Gα15 expression at physiological levels (promoted by a tetracycline-induced transactivation system) exerted a similar effect by blunting the $Ca^{2+}$ transient induced through the Gq/11-coupled thyrotropin-releasing hormone receptor (Offermanns *et al.* 2001). G15 expression also inhibited the signaling of β2AR to Gs (Innamorati *et al.* 2009).

Whether the ability of G15 to compete for activated GPCRs relates to its resistance to GPCR desensitization is still to be addressed.

## Getting further insights on G15 biological function

The high degree of promiscuity, combined with the functional redundancy within the Gq/11 subclass and with the absence of specific pharmacological inhibitors, restricts the number of approaches that can be used to define G15 function. For this reason, many researchers took advantage of Gα15-Q212L, a constitutively active mutant incapable of efficient GTP hydrolysis. By this approach, direct activation of downstream effectors is achieved bypassing the GPCR. Gα15-Q212L promoted the activity of transcription factors like nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and signal transducer and activator of transcription 3 (STAT3) via PKC (Lo & Wong 2006, Lee & Wong 2009) and c-Src/mitogen-activated protein kinase (MAPK)-dependent pathway (Wu *et al.* 2003, Liu & Wong 2004; Fig. 2). As a member of a family of latent cytoplasmic transcription factors, STAT3 has long been implicated in cell growth and development relaying signals from the plasma membrane to the nucleus. It is therefore tempting to speculate that G15 promotes quiescence and initiates differentiation programs in transient amplifying cells. Experiments produced in various cell lines suggested that G15 regulates cell maturation but, at the same time, revealed several contradictory aspects. In a neuronal maturation model (PC12 cells) G15 promoted cell differentiation (Heasley *et al.* 1996*a*), and similar results were observed in a model of erythroid differentiation (MB-02 erythroleukemia



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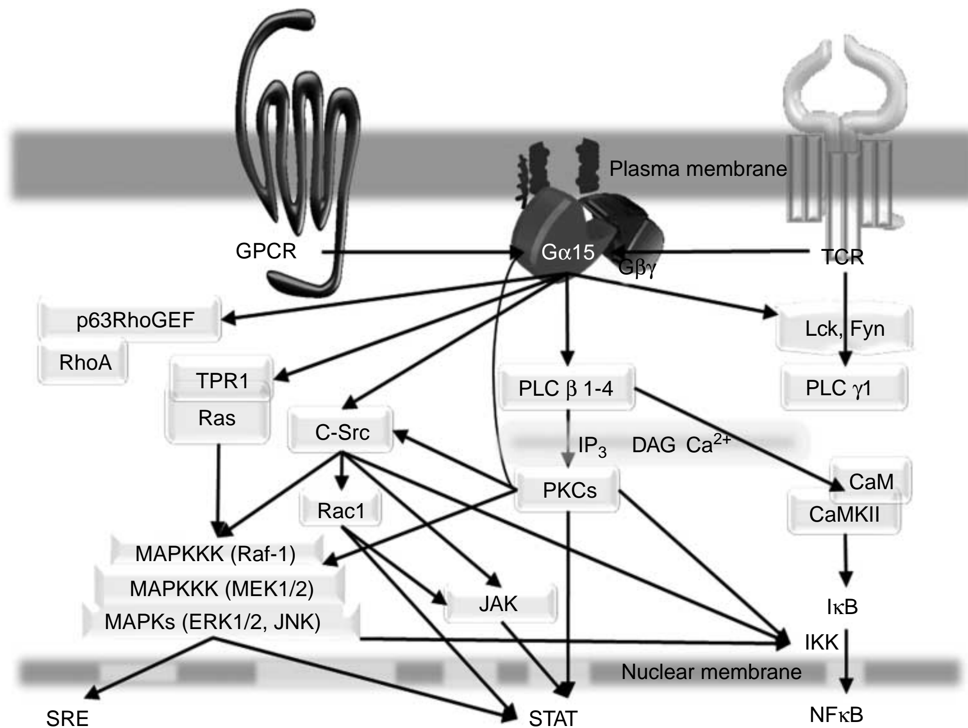


**Figure 2** Generalized scheme of G15 signaling. This scheme represents an overlay of signaling pathways that were pointed out by specifically modulating G15 activity. Different approaches were used to achieve this objective including promoting gain of function (by recombinant expression of Gα15 as the wild type form or as a constitutively active mutant) or inducing loss of function in cells endogenously expressing Gα15 (by RNA interference or by competing deficient mutant). In many cases, a signaling knot represents more than one protein isoform. Like any other Gq/11 family member, G15 activates different isoforms of PLCβ promoting PIP2 hydrolysis in response to GPCR stimulation (Wu *et al.* 1993, Offermanns & Simon 1995). The activation of CaMKII (Lo *et al.* 2003, Liu & Wong 2004) and $Ca^{2+}$-dependent (Offermanns *et al.* 2001) or independent (PKCμ in COS cells unpublished) PKC isoforms triggers several pathways, including those stimulating NFκβ (Yang *et al.* 2001), ERK (Lo *et al.* 2003), JNK (Heasley *et al.* 1996) JAK (Lo & Wong 2006, Lo *et al.* 2008), cSrc (Lee *et al.* 2009). Small GTPases, such as Ras and Rho are indirectly modulated by G15, via TPR1 (Yu *et al.* 2008, Su *et al.* 2009) and p63RhoGEF (Yeung & Wong 2009) respectively. The best characterized effector of Ras is the MAPK pathway that proceeds through ERK1/2 (Fehrenbacher *et al.* 2009). MAPKs activated by G15 (Buhl *et al.* 1995, Tian *et al.* 2008) lead to the activation of transcription factors such as STAT (Lo *et al.* 2003, Lo & Wong 2006), NFκB (Yang *et al.* 2001, Lee & Wong 2009) and SRE (Mao *et al.* 1998, Yeung & Wong 2009). G15 phosphorylation by PKC modulates GPCR coupling providing feedback regulation (Aragay & Quick 1999, Gu *et al.* 2003). In addition, like other Gq/11 family members (Zamoyska 2006), G15 was shown downstream the large signalosoma assembled upon T cell receptor activation (Zhou *et al.* 1998), G15 was reported as an intermediate effector towards the activation of Lck and Fyn. As a result, the inhibition G15 function reduced lymphocyte activation in response to T cell receptor engagement. In addition, G15 may affect other physiological functions such as transcription, proliferation (Qian *et al.* 1994), differentiation (Ghose *et al.* 1999), secretion (Lippert *et al.* 1997, Offermanns *et al.* 2001). CaM, calmodulin; CaMKII, CaM kinase II; ERK, extracellular signal-regulated kinases; GPCR, G protein coupled receptor; JAK, janus kinase; JNK, cJun NH2-terminal kinase; Lck, leukocyte-specific protein tyrosine kinase; MAPK, mitogen-activated protein kinases; MAPKK, MAPK kinase; MAPKKK, MAPKK kinase; MEK, MAPK/ERK kinase; p63, Rho GEF p63 Rho G protein exchange factor; PLC, Phospholipase C; PKC, protein kinase C; PIP2, phosphatidylinositol bisphosphate; Rac1, Ras-related C3 botulinum toxin substrate 1; Raf-1, V-Raf-1 murine leuk. viral oncog. homolog 1; RhoA, Ras homolog gene family, member A; STAT, signal transducers and activators of transcription; TPR1, Tetratricopeptide repeat 1; TCR, T cell receptor.

cells; Ghose *et al.* 1999). However, in the latter case, overexpression or downmodulation of Gα15 sorted out the same effect. Likewise, in lymphoid Jurkat cells, both sense and antisense DNAs produced a similar reduction in CD69 and IL2 expression (Lippert *et al.* 1997). Reduced cell growth was obtained in MB-02 and in 'small cell lung carcinoma' (SCLC) cells (Heasley *et al.* 1996*a*) upon overexpression of Gα15-Q212L. In vascular smooth muscle cells, only Gα15 did not produce pro-apoptotic effects among the Gq/11α family members that were tested (Peavy *et al.* 2005). In SCLC cell lines, constitutively active Gα15 inhibited cloning efficiency, but no effect was observed in 'non-cell lung carcinoma' clones (Heasley *et al.* 1996*a*).

Thus, it is far too premature to draw any conclusion. In particular, results based on constitutively active Gα15 are particularly questionable because the signal triggered by a permanently active G α-subunit is clearly different from the signal triggered by an activated receptor. First, it does not support transient events,



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such as the acute increase of the intracellular $Ca^{2+}$ concentration that is normally produced by a receptor. Secondly, signals elicited by constitutively active G proteins lack parallel coordinated pathways initiated by GPCRs that sometimes are even G protein-independent (Brzostowski & Kimmel 2001) and, anyway, always include βγ. For example, when G15 is stimulated by the adenosine A1 receptor in HEK cells, the βγ-subunit activates NFκB (Liu & Wong 2004). Thirdly, the sustained basal inositol phosphate turnover achieved in clones expressing Gα15-Q212L produced loss of responsiveness to agonist-dependent $Ca^{2+}$ mobilization (Heasley *et al.* 1996*a*, Lobaugh *et al.* 1996, Quick *et al.* 1996), probably due to a partial depletion of $Ca^{2+}$ stores together with a reduction of inositol trisphosphate receptor number. Exogenous expression of Gα15-Q212L inhibited cell growth in NIH-3T3 (Lobaugh *et al.* 1996) and Swiss 3T3 fibroblasts (Qian *et al.* 1994), but at the same time inhibited the responsiveness of platelet-derived growth factor (PDGF), ATP, and bombesin towards effectors like PKC, Raf, MEK, thus indicating a general distortion of the signaling network. Finally, cells tonically exposed to G protein signaling might compensate by counteracting downstream signaling steps or even suffer undesired long-term consequences as shown for the same G15 (Lobaugh *et al.* 1996). Constitutively active Gq/11 produces PLCβ signals capable of inducing cell transformation at low levels of expression, but it becomes eventually noxious at higher levels (Kalinec *et al.* 1992). The type of response to G15 signaling is also likely to depend upon the intensity of the stimulus, and activity levels promoted by overexpression of Gα15-Q212L exceed what is normally effective in the cell.

In summary, G15 physiological activity certainly relies on coordinated multibranched signals that are flawed by the chronic activation of a single pathway.

As mentioned above, the assumption that G15 biological role strictly relates to hematopoietic cell growth/differentiation and to lymphocyte activation (Su *et al.* 2009) is mostly inferred on its distribution and poorly supported by knockout mice that are substantially normal and capable of responding to several inflammatory challenges (Davignon *et al.* 2000). Normal hematopoiesis was also observed in Gα15 and Gαq double knockout mice that in most hematopoietic cells only express Gα11. Gα11 knockout mice exhibit as well normal hematopoiesis suggesting functional redundancy in Gq/11 subclass signaling (Davignon *et al.* 2000; double Gαq and Gα11 knockout is lethal). G15 function remains therefore substantially unknown.

G15 activity may become specifically important when GPCR stimulation is particularly intense and prolonged. Under these conditions, desensitization is expected to silence other pathways that are instead more strictly regulated. Retroviral transduction of silencing RNA and conditional knockout models will probably turn out to be determinant in the near future to clarify G15-specific functions: by this mean compensatory mechanisms should be avoided shutting off only G15-dependent branches.

## Conclusions

A number of experimental observations support the hypothesis that G15 appeared late in evolution (Fig. 1C) to fulfill highly specialized functions. A loose selectivity combined to high affinity and atypical resistance to GPCR desensitization could provide a strategy to deliver stimuli that are particularly intense. Such a powerful action is likely to develop along specific intracellular pathways. For instance, only G15 among Gq/11 family members efficiently activates NFκB in HeLa cells (in response to fMLP, C5a, C3a, receptors, CCR8, and CXCR2; Yang *et al.* 2001) and in HEK (in response to adenosine A1 receptor; Liu & Wong 2004). More in general, different genes were transcribed upon transfection of the constitutively active Gα15 (Peavy *et al.* 2005). Unfortunately, thus far, no clear physiological outcome has convincingly been associated to G15 activity.

Treasuring on indications provided by studies in signal transduction, future research will identify circumstances where G15 atypical signaling is matched by evident phenotypic outcomes. G15 is expressed in tissues characterized by a high rate of cell turnover (bone marrow and epithelia (Lippert *et al.* 1997)). We suggest that research focus should be extended beyond the immune response (epithelial and other intermediate maturation stages) and that experimental conditions should highlight G15 function peculiarities so that its effects emerge over the redundant functions of the other Gq family members (i.e. under prolonged/intense GPCR stimulations).

## Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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