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 (Gz15) 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)). Gz15 was originally cloned in mouse (Wilkie et al. 1991). The human isoform was named Gz16 assuming it represented a novel subtype (Amatruda et al. 1991), but later on it was recognized as the poorly conserved ortholog of Gz15 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 (PLCb; 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. 2001) and smootherned (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.
inducible cellular models, such as HL60 or WB4 cells. The expression of Gα15 maturation. This was reproduced in vitro (Ghose et al. 2001).

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, Pfeilstöcker et al. 2000), erythroid precursors (Ghose et al. 1999), megakaryocytic (den Dekker et al. 2001a,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).

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

<table>
<thead>
<tr>
<th>Organ, cells</th>
<th>Species</th>
<th>References</th>
<th>EST (m)</th>
<th>EST (H)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tonsil: B and T cells</td>
<td>H</td>
<td>Grant et al. (1997)</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Thymus (decreasing in adult)</td>
<td>m</td>
<td>Wilkie et al. (1991)</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Lymph nodes</td>
<td>m</td>
<td>Davignon et al. (2000)</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Blood</td>
<td>H</td>
<td>Lippert et al. (1997)</td>
<td>-</td>
<td>++</td>
</tr>
<tr>
<td>Activated peripheral blood cells</td>
<td>H</td>
<td>Mapara et al. (1995) and Grant et al. (1997)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>H</td>
<td>Lippert et al. (1997)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Pre-B cells</td>
<td>H</td>
<td>Lippert et al. (1997)</td>
<td>+</td>
<td>++</td>
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<tr>
<td>HSC</td>
<td>H</td>
<td>Lippert et al. (1997)</td>
<td>+</td>
<td>++</td>
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<tr>
<td>γδ</td>
<td>H</td>
<td>Davignon et al. (2000)</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Activated T cells</td>
<td>H</td>
<td>Tenailleau et al. (1997) and Su et al. (2009)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>H</td>
<td>Tenailleau et al. (1997) and Su et al. (2009)</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Megakaryocytes</td>
<td>H</td>
<td>Tenailleau et al. (1997) and Su et al. (2009)</td>
<td>+</td>
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<tr>
<td>Neutrophils</td>
<td>m</td>
<td>Davignon et al. (2000)</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Monocytes</td>
<td>m</td>
<td>Tenailleau et al. (1997) and Su et al. (2009)</td>
<td>+</td>
<td>++</td>
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<tr>
<td>Bone marrow: HSC and erythroid cells</td>
<td>H</td>
<td>Tenailleau et al. (1997) and Pfeilstöcker et al. (2000)</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

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: yolk (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 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, Pfeilstöcker et al. 2000), erythroid precursors (Ghose et al. 1999), megakaryocytic (den Dekker et al. 2001a,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).

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Epithelia

Despite broadly described as hematopoietic specific (Amatruda et al. 1991, Hubbard & Hepler 2006, Su et al. 2009), Gα15 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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**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.o.) 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 A431 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.
indication pointing to an involvement of G15 in tumor cell growth (Heasley et al. 1996b), Gz15 was coinmunoprecipitated 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 Gz15 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 Gz15 expression in several organs. We confirmed by quantitative PCR (Fig. 1A) that very low, albeit significant, levels of Gz15 are present in heart, kidney, and almost all tissues analyzed. Though abundance was much reduced compared with HSCs or TECs, a small number of Gz15 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 Gz15 isoforms?

Another puzzling aspect about Gz15 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. 2001a,b). During megakaryocytic maturation (den Dekker et al. 2001a,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 Imnammorati 2009, unpublished observation) proves that both represent Gz15 rather than a cross-reacting z-subunit, or any other protein sharing a common epitope.

For other G protein z-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 Gz15, 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 Gz15) 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 b2AR (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 b y-subunit, or by indirect activation through other intermediate effectors (see for instance the activation of PLCz by Gs via PKA (Schmidt et al. 2001)). The lack of pharmacological inhibitors specific for Gz15 further complicates the analysis.

Thus, it is not surprising that only very few examples describe GPCR signaling through naive G15. Knocking out Gz15 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 Gz15 led to reduced mobilization of intracellular Ca2+ upon stimulation of
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 Gz15 in HSCs suggested that it may serve as a specific effector of GPCRs involved in immunity (Aamatruda 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 Gz15 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 Gz 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 Gz15 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. Gz15 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 Gz15-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).
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 Gz-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 Gz15 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 Gz15, but not with Gzq or Gzs, as if Gz15 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 stages), 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 stability than the interaction with other G proteins. A steady interaction of wild-type P2Y2 receptor with Gz15 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 Gz15 expression at physiological levels (promoted by a tetracycline-induced transactivation system) exerted a similar effect by blunting the Ca2+ 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 Gz15-Q212L, a constitutively active mutant incapable of efficient GTP hydrolysis. By this approach, direct activation of downstream effectors is achieved bypassing the GPCR. Gz15-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. 1996a), and similar results were observed in a model of erythroid differentiation (MB-02 erythroleukemia
In vascular smooth muscle cells, only Gα15 produce pro-apoptotic effects among the Gq/11 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. 1996a).

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 Gαz-subunit is clearly different from the signal triggered by an activated receptor. First, it does not support transient events,
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 G15-Q212L produced loss of responsiveness to agonist-dependent Ca$^{2+}$ mobilization (Heasley et al. 1996a, 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 Gz15-Q212L inhibited cell growth in NIH-3T3 (Lobaugh et al. 1996), 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 countering downstream signaling steps or even suffer undesired long-term consequences as shown for the same G15 (Lobaugh et al. 1996). Constitutively active Gq/11 produced 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 Gz15-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 Gz15 and Gzq double knockout mice that in most hematopoietic cells only express Gz11. Gz11 knockout mice exhibit as well normal hematopoiesis suggesting functional redundancy in Gq/11 subclass signaling (Davignon et al. 2000; double Gzq and Gz11 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 Gz15 (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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References


Offermanns S & Simon MI 1995 Ga15 and Ga16 couple a wide variety of receptors to phospholipase C. *Journal of Biological Chemistry* 270 15175–15180.


Robillard L, Ethier N, Lachance M & Hebert TE 2000 Gbetagamma
subunit combinations differentially modulate receptor and effector
Robishaw JD, Smigel MD & Gilman AG 1986 Molecular basis for two
forms of the G protein that stimulates adenylyl cyclase. Journal of
Biological Chemistry 261 9587–9590.
Rock BM, Xin L & Wilcox JN 1997 Two Gq class G proteins are
expressed in human keratinocytes. Journal of Investigative Dermatology
109 645–649.
Rozenfeld-Granot G, Toren A, Amariglio N, Nagler A, Rosenthal E,
Identification of hematopoietic cell populations from the dermal papillae
Tenailleau S, Corre I & Hermouet S 1997 Specific expression of
the hematopoietic G protein G16 GTP-binding protein.
Yu MY, Ho MK, Liu AM & Wong YH 2008 Mutations on the Switch III
region and the 7 helix of Gq16 differentially affect receptor
binding and regulation of downstream effectors. Journal of
Molecular Signalling 3 17.
Zamoyska R 2006 Superantigens: supersignalers? Science's STKE
358 pe45.
Zhou J, Stanners J, Kabouridis P, Han H & Tsoukas CD 1998 Inhibition
of TCR/CD3-mediated signaling by a mutant of the hematopoietically
expressed G16 GTP-binding protein. European Journal of
Immunology 28 1645–1655.
Zhu X & Birnbaumer L 1996 G protein subunits and the stimulation of
phospholipase C by Gα-and Gi-coupled receptors: lack of receptor
selectivity of Gq(16) and evidence for a synergic interaction
between Gbeta gamma and the α subunit of a receptor activated G
protein. PNAS 93 2827–2831.

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