An intact helical domain is required for Gα₁₄ to stimulate phospholipase Cβ

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Abstract

Background: Stimulation of phospholipase Cβ (PLCβ) by the activated α-subunit of Gα₁₄ (Gα₁₄) constitutes a major signaling pathway for cellular regulation, and structural studies have recently revealed the molecular interactions between PLCβ and Gα₁₄. Yet, most of the PLCβ-interacting residues identified on Gα₁₄ are not unique to members of the Gα₁₄ family. Molecular modeling predicts that the core PLCβ-interacting residues located on the switch regions of Gα₁₄ are similarly positioned in Gα₁₄ which does not stimulate PLCβ. Using wild-type and constitutively active chimeras constructed between Gα₁₄ and Gα₁₄, a member of the Gα₁₄ family, we examined if the PLCβ-interacting residues identified in Gα₁₄ are indeed essential.

Results: Four chimeras with the core PLCβ-interacting residues composed of Gα₁₄ sequences were capable of binding PLCβ2 and stimulating the formation of inositol trisphosphate. Surprisingly, all chimeras with a Gα₁₄ N-terminal half failed to functionally associate with PLCβ2, despite the fact that many of them contained the core PLCβ-interacting residues from Gα₁₄. Further analyses revealed that the non-PLCβ2 interacting chimeras were capable of interacting with other effector molecules such as adenylyl cyclase and tetratricopeptide repeat 1, indicating that they could adopt a GTP-bound active conformation.

Conclusion: Collectively, our study suggests that the previously identified PLCβ-interacting residues are insufficient to ensure productive interaction of Gα₁₄ with PLCβ, while an intact N-terminal half of Gα₁₄ is apparently required for PLCβ interaction.

Background

The superfamily of G protein-coupled receptors (GPCRs) constitutes the largest group of cell surface detectors for extracellular signals. Upon ligand binding, conformational changes in the receptor trigger the activation of heterotrimeric G proteins, which consists of α, β, and γ subunits, and results in the activation of various downstream effectors [1, 2]. Gα proteins are classified into four main families named as Gαq, Gαi, Gαz, Gα₁₂/₁₃, while five Gβ and twelve Gγ isoforms have been identified to date. The diversity in G protein subunits allows disparate signaling pathways to be regulated by different receptors. Robust stimulation of phospholipase Cβ (PLCβ) is primarily mediated by GPCRs that utilize Gα proteins for signaling [3], thereby leading to diverse cellular responses that range from cell proliferation to differentiation. The four known isoforms of PLCβ (PLCβ1-4) [4] are all stimulated by GTP-bound Gαq subunits [5], even though they are either enriched in the cytosol (PLCβ2 and PLCβ3) or at the plasma membrane (PLCβ1 and PLCβ4) [6]. PLCβ catalyzes the hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP₂) into diacylglycerol and inositol 1,4,5-trisphosphate (IP₃), and reciprocally acts as a GTPase activating protein (GAP) of Gαq [7, 8]. Since there are several members within the Gαq subfamily (Gαq, Gα₁₁, Gα₁₃a, and Gα₁₃b/ Gα₁₃c) and all are fully capable of stimulating PLCβ [5], numerous GPCRs employ the Gαq/PLCβ pathway to regulate different cellular functions. Moreover, the Gβγ complex released upon G protein activation can also stimulate PLCβ2 and PLCβ3 isoforms [9, 10]. Given the importance of the Gαq/PLCβ axis in cell growth [11], its dysregulation is expected to contribute to the pathophysiology of various diseases. Indeed, somatic mutations causing constitutive activation of Gα₁₄ drive ~50% of all uveal melanomas [12].

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Despite intense efforts directed at understanding the interactions of Gαq and PLCβ, the structure of a PLCβ-Gαq complex has only recently been solved by molecular replacement manipulations using the crystal structures of PLCβ3 and an activated Gαq [13]. The predicted structure of the PLCβ3-Gαq complex has identified a series of discrete residues that form the interacting surfaces (Fig. 1a). According to the structural data, PLCβ3 binding occurs mainly at the switch regions of Gαq (Fig. 1a and b). The switch I and II residues of Gαq (green) interact with PLCβ3 through an extended loop region between EF hands 3/4, which is conserved in all PLCβ isoforms (Additional file 1: Figure S1), and the region between the catalytic TIM barrel and C2 domain of PLCβ3, providing an interface between PLCβ3 and Gαq for interaction between a series of charged residue pairs. The highly conserved helix-turn-helix segment (Hα1/Hα2) at the C-terminus of the C2 domain of PLCβ3 resides on the surface region formed by switch II (α2-β4) and the α3 helix of Gαq and allows the formation of various contacts with Gαq in the large binding interface (Fig. 1c). More recently, discovery of the full-length structures of both PLCβ3 and Gαq in complex has highlighted additional domains of PLCβ3 and Gαq necessary for activation of lipid hydrolysis and protein interactions [14]. The crystallized full-length PLCβ3 contains a distal C-terminal domain (CTD) which is considered to be important for activation, membrane localization, and regulation by Gαq proteins [15, 16]. The distal CTD adopts an orientation that makes direct contacts with the αN helix of Gαq and most likely participates in binding with Gαq proteins.

Regions of the Gαq necessary for PLCβ interaction (namely, Ile211 to Lys276 which encompass the α2-β4-α3-β5 regions) have previously been identified by alanine-scanning mutagenesis [17] and they are appropriately positioned for the interaction with PLCβ3 (Fig. 1a). A total of 33 amino acids in small clusters along the β2 to α4 regions (except Ile23, Ile25, and Leu29, which lies in the αN helix, and Lys41 which lies on the β1 strand) of Gαq are predicted to form intermolecular bonds with PLCβ [13, 14]. As expected, most interacting residues in the core regions are conserved in all other Gαq members including Gα11, Gα14, and Gα16 (Fig. 1a). However, between 36 and 60 % of the identified PLCβ-interacting residues are also found in other Gαq protein families, with members of the Gαq family having the highest homology to Gαq (Additional file 1: Figure S2) [18, 19]. For instance, Gαs of the Gαq subfamily exhibits close to 60 % identity with Gαq in the core PLCβ-interacting regions (Fig. 1a). Such a high degree of identity is rather surprising especially when Gα16, which stimulates PLCβ, is only 74 % identical to Gαq in the PLCβ-interacting regions (Fig. 1a). More interestingly, molecular modeling between Gαq and Gαq predicts that their differences in the PLCβ-interacting regions form a ring around a central core domain (Fig. 1b, space filled models), with most of the PLCβ contact points conserved between the two Gα subunits (Fig. 1c). This calls into question whether the residues identified by molecular replacement [13] are sufficient to provide PLCβ binding selectivity to Gαq members. Although the identified residues are involved in the formation of the PLCβ3-Gαq complex and are necessary in PLCβ3 activation as confirmed in IP3 studies [13], there may be additional regions in Gαq members that determine selectivity for PLCβ.

It has been well established that constitutively active Gαq subunits can efficiently stimulate PLCβ [20] but has no regulatory effect on adenylyl cyclase [21]. Early studies have employed chimeric Gαq/Gαz and Gα16/Gαz constructs to map the PLCβ and receptor interacting domains on the Gαq and Gα16 subunits [17, 22]. It has not been demonstrated whether other Gαq members such as Gα14 (with over 80 % sequence similarity with Gαq) utilize the same regions to interact with PLCβ. Likewise, it remains to be determined if other PLCβ isoforms such as PLCβ2 (with the highest resemblance to PLCβ3; Additional file 1: Figure S1) employ similar structural regions as PLCβ3 for coupling to active Gαq. By generating a series of Gα subunit chimeras and testing their abilities to functionally associate with PLCβ2 in HEK293 cells, we have demonstrated that an intact helical domain in the N-terminus of Gα14 is necessary for productive interaction with PLCβ.

Results
The PLCβ-interacting core regions of Gα14 are insufficient to stimulate PLCβ2
The PLCβ-interacting surfaces of Gαq have been generally mapped to the β2-β3-α2-β4-α3 regions [13, 17], and these residues are mostly conserved among Gα11, Gα14, and Gα16 (Fig. 1a). Given that most of the PLCβ contact sites of Gαq appear to be similarly present in Gα subunits (Additional file 1: Figure S2), it is possible to confer PLCβ-stimulating function upon a Gα subunit by incorporating Gαq-specific residues. This will also allow for the identification of any additional structural determinant on Gαq which may specify interaction with PLCβ. In order to distinguish exogenous from endogenous Gα subunits, we have opted to use Gα14 as the backbone for constructing chimeras instead of Gαq or Gα11. Unlike Gαq/Gαz, Gα14 is not expressed in HEK293 cells [23] and it differs from Gαq by only two amino acids (Lys256 and Thr260 in Gα14) in the PLCβ-interacting regions (Fig. 1a, b). To determine if Gα14 utilizes the same regions as Gαq for PLCβ interaction, Gα14/Gαz chimeras were made by swapping specific domains between Gα14 and Gαz. Gαz was selected because it does not interact with PLCβ [24] or other Gαq regions.
Fig. 1 Alignment of PLCβ-interacting residues in Gαq family and Gαz.  

**a** Schematic view of Gαq divided into helical (light blue) and GTPase (light green) domains with α-helices and β-strands represented by rectangles and ovals, respectively. Interacting domains of Gαq with PLCβ are indicated by yellow boxes below the Gαq sequence; the three bold segments indicate the relative positions of the three switch regions (Sw1 to Sw3 from left to right). Sequence alignment of PLCβ-interacting domains in the Gαq family as compared to that of Gαz, conserved (green) or divergent (red) PLCβ-interacting residues are interspersed by conserved residues which are not implicated in interaction with PLCβ (grey). Residues forming direct interactions with PLCβ3 as identified by Waldo et al. [13] are indicated by an asterisk.

**b** Structural representation of Gαq, Gα14, and Gαz alignments with switch regions (Sw1-3) and the α3 region. PLCβ3-interacting residues revealed in the sequence alignment are colored as indicated in A. Space filling models are showing interacting surfaces. Structural models of Gαq and Gαz are generated based on Gαq-PLCβ3 (PDB code: 3OHM) using SWISS-MODEL [65, 66]. Structure alignments are carried out with PyMOL (The PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC).  

**c** Complex of Gαq/Gαz-PLCβ3. The Gαq/Gαz aligned model is represented as indicated in (B). PLCβ3 (yellow) is depicted as a cartoon ribbon, containing the helix-turn-helix segment (Hα1/Hα2), the N-terminal PH domain, four EF hands, the catalytic TIM barrel, and a C2 domain. The switch regions of Gαq interact with PLCβ3 through an extended loop region between EF hands 3/4 and the region between the catalytic TIM barrel and C2 domain. The helix-turn-helix segment (Hα1/Hα2) at the C-terminus of PLCβ3 resides on the surface region formed by switch 2 and α3 of Gαq. The αN helix of Gα proteins and carboxy-terminal (CT) domain of PLCβ3 are not included in the structural models.
effectors such as TPR1 [25]. The chimeric approach is well suited for mapping functional domains on the \( \alpha \) subunits because their tertiary structures highly resemble one another. Moreover, chimeras made with \( \alpha_z \) and \( \alpha_{16} \) proteins are structurally viable [22, 25].

Molecular modeling predicts that the differences between \( \alpha_{14} \) and \( \alpha_z \) in the PLC\( \beta \)-interacting regions are distributed at the perimeters of the interacting surfaces, in much the same way as those of \( \alpha_q \) versus \( \alpha_z \) (Fig. 1b). Since most of the \( \alpha_z \)-specific sequences in the PLC\( \beta \)-interacting domain reside in the \( \alpha_2-\beta_4-\alpha_3 \) regions (Fig. 1a), we began by testing the importance of these regions by swapping the C-terminal half of \( \alpha_z \) with \( \alpha_{14} \) and assaying for the ability of the chimeras to interact with PLC\( \beta \). PLC\( \beta \) was chosen on the basis that it shares 76% identity with PLC\( \beta \) at the \( \alpha_q \)-interacting residues [13, 14]. The 14z151 chimera was constructed with the \( \alpha_2-\beta_4-\alpha_3 \) regions together with the rest of the C-terminus of \( \alpha_{14} \) (151 residues) replaced by the cognate sequence from \( \alpha_z \); the mirror image of 14z151 was also constructed and named as 203z14 (Fig. 2a). Construction of the chimeras was guided by the predicted tertiary structure of the \( \alpha \) subunits as well as by our previous experience in determining the receptor and effector interacting domains of various \( \alpha \) subunits [22, 26–28]. A glutamine to leucine point mutation (QL) was introduced at Gln\(^{205} \) (equivalent to Gln\(^{209} \) in \( \alpha_q \)) to generate constitutively active mutants [29]. HEK293 cells were co-transfected with PLC\( \beta \) in combination with pcDNA1, wild-type or constitutively active mutant of \( \alpha_{14} \), \( \alpha_z \), 14z151 or 203z14. As illustrated in Fig. 2b (upper panels), wild-type and constitutively active \( \alpha_{14} \), but not those of \( \alpha_z \), were successfully co-immunoprecipitated by anti-

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**Fig. 2** The putative PLC\( \beta \) domain of \( \alpha_{14} \) is not required for PLC\( \beta \) interaction and activation. **a** Schematic representation of the 14z151, 203z14, 14z173, and 182z14 chimeras. Predicted secondary structures are illustrated as boxes (\( \alpha \)-helic) or circles (\( \beta \)-strands) above the chimeras. Black areas represent human \( \alpha_{14} \) sequence while those in grey signify the corresponding sequence of human \( \alpha_z \). **b** HEK293 cells were co-transfected with PLC\( \beta \) in combination with pcDNA1, wild-type or constitutively active mutant of \( \alpha_{14} \), \( \alpha_z \), 14z151 or 203z14. Cells were then labelled and assayed for IP\(_3\) formation. Fold stimulations were calculated as the ratios of QL-induced to wild-type IP\(_3\) accumulations. Data represent the mean ± S.E.M. of three independent experiments, \( n = 3 \). * IP\(_3\) production was significantly enhanced as compared to corresponding wild-type transfected cells; Dunnett’s test, \( p < 0.05 \).
PLCβ2 antiserum and protein G sepharose. Chimera 203z14 did not interact with PLCβ2 despite having the α2-β4-α3 regions of Ga14 (Fig. 2b). This indicates that the other PLC-interacting regions of Ga14 (e.g., β2 and β3 regions) might be required for PLCβ2 interaction. More surprisingly, 14z151 was pulled down by anti-PLCβ2 even though its α2-β4-α3 regions were composed of Ga2 sequences.

To test the possibility that the β2-β3 regions are needed to maintain the overall structural integrity of the PLCβ-interacting surfaces, we further constructed a pair of chimeras with the junction extended forward to include the β2-β3 regions (Fig. 2a). Again, both the wild-type and constitutively active mutant of the chimera which harbored the β2-β3-α2-β4-α3 regions of Ga14 (182z14 and 182z14QL) failed to associate with PLCβ2, while their mirror images (14z173 and 14z173QL) co-immunoprecipitated with PLCβ2 (Fig. 2b). All of the chimeras and PLCβ2 were expressed at detectable and comparable levels in the total cell lysates (Fig. 2b, lower panels). These results suggest that the β2-β3-α2-β4-α3 regions, which are known to be important in Gaq for PLCβ interaction, might not be sufficient for Ga14 to interact with PLCβ.

The co-immunoprecipitation results were subsequently confirmed by PLCβ functional assays. HEK293 cells were transfected with pcDNA1, Ga14, Ga2, the various chimeras or their constitutively active mutants and then subjected to IP3 accumulation assay. In agreement with previous reports [24, 30], expression of Ga14QL but not Ga2QL significantly stimulated IP3 formation (Fig. 2c). Both 14z151QL and 14z173QL also stimulated IP3 production whereas 182z14QL and 203z14QL failed to do so (Fig. 2c). None of the wild-type chimeras significantly affected IP3 production as compared to the vector controls (results not shown). Hence, these results demonstrate that the mere presence of the β2-β3-α2-β4-α3 regions of Ga14 does not necessarily confer upon the Ga subunit an ability to stimulate PLCβ. More interestingly, these regions can be functionally replaced by those from Ga2.

To test if the replacement of the PLC-interacting regions of Ga14 by cognate sequences from Ga2 can indeed support PLCβ activation, we swapped the β2-β3 or the α2-β4-α3 regions independently between the two Ga subunits (Fig. 3a). Among the various PLC-interacting regions, the α2 and α3 helices harbor most of residues that have been identified to form intermolecular bonds

**Fig. 3** Role of β2-β3 and α2-β4-α3 regions of Ga14 in interaction and activation of PLCβ. **a** Schematic representation of zα2β4-α3, 14α2β4-α3, zβ2β3 and 14β2β3 chimeras. **b** Cells were co-transfected with PLCβ and Ga protein or the indicated chimeras. Co-immunoprecipitation assays were performed and analyzed as in Fig. 2. Data shown represent one of three sets of immunoblots; two other sets yielded similar results. **c** HEK293 cells were transiently transfected with the wild-type or constitutively active mutants (QL) of Ga proteins or chimeras and then subjected to IP3 accumulation assay and analyzed as in Fig. 2. *, IP3 production was significantly enhanced as compared to corresponding wild-type transfected cells; Dunnett t test, p < 0.05
with PLCβ3 (Fig. 1a; [13]). Hence, substitution of the α2-β4-α3 regions in Gα14 with those of Gαz might severely disrupt the ability of the resultant chimera (named as za2β4α3) to interact with PLCβ. Although za2β4α3 was co-immunoprecipitated by anti-PLCβ2 (Fig. 3b), its constitutively active mutant displayed a much weaker ability to induce IP3 formation as compared to Gα14QL (Fig. 3c). In contrast, chimera 14z2β4α3 (the mirror image of za2β4α3) failed to associate or stimulate PLCβ2, suggesting that the α2-β4-α3 of Gα14 alone was insufficient to ensure PLCβ interaction. Likewise, we examined the role of the β2-β3 strands of Gα14 in PLCβ interaction. Chimera of Gα14 with the β2-β3 domain replaced by Gαz (zβ2β3), and its mirror image (14β2β3), were constructed to determine if β2-β3 alone would affect PLCβ interaction with Gα14 (Fig. 3a). Our results showed that zβ2β3 remained capable of interacting with PLCβ and stimulating its activity (Fig. 3b, c), suggesting that the Gαz-specific residues in this region are sufficiently similar to those of Gα14 to allow productive interaction with PLCβ. On the other hand, 14β2β3 with most of the C-terminal and N-terminal of Gα14 replaced by Gαz, failed to interact with PLCβ2 or mediate IP3 production (Fig. 3b, c).

The N-terminal helical domain of Gα14 is important for PLCβ interaction and activation

The preceding results suggested that the N-terminal half (αN-αF) of Gα14 is seemingly important for PLCβ interaction and activation. Substituting the N-terminal of Gα14 from αN to αF with Gαz completely abolished the ability of Gα14 to activate PLCβ even though the chimeras (182z14 and 203z14) can be successfully expressed (Fig. 2). To narrow down the residues in αN-αF which are involved in PLCβ activation, the N-terminal helical domain (αA-αF) of Gα14 was split into two halves and replaced by cognate sequences from Gαz (Fig. 4a). The helical domain is essential for maintaining the overall structure of the Ga subunit and participates in effector regulation [31]. In order to minimize possible disruption to the Ga structure, the chimeras were designed to switch from Gα14 to Gαz or vice versa at a position in the middle of the helical domain (Fig. 4a) where the residues of the two templates have high homology. Chimera 14z224 harboring the αN-αCo of Gα14 was expressed efficiently but was unable to functionally associate with PLCβ (Fig. 4b, c). The mirror image of 14z224, chimera 131z14, also failed to interact with PLCβ or stimulate IP3 formation (Fig. 4b, c). Replacement of the

![Fig. 4](image-url)
second half of the helical domain (αD-αF) of Gα14 by Gα3, sequences, or vice versa, produced chimeras zaDEF and 14αDEF that neither interacted with PLCβ nor stimulated IP3 formation (Fig. 4b, c). It should be noted that chimeras 131z14 and zaDEF contained the putative PLCβ-interacting core domain (Fig. 4a).

The extreme N-terminus of the Gα subunit contains motifs for membrane localization and is thus often removed prior to crystallization [13]. By superimposing the N-terminal αN helix onto the crystal structure of Gαq, molecular modeling of the Gαq/PLCβ3 complex predicts that the αN helix may represent a contact site for PLCβ3 (Fig. 5a). Given that several chimeras (14z151, 14z173, za2β4αα3 and zβ2β3) were able to stimulate PLCβ activity despite having the PLCβ-interacting core region from Gαq, the provision of a Gα14 αN helix on a Gα backbone (14αN) might allow the resulting chimera to interact with PLCβ. However, chimera 14αNQL (Fig. 5b) did not stimulate IP3 formation whereas chimera zaNQL. (Gα14 backbone with a GααN helix) functionally interacted with PLCβ (Fig. 5c). Collectively, these results suggest that the αN helix is not a critical determinant in the recognition of PLCβ by Gα14.

Non-PLCβ-interacting Gα14 chimeras can interact with other effectors

Since nine chimeras (181z14, 203z14, 14αβ4α3, 14β2β3, 14z224, 131z14, zaDEF, 14αDEF, and 14αN) failed to interact with PLCβ despite clear evidence of expression, we sought to determine if these chimeric Gα subunits were in fact functional. Those chimeras harboring large segments of Gαq sequence may behave like Gαq and thus be capable of inhibiting adenylyl cyclase. The panel of chimeras was therefore subjected to cAMP accumulation assay. The ability of the constitutively active mutant of each chimera to inhibit forskolin-induced cAMP accumulation was compared to its corresponding wild-type chimera (Fig. 6a). Like GαQL, the constitutively active mutants of 14β2β3, 14z224, 14αDEF, and 14αN inhibited the forskolin response by 55-80 %, thereby confirming that these chimeras can adopt an active conformation. With four of the nine non-PLCβ-interacting chimeras demonstrating an ability to inhibit adenylyl cyclase, only five chimeras remained functionally unaccounted for.

Apart from being able to stimulate PLCβ by direct association [32, 33], Gα14 can also activate the Ras/ERK signaling pathway by interacting with TPR1 [34]. The Gα/TPR1 interaction is important for IKK and STAT3 phosphorylation via the Ras/ERK pathway [35, 36] and is apparently independent of PLCβ [25]. Since five chimeras (203z14, 182z14, 131z14, zaDEF, and 14z2β4α3) failed to exhibit any functional response in either IP3 or cAMP accumulation assays, we tested if these chimeras can associate with TPR1. HEK293 cells were co-transfected with an N-terminal Flag-tagged TPR1 (Flag-TPR1) and either the wild-type or the constitutively active mutant of Gα14, Gα3, or a chimera. Transfectants were subjected to co-immunoprecipitation using an anti-Flag affinity gel and protein G sepharose. The immunoprecipitates and cell lysates were then examined by western blot analysis using anti-Flag and either anti-Gαq or anti-Gα3 antisera, depending on whether the N-terminus of the chimera is made up of Gα14 or Gα3 sequences. In agreement with previous studies [25, 34], neither Gαq nor GαQL interacted with Flag-TPR1 whereas both Gα14 and Gα14QL co-immunoprecipitated with Flag-TPR1; noticeably more Gα14QL was associated with Flag-TPR1 (Fig. 6b). In contrast to Gαq, chimeras 203z14, 182z14, 131z14, and 14α2β4αα3 were clearly detectable in the Flag-TPR1 immunoprecipitates (Fig. 6b); TPR1 interaction with 14α2β4αα3 appeared to be weaker than the other chimeras. However, zaDEF could not be co-immunoprecipitated by Flag-TPR1 (Fig. 6b). Hence, only zaDEF did not exhibit any response in all of the functional assays. The ability of other chimeras to interact with Flag-TPR1 was similarly examined (Additional file 1: Figure S3) and the results are summarized in Table 1. Besides the inability of zaDEF to interact with Flag-TPR1, 14β2β3 and 14αN also exhibited negligible association with Flag-TPR1 but they were capable of coupling to adenylyl cyclase (Fig. 6a).

Results obtained from the various assays are summarized in Table 1. Collectively, these results suggest that the core PLCβ-interacting regions are insufficient to ensure productive interaction with PLCβ and, more surprisingly, some of these regions can be functionally substituted by cognate residues from Gαq. Interestingly, an intact N-terminal helical domain (αA-αF) of Gα14 are seemingly important for Gα14-mediated PLCβ activation. Gα14 chimeras with αA-αF replaced entirely or in part by Gαq can be expressed at a detectable level but failed to interact with PLCβ2 or stimulate IP3 production.

Discussion

Structure and function correlations of members within the same protein family are often based on extensive analyses of a prototypical member. In the case of the Gαq family, it is generally assumed that all members interact with the canonical effector PLCβ in much the same way as Gαq. The biochemical functions of Gαq family members are almost indistinguishable [33, 37] except for their ability to recognize specific receptors [38]. Hence, it is rather surprising that the putative PLCβ-interacting domains identified from studies on Gαq [13, 14, 17, 39] are simply insufficient to support efficient regulation of PLCβ by Gα14. Although detailed structural comparison between Gαq and Gα14 is not feasible because of the lack of Gα14 structural data, the overall sequence similarity of over 80 % indicates a highly conserved three-dimensional
structure shared by both proteins [18, 19]. Since the structural homology and the residues responsible for PLCβ interaction and activation are presumably conserved from Gαq to Gα16, one would expect that Gα16 may utilize the same residues for PLCβ activation. It should also be noted that sequence variations in interacting residues of PLCβ2 and PLCβ3 may affect the ability of Gα16 to efficiently stimulate PLCβ2. In particular, conservative substitutions such as D973E and Q1066S in the distal CTD may have limited consequences for Gα16 binding, whereas more severe mutations in other interacting regions (E261S, Y855L, and R1062A) may significantly affect efficient activation by Gα proteins. Nonetheless, the substantial amount of conserved Gαq-interacting residues (76%) in PLCβ2 should

Fig. 5 Role of the N-terminal helix (αN) in the Gαq-PLCβ3 complex. a The model of Gαq (light orange) is shown as a space filling structure and contains the αN-helix and other regions as indicated. PLCβ3 (yellow) is depicted as a cartoon ribbon, containing the helix-turn-helix segment (Ha1/Ha2), the N-terminal PH domain, four EF hands, the catalytic TIM barrel, and a C2 domain. PLCβ3-interacting residues of Gαq are colored in magenta. The carboxy-terminal (CT) domain of PLCβ3 is not included in the structural model. The structure of the αN-helix is generated by replacing the amino acid sequence of Gαi (Gαi1γ2, PDB code: 1GP2) with the Gαq sequence. The final model is generated by alignment of Gαq-PLCβ3 (PDB code: 3OHM) and the modified heterotrimer Gαqβ1γ2 using PyMOL (The PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC). The orientation of the αN-helix represents the conformation in the heterotrimer and is not optimized for the Gαq-PLCβ3 complex. In this case, the αN-helix points towards the cell membrane and clashes with PLCβ3, but in fact may exist in a conformation which interacts with PLCβ3. b Schematic representation of αN and zaN chimeras. c Cells were co-transfected with PLCβ2 and Gα protein or the indicated chimeras. Co-immunoprecipitation assays were performed and analyzed as in Fig. 2. Data shown represent one of three sets of immunoblots; two other sets yielded similar results. For the IP3 accumulation assay, HEK293 cells were transiently transfected with the wild-type or constitutively active mutants (QL) of Gα proteins or chimeras and analyzed as in Fig. 2. *, IP3 production was significantly enhanced as compared to corresponding wild-type transfected cells; Dunnett t test, p < 0.05.
provide sufficient interacting regions for effector activation. However, Ga14/Gaz chimeras lacking the putative PLCβ interacting domain (e.g., 14z151 and 14z173) are fully capable of interacting and stimulating PLCβ. Since these gain-of-function results do not correspond with current structural information on Gaq/PLCβ interaction [13, 14], it would appear that our understanding on how G proteins stimulate the PLCβ pathway is far from complete.

Ga14 and Gaz resemble each other both structurally and biochemically. Both proteins are able to stimulate PLCβ2 and exhibit similar profiles of IP3 production [32]. Similar to other members of the Gaq subfamily, Ga14 links a variety of Gq-, Gs-, and Gi- coupled receptors to stimulate PLCβ3 [40–42]. In addition, palmitoylation of cysteine residues in the N termini of Gaq and Ga14 is essential for membrane localization and efficient PLCβ activation [43, 44]. However, co-immunoprecipitation and PLCβ activation studies using Ga14/Gaz chimeras suggested that an intact helical domain (αN-αF) of Ga14, but not the previously identified PLCβ3 interacting regions (α2-β4-α3-β5), is required for PLCβ interaction and activation. It should be noted that each Ga protein can be divided into the GTPase domain that comprises the PLCβ interacting regions and the helical domains composed of αA to αF.

**Fig. 6** Ability of different chimeras to interact with AC and TPR1. **a** HEK293 cells were transiently transfected with the wild-type or constitutively active mutants of Ga protein and chimeras indicated in the figure. The transfectants were labelled with [3H]adenine (1 μCi/ml) in 1 % FBS/MEM. The labelled cells were treated with 50 μM of FSK for 30 min before subjected to cAMP accumulation assay. cAMP fold inhibition was calculated as the ratios of QL-induced to wild-type cAMP inhibition. Data represent the mean ± S.E.M. of three independent experiments, n = 3. *, cAMP accumulation was significantly inhibited as compared to corresponding wild-type transfected cells; Dunnett t test, p < 0.05. **b** HEK293 cells were transiently co-transfected with FLAG-TPR1 in combination with Ga proteins and the indicated chimeras. Cell lysates were immunoprecipitated by anti-FLAG affinity agarose gel. The immunoprecipitates were immunoblotted with anti-Ga14, anti-Gaz, or anti-FLAG antiserum. The crude lysates were used to examine the expression levels of Ga14, Gaq, Ga14/Gaz, or FLAG-TPR1 by Western blot analysis. The immunoblots shown represent one of three sets of immunoblots; two other sets yielded similar results.
helices [31]. There is increasing evidence to suggest that the helical domain participates in the activation and regulation of the Gα subunit [45]. For instance, the helical domain of Gα16 is known to bind GRK2 [46]. Substitution of the previously identified PLCβ3 interacting regions (α2-β4-α3-β5) of Gα14 by Gαz is expected to abolish PLCβ interaction and activation. However, Gα14/Gαz chimeras consisting of varying combinations of the interacting regions were able to interact and activate PLCβ. Most surprisingly, the 14z151QL chimera, consisting of an entire α2-β4-α3-β5 region of Gαz, was able to stimulate IP3 production to similar levels as Gα14, indicating that this region is not responsible for specifying interaction with PLCβ. The reduction in PLCβ activity of the 14z173QL chimera, consisting of an additional substitution of the adjacent β2-β3 region by Gαz, could be caused by its weaker binding with PLCβ, as lower protein levels of the chimera were observed in complex with PLCβ in the co-immunoprecipitation assay (Fig. 2b). Also, a GTPase domain consisting of an intact α2-β4-α3-β5 region of either Gα14 or Gαz seems important for maximal activation of PLCβ. The zα2β4α3QL chimera disrupts this region and decreased IP3 production was observed without obvious binding defects as determined in the co-immunoprecipitation assay. In general, chimeras with substitutions in the GTPase domain of Gα14 showed limited binding defects but significant effects on IP3 production, which emphasizes the importance of the GTPase domain in PLCβ activation as compared to its less prominent role in protein binding.

Further co-immunoprecipitation and PLCβ activation studies using Gα14/Gαz chimeras suggested that the helical domain of Gα14 is required for PLCβ activation. Replacing either half (amino acids 1–131, or 132–181) of the N-terminus of Gα14 by Gαz disrupted the ability of the chimeras (14z224, 131z14, zaDEF, and 14aDEF) to interact with PLCβ, suggesting that an intact helical core is necessary for PLCβ binding. To date, the sites for effector binding have been mostly mapped to the GTPase domain [13, 14, 17, 39, 47, 48], while much less is known about the function of the helical domain. The helical domain is the most divergent among Gα subunits [49]. Early structural and sequence analyses on Gα predicted that the helical domain is involved in effector interaction and may act as a regulatory entry point for GPCRs and Gβγ subunits [49, 50]. Together with the GTPase domain, it forms the nucleotide binding pocket and regulates GDP/GTP exchange by altering the binding affinity of Gα and its substrate [51, 52]. It has been proposed to participate in G protein oligomerization [53] and in the transition between the inactive and active conformations of Gα [54]. Furthermore, the helical domain of Gαz has been proposed to accelerate GTP hydrolysis by the GTPase domain, functioning as a GTPase-activating protein (GAP) [55]. A study using human/Xenopus chimeras of Gαz subunit revealed that the helical domain of Gαz is also important for

| Table 1 Functional characterizations of the chimeras and their correlation with intact PLCβ or Gα14 helical domains |
|---------------------------------------------------------------|
|                  | PLCβ stimulation | Adenylyl cyclase inhibition | PLCβ interaction | TPR1 interaction | Intact PLCβ domain | Intact Gα14 helical domain |
|------------------|------------------|----------------------------|-----------------|-----------------|-------------------|-----------------------------|
| Gα14             | Yes              | No                         | Yes             | Yes             | Yes               | Yes                        |
| 203z14           | No               | No                         | No              | Yes             | Yes               | No                         |
| 14z151           | Yes              | No                         | Yes             | Yes             | Yes               | Yes                        |
| 182z14           | No               | No                         | No              | Yes             | Yes               | No                         |
| 14z173           | Yes              | No                         | Yes             | Yes             | No                | Yes                        |
| 14zββ3           | No               | No                         | No              | No              | No                | No                         |
| zβ2β3            | Yes              | No                         | Yes             | Yes             | Yes               | Yes                        |
| 14z224           | No               | No                         | No              | Yes             | No                | No                         |
| 131z14           | No               | No                         | Yes             | Yes             | No                | No                         |
| 14aDEF           | No               | No                         | Yes             | Yes             | No                | No                         |
| zaDEF            | No               | No                         | No              | Yes             | No                | Yes                        |
| 14aββ4α3         | No               | No                         | Yes             | Yes             | No                | No                         |
| zaββ4α3         | Yes              | No                         | Yes             | Yes             | No                | Yes                        |
| 14aN             | No               | No                         | No              | No              | No                | No                         |
| zaN              | Yes              | No                         | Yes             | Yes             | No                | No                         |
| Gαz              | No               | No                         | No              | No              | No                | No                         |

Results of Gα proteins and chimeras in functional studies and co-immunoprecipitation assays are summarized. PLCβ stimulation was determined by measuring IP3 production by constitutively active (QL) chimeras as compared to their corresponding wild type activity (Figs. 2c, 3c, 4c, and 5c). The ability of QL-chimeras to inhibit adenylyl cyclase was determined in FSK-induced cAMP accumulation assays (Fig. 6a). Co-immunoprecipitation assays were performed using anti-PLCβ and anti-FLAG for the detection of PLCβ (Figs. 2b, 3b, 4b, and 5c) and TPR1 (Fig. 6b), respectively. Constructs containing an intact PLCβ binding domain (α2-β4-α3-β5 region) or an intact helical domain (αA-αF region) of Gα14 are also shown.
the activation of adenyl cyclase [56]. More recently, crystallization studies suggest that major displacement of the helical domain is required for receptor coupling [57], thereby proposing a role for the helical domain as the inhibitory barrier for receptor-dependent activation. Considering the potential functions of the helical domain, our present study supports the involvement of the helical domain in effector interaction and regulation.

According to recent structural data, the αN helix is an important structure of Gα subunits in many aspects [14]. It is required for the binding of Gβγ [58, 59] and GPCRs [27, 60, 61]. It is also the site of lipid modification which enables proper localization of the G proteins to the plasma membrane (reviewed in [62]). Truncation of the αN helix of Gαq decreased Goαq-stimulated PLCβ3 activity without affecting the binding affinity (Kd) with PLCβ3 [14]. Likewise, mutations in the hydrophobic surface of the distal CTD of PLCβ3, which is thought to form interactions with the αN helix of Goαq, did not decrease the binding affinity while IP3 production was still observed at considerable levels [14]. Consistently, our zaNQL chimera with the αN helix of Go14 replaced by Gα did not show binding defects in co-immunoprecipitation assays and successfully induced IP3 production. Moreover, removal of the distal CTD domain of PLCβ3 has been shown to significantly inhibit IP3 production while only modestly affecting the binding affinity with Goαq [14]. These results suggest that interaction between the distal CTD of PLCβ3 and the αN helix of Goαq or Go14 is important for maximal stimulation of PLCβ3 and plays a less prominent role in Gα protein binding.

Gαq belongs to the Gα subfamily and is able to inhibit adenyl cyclase (AC) activity and subsequent cAMP production by direct association with AC (Fig. 6). Accordingly, substitution of Gα by portions of Go14 structure may affect the level of inhibition of cAMP production. Disruption of the GTPase domain of Gαq, as demonstrated by the 14α2β4α3 chimera, abolishes the ability of Gαq to inhibit AC. The 14z224QL chimera significantly inhibited cAMP production as compared to 14z173QL, which lacks the αDEF region of Gαq and was unable to inhibit AC. However, the 14αDEFQL chimera with an αDEF region of Gαq significantly inhibited AC at comparable levels. Moreover, chimeras lacking the entire helical domain of Gαq (14z151 and 14z173) were unable to inhibit AC. These results suggest that both an intact GTPase domain of Gαq, which agrees with previously reported literature [39, 48], and at least a portion of the helical domain of Gαq is required for AC inhibition.

Go14 and Go16 belong to the same family and share high homology in terms of their amino acid sequence and signaling properties. Both Go14 and Go16 have been shown to activate Ras and downstream transcription factors such as NFKB and STAT3 through interaction with TPR1 [25, 34]. Previous studies using Gα16/Gαz chimeras suggested that the β2 and β3 strands of Gα16 are important for the interaction with TPR1 but this is not necessarily the case for Gα14. Co-immunoprecipitation studies using Gα14/Gαz chimeras (summarized in Table 1) suggest that TPR1 interacts with Gα14 and Gα16 through different structural regions. As demonstrated by the zaDEF chimera, the αDEF region of Gα14 seems necessary for interaction with TPR1. However, several chimeras lacking this region, including 182z14, 203z14, and 131z14, are able to interact with TPR1. The inability of the zaDEF chimera to interact with TPR1 could be caused by disruption of the helical domain resulting in instability of the protein structure. Disruption of the GTPase domain and substitution of the helical domain by Gαq, as demonstrated by the za2β4α3 chimera, did not completely abolish TPR1 interaction. Moreover, the mirror image pairs 14z151 and 203z14, as well as 14z173 and 182z14, were able to interact with TPR1. These results indicate that the presence of either the α2-β4-α3 region of the GTPase domain or an intact helical domain of Gα14 is sufficient for TPR1 interaction.

Conclusion

The present study has successfully used chimeric Gα14/Gαq to map critical regions for effector regulation and demonstrates the insufficiency of previous structural information in supporting efficient effector regulation by Gα proteins. Although the roles of the αN helix and helical domain of Gα subunits in G protein-mediated signal transduction have mostly been neglected, our results designate important roles for these domains of Gα14 in effector interaction and activation.

Methods

Reagents

The human cDNAs of Gα14, Gα16, and Gα16Q were obtained from Guthrie Research Institute (Sayre, PA, USA). Cell culture reagents, including LipofectAMINE PLUS reagents, and Lipofectamid 2000 were purchased from Invitrogen (Carlsbad, CA, USA). Anti-Gα14 targeting the N-terminal was obtained from Gramsch Laboratories (Schwabhausen, Germany). Anti-FLAG antibody and anti-FLAG affinity gel were from Sigma-Aldrich (St. Louis, MO, USA). Other antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Protein G-agarose and dithiobis[succinimidylpropionate] (DSP) cross-linker were from Pierce Biotechnology (IL, USA). Osmosins nitrocellulose membrane and ECL kit were from Westborough (MA, USA) and Amersham (Piscataway, NJ, USA), respectively. Pertussis toxin (PTX) was obtained from List Biological Laboratories (Campbell, CA, USA), and octreotide (OCT) was from Sigma-Aldrich (St. Louis, MO, USA).
Cell culture and co-immunoprecipitation

HEK293 cells were obtained from the American Type Culture Collection (CRL-1573, Rockville, MD). They were maintained in Eagle’s minimum essential medium at 5 % CO₂, 37 °C with 10 % fetal bovine serum, 50 units/mL penicillin and 50 μg/mL streptomycin. For co-immunoprecipitation experiments, HEK293 cells were grown to 80 % confluency in 100 mm tissue culture plates and then co-transfected with 200 ng Gα and 200 ng FLAG-TPR1 cDNAs using 15 μL PLUS and Lipofectamine-2000 as template reagents in Opti-MEM. Serum was replenished 3 h after transfection. Cross-linking was performed one day after transfection; transfected HEK293 cells were washed with PBS twice and then treated with 0.5 mM DSP in PBS for 15 min at room temperature. Cells were then washed again with PBS and maintained in quenching solution (50 mM glycine in PBS, pH 7.4) for 5 min. Subsequently, cells were lysed in ice-cold RIPA buffer (25 mM HEPES at pH 7.4, 0.1 % SDS, 50 mM Na₃VO₄, 4 μg/mL aprotinin, 100 μM phenylmethylsulfonyl fluoride, and 2 μg/mL leupeptin). Cell lysates were gently rocked with an anti-Gα₁₄ antiserum at 4 °C overnight, and then incubated in 30 μL protein G-agarose (50 % slurry) at 4 °C for 2 h. Alternatively, the cell lysates were incubated in 30 μL anti-FLAG affinity agarose gel (50 % slurry) at 4 °C overnight. Immunoprecipitates were washed with ice-cold RIPA buffer (400 μL) for four times, resuspended in 50 μL RIPA buffer and 10 μL 6× sample buffer and then boiled for 5 min. Gα₁₄ and FLAG-TPR1 proteins in the immunoprecipitates were analyzed by Western blots.

Construction of chimeras

Gα chimeras were constructed from cDNAs encoding human Gα₁₄ and Gα₃ by using polymerase chain reaction (PCR) techniques. The N-terminal 37, 131, 182 and 203 residues of Gα₁₄ were substituted by the corresponding amino acids of Gα₃ to generate zuN, 131z14, 182z14 and 203z14 chimeras, respectively. Primers were designed to produce two half-length fragments with overlapping regions; the forward fragment was generated with the antisense and T7 primers, whereas the backward fragment was made with the sense and reverse primers which target a BGH polyadenylation signal (BGH primers). The two half-products were then annealed together to generate a full-length fragment by another round of PCR using T7 and BGH primers. Mirror images of these constructs were generated analogously and were named 14z173, 14z224, 14z173 and 14z151 chimeras. PCR (30 cycles each with 94 °C for 60 s, 58 °C for 60 s and 72 °C for 90 s) was carried out using AccuPrime PCR mix. The 14β2β3 chimera was constructed using 182z14 as the DNA template for the forward half-product and 14z151 DNA template for the backward half-product. The 14z2β3 chimera was constructed using 14z173 as the DNA template for the forward half-product and 203z14 as the DNA template for the backward half-product. 14α2β4α3 was constructed using 203z14 as the template for the forward half product and Gα₃ as the template for the backward half product. Its mirror image zα2β4α3 was constructed using 14z151 as the template for the forward half product and Gα₁₄ for the backward half product. Finally, 14αDEF was constructed using 131z14 as the DNA template for the forward half-product and Gα₃ as template for the backward half-product. Its mirror image zαDEF was constructed using 14z224 as the DNA template for the forward half-product and Gα₁₄ as template for the backward half-product. Primers for chimera construction are listed in Table 2. All Gα chimeras were checked by restriction mapping and then subcloned into pcDNA3 at HindIII and XbaI sites. The constructs were confirmed by dideoxynucleotide sequencing using Applied Biosystem Big Dye Terminator v3.1 Cycle Sequencing Kits (Foster City, CA, USA).

Inositol Phosphates (IP₃) accumulation assay

HEK293 cells were seeded on a 12-well plate at 2 × 10⁵ cells/well one day prior to transfection. Cells were then transfected with 200 ng Gα using 2 μL PLUS and Lipo-fectamine reagents in Opti-MEM. On the next day, cells were labeled with inositol-free Dubecco’s modified Eagle’s medium (DMEM; 750 μL) containing 5 % FBS and 2.5 μCi/mL myo-[³H]inositol overnight. Labeled cells were washed twice with the inositol phosphates assay medium (DMEM buffered with 20 mM HEPES, pH 7.5 and 5 mM LiCl) and were incubated for 1 h at 37 °C. Reactions were stopped by replacing the assay medium with 750 μL ice-cold 20 mM formic acid and the lysates were kept in 4 °C for 30 min before the separation of [³H]IP from other labeled species by sequential ion-exchange chromatography as described previously [63].

cAMP accumulation assay

HEK293 cells were labeled overnight with [³H]adenine (1 μCi/ml) in culture medium containing 1 % FBS. The labeled cells were rinsed once with 2 ml of assay medium (MEM containing 20 mM HEPES, pH 7.4) and incubated at 37 °C for 30 min with 1 ml of assay medium containing 1 mM 1-methyl-3-isobutylxanthine in the absence or presence of 50 μM forskolin. The cells were lysed with 1 ml 5 % trichloroacetic acid with 1 mM ATP to terminate the reaction and were stored at 4 °C for 1 h. Intracellular [³H]cAMP was isolated by sequential chromatography as described previously [64]. The level of [³H]cAMP was estimated by determining the ratios of [³H]cAMP to total [³H]ATP and [³H]ADP pools.
Molecular modeling

Ga\(\alpha\) in a complex with PLC\(\beta\)3 (PDB ID: 3OHM, [13]) was employed to illustrate the interaction between Ga and PLC\(\beta\), and for creating a molecular model of Ga\(\alpha\) by homologous modeling using SWISS-MODEL [65, 66]. Visualization of various structures was accomplished using PyMOL (The PyMOL Molecular Graphics System, Version 1.3 Schrödinger, LLC).

Western blotting analysis

Protein samples were resolved on 12 % SDS-polyacrylamide gels and transferred to Osmonics nitrocellulose membrane. Resolved proteins were detected by their specific primary antibodies and horseradish peroxidase-conjugated secondary antisera. The immunoblots were visualized by chemiluminescence with the ECL kit from Amersham, and the images detected in X-ray films were analyzed and interpreted using the Eagle Eye II still video system (Stratagene, La Jolla, CA, USA).

Table 2 Primer sequences for constructing various Ga\(\alpha\)2/\(\beta\)3 chimeras

| Chimera | Templates | Primers |
|---------|-----------|---------|
| 203z14  | Ga\(\alpha\)/Ga\(\beta\) | F: 5'- ATGGTGACGCGCGGCGCAACGATCGGG -3'  
|         |           | R: 5'- TCCCTGACGTGGCCCGCCCGCCCGCCCGC -3'  
| 14z151  | Ga\(\alpha\)/Ga\(\beta\) | F: 5'- ATGGTGACGCGCGGCGCAACGATCGGG -3'  
|         |           | R: 5'- CTCTGACGTGGCCCGCCCGCCCGCCCGC -3'  
| 182z14  | Ga\(\alpha\)/Ga\(\beta\) | F: 5'- ATGGTGACGCGCGGCGCAACGATCGGG -3'  
|         |           | R: 5'- TCCCTGACGTGGCCCGCCCGCCCGCCCGC -3'  
| 14z173  | Ga\(\alpha\)/Ga\(\beta\) | F: 5'- ATGGTGACGCGCGGCGCAACGATCGGG -3'  
|         |           | R: 5'- CACAATGCGTGCGGAGCGGAGGAGGAGGAGGAGG -3'  
| 14z2\(\beta\)3 | 182z14/14z151 | F: 5'- ATGGTGACGCGCGGCGCAACGATCGGG -3'  
|         |           | R: 5'- CTCTGACGTGGCCCGCCCGCCCGCCCGC -3'  
| z\(\beta\)3 | 14z2/303z14 | F: 5'- ATGGTGACGCGCGGCGCAACGATCGGG -3'  
|         |           | R: 5'- TCCCTGACGTGGCCCGCCCGCCCGCCCGC -3'  
| 14z224  | Ga\(\alpha\)/Ga\(\beta\) | F: 5'- GCCCATGAAGTGGGACTCCCTGCCC -3'  
|         |           | R: 5'- CACAATGCGTGCGGAGCGGAGGAGGAGGAGGAGG -3'  
| 131z14  | Ga\(\alpha\)/Ga\(\beta\) | F: 5'- GCCCATGAAGTGGGACTCCCTGCCC -3'  
|         |           | R: 5'- CACAATGCGTGCGGAGCGGAGGAGGAGGAGGAGG -3'  
| 14aDEF  | 131z14/Ga\(\beta\) | F: 5'- GCCCATGAAGTGGGACTCCCTGCCC -3'  
|         |           | R: 5'- CACAATGCGTGCGGAGCGGAGGAGGAGGAGGAGG -3'  
| zaDEF   | 14z224/Ga\(\beta\) | F: 5'- GCCCATGAAGTGGGACTCCCTGCCC -3'  
|         |           | R: 5'- CACAATGCGTGCGGAGCGGAGGAGGAGGAGGAGG -3'  
| 14a2\(\beta\)3 | 203z14/Ga\(\beta\) | F: 5'- ATGGTGACGCGCGGCGCAACGATCGGG -3'  
|         |           | R: 5'- TCCCTGACGTGGCCCGCCCGCCCGCCCGC -3'  
| za2\(\beta\)3 | 14z151/Ga\(\beta\) | F: 5'- ATGGTGACGCGCGGCGCAACGATCGGG -3'  
|         |           | R: 5'- CACAATGCGTGCGGAGCGGAGGAGGAGGAGGAGG -3'  
| 14aN    | Ga\(\alpha\)/Ga\(\beta\) | F: 5'- GCCCATGAAGTGGGACTCCCTGCCC -3'  
|         |           | R: 5'- CACAATGCGTGCGGAGCGGAGGAGGAGGAGGAGG -3'  
| zaN     | Ga\(\alpha\)/Ga\(\beta\) | F: 5'- GCCCATGAAGTGGGACTCCCTGCCC -3'  

Additional file

**Additional file 1:** Figure S1. Alignment of Ga\(\alpha\)/Ga\(\beta\)3-interacting residues in the PLC\(\beta\) family. Figure S2. Alignment of PLC\(\beta\)-interacting residues in the Ga protein family. Figure S3. Interaction of Ga\(\alpha\)/Ga\(\beta\)3 chimeras with Flag-TPR1. (PPTX 306 kb)

**Abbreviations**

AC: Adenylyl cyclase; ERK: Extracellular signal-regulated kinase; GPCR: G protein-coupled receptors; HRK293: Human embryonic kidney 293; IP\(\beta\): Inositol trisphosphate; MAPK: Mitogen-activated protein kinase; PLC\(\beta\): Phospholipase C\(\beta\); TPR1: Tetratricopeptide repeat 1.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

DHTK carried out most of the experiments and analyzed and interpreted the results. LYY performed several co-immunoprecipitation experiments. ASLC helped to design the mutant constructs and KMW performed molecular model constructions and participated in drafting the manuscript. YHW conceived the study and participated in its design and coordination as well as drafted the manuscript. All authors have read and approved the final version of the manuscript.

**Authors’ information**

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References
1. Oldham WM, Hamm HE. Heterotrimetric G protein activation by G-protein-coupled receptors. Nat Rev Mol Cell Biol. 2008;9(1):60–71.
2. Tesmer JJ. The quest to understand heterotrimetric G protein signaling. Nat Struct Mol Biol. 2010;17(6):650–2.
3. Sandal M, Patrinier D, Carlani P, Musiani F, Giorgetti A. Structure/function relationships of phospholipases C Beta. Curr Protein Pept Sci. 2013;14(6):650–7.
4. Jhon DY, Lee HH, Park D, Lee CW, Lee KH, Yoo OJ, et al. Cloning, sequencing, purification, and Gq-dependent activation of phospholipase C beta 3. J Biol Chem. 1993;268(9):6564–61.
5. Lyon AM, Tesmer JJ. Structural insights into phospholipase C-B2 function. Mol Pharmacol. 2013;84(4):488–500.
6. Adjobo-Hermans MJ, Crosby KC, Purvisy M, Bhageloe A, van Weeren L, Schultz C, et al. PLCB3 isoforms differ in their subcellular location and their CT-domain dependent interaction with Goa. Cell Signal. 2013;25(1):255–63.
7. Berridge MJ, Irvine RF. Inositol trisphosphate, a novel second messenger in cellular signal transduction. Nature. 1984;312:315–21.
8. Ross F, Wilkie T. GIIa-activating proteins for heterotrimetric G protein signaling: regulators of G protein signaling (RGS) and RGS-like proteins. Annu Rev Biochem. 2000;69:795–827.
9. Dorm GW, Oswald KJ, McCluskey TS, Kuhel DG, Liggett SB. a2u-adrnergic receptor stimulated calcium release is transduced by Gq-associated Gβγ-mediated activation of phospholipase C. Biochemistry. 1997;36:6415–24.
10. Park D, Jhon DY, Lee CW, Lee KH, Rhee SG. Activation of Gαq signaling by PH domain of Gαq isoforms. J Biol Chem. 1995;268:4573–4.
11. Rozengurt E. Mitogenic signaling pathways induced by G protein-coupled receptors. Annu Rev Cell Biol. 1992;8:109–49.
12. Gaugler L, Raamsdonk CD, Bezrookove V, Green G, Brien JMO, Simpson EM, et al. Kinetic spectroscopy reveals interfaces of the C-terminal coiled-coil domain of Gqα. J Biol Chem. 2002;277(23):21279–93.
13. Waldo GL, Ricks TK, Hicks SN, Cheever ML, Kawano T, Tsuboi K, et al. Kinetic spectroscopy reveals interfaces of the C-terminal coiled-coil domain of Gqα. J Biol Chem. 2002;277(23):21279–93.
14. Lyon AM, Dutta S, Boguth CA, Skiniotis G, Tesmer JJG. Full-length Gqα subunit multigene family. Nat Struct Mol Biol. 2010;17(6):650–7.
15. Park D, Jhon DY, Lee CW, Ryu SH, Rhee SG. Removal of the carboxyl-terminal region of phospholipase C-β1 isozyme by Gαq activation. J Biol Chem. 2000;275(31):23919–26.
16. Kim C, Park D, Rhee SG. The role of the carboxyl-terminal basic amino acids in Gαq-dependent activation, particulate association, and nuclear localization of phospholipase C-B1. J Biol Chem. 1996;271:2187–92.
17. Venkatakrishnan G, Eton J. Identification of determinants in the a-subunit of Gαq required for phospholipase C activation. J Biol Chem. 1996;271:5066–72.
18. Wilkie TM, Scherke PA, Strathmann MP, Siepak VZ, Simon MJ. Characterization of G-protein α subunits in the Gαq class: expression in murine tissues and in somatostatin and hematoietic cell lines. Proc Natl Acad Sci. 1991;88(22):10049–53.
19. Wilkie TM, Gilbert DJ, Olsen AS, Chen XN, Amatruda TT, Korenberg JR, et al. Evolution of the mammalian G protein α subunit multigene family. Nat Genet. 1992;1(2):85–91.
20. Conklin BR, Chabukswar M, Wong YH, Federman A, Bourne HR. Recombinant Gqα: mutational activation andcoupling to receptors and phospholipase C. J Biol Chem. 1992;267:31–4.
21. Hepler JR, Kozasa T, Smrcka AV, Simon MI, Rhee SG, Stemwinder PC, et al. Purification from SF9 cells and characterization of recombinant Gαq and Gγα. Activation of purified phospholipase C isoforms by Goa subunits. J Biol Chem. 1993;268(19):14367–75.
47. Berlot CH, Bourne HR. Identification of effector-activating residues of \( \alpha_\text{s} \). Cell. 1992;68:911–22.
48. Grishina G, Berlot CH. Identification of common and distinct residues involved in the interaction of \( \alpha_2 \) and \( \alpha_5 \) with adenylyl cyclase. J Biol Chem. 1997;272:20619–26.
49. Coleman DE, Berghuis AM, Lee E, Linder ME, Gilman AG, Sprang SR. Structures of active conformations of \( \alpha_\text{i} \) and the mechanism of GTP hydrolysis. Science. 1994;265:1405–12.
50. Masters SB, Stroud RM, Bourne HR. Family of G protein \( \alpha \) chains: amphipathic analysis and predicted structure of functional domains. Protein Eng. 1986;1:47–54.
51. Noel JP, Hamm HE, Sigler PB. The 2.2 Å crystal structure of \( \alpha_\text{transducin} \) complexed with GTP\( \gamma \)S. Nature. 1993;366:654–63.
52. Remmers AE, Engel C, Liu M, Neubig RR. Interdomain interactions regulate GDP release from heterotrimeric G proteins. Biochemistry. 1999;38:13795–800.
53. Mixon MB, Lee E, Coleman DE, Berghuis AM, Gilman AG, Sprang SR. Tertiary and quaternary structural changes in \( \alpha_\text{Gi} \) induced by GTP hydrolysis. Science. 1995;270:954–60.
54. Codina J, Birnbaumer L. Requirement for intramolecular domain interaction in activation of G protein \( \alpha \) subunit by aluminum fluoride and GDP but not by GTP\( \gamma \)S. J Biol Chem. 1994;269:29339–42.
55. Markby DW, Onrust R, Bourne HR. Separate GTP binding and GTPase activating domains of a G alpha subunit. Science. 1993;262(5141):1895–901.
56. Antonelli M, Bimbaumer L, Allende J, Olate J. Human-Xenopus chimeras of \( \alpha_\text{Gi} \) reveal a new region important for its activation of adenylyl cyclase. FEBS Lett. 1994;340:249–54.
57. Rasmussen SG, DeVree BT, Zou Y, Kruse AC, Chung KY, Kobilka TS, et al. Crystal structure of the \( \beta \) adrenergic receptor-G protein complex. Nature. 2011;477(7366):549–55.
58. Lambright DG, Sondek J, Bohrm A, Skiba NP, Hamm HE, Sigler PB. The 2.0 Å crystal structure of a heterotrimeric G protein. Nature. 1996;379:311–9.
59. Wall MA, Coleman DE, Lee E, Iniguez-Lluhi JA, Posner BA, Gilman AG, et al. The structure of the G protein heterotrimer \( \alpha_\text{Gi} \beta_\text{i} \gamma_\text{2} \). Cell. 1995;83:1047–58.
60. Hamm HE, Deretic D, Arendt A, Hargrave PA, Koenig B, Hofmann KP. Site of G protein binding to rhodopsin mapped with synthetic peptides from the \( \alpha \) subunit. Science. 1988;241:832–5.
61. Onrust R, Herzmark P, Chi P, Garcia PD, Lichtarge O, Kingsley C, et al. Receptor and \( \beta \text{y} \) binding sites in the \( \alpha \) subunit of the retinal G protein transducin. Science. 1992;275:381–4.
62. Marrari Y, Crouchman M, Iannejad R, Wedegaertner PB. Assembly and trafficking of heterotrimeric G proteins. Biochemistry. 2007;46:7665–77.
63. Tsu RC, Chan JS, Wong YH. Regulation of multiple effectors by the cloned \( \delta \) opioid receptor: stimulation of phospholipase C and type II adenylyl cyclase. J Neurochem. 1995;64:2700–7.
64. Wong YH. G assays in transfected cells. Methods Enzymol. 1994;238:881–94.
65. Arnold K, Bordoli L, Kopp J, Schwede T. The SWISS-MODEL workspace: a web-based environment for protein structure homology modelling. Bioinformatics. 2006;22:195–201.
66. Bordoli L, Kiefer F, Arnold K, Benkert P, Battey J, Schwede T. Protein structure homology modelling using SWISS-MODEL. Nat Protoc. 2009;4:1.