Functions and Regulatory Mechanisms of Gq-Signaling Pathways

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Key Words
Gq · Phospholipase Cβ · Interacting proteins · Ric-8 · Flotillin · Pasteurella multocida toxin · YM-254890 · Neural progenitor cells · Proliferation · Differentiation · Migration

Abstract
Gq family members of heterotrimeric G protein activate β isoforms of phospholipase C that hydrolyzes phosphatidylinositol phosphate to diacylglycerol and inositol trisphosphate, leading to the protein kinase C activation and intracellular Ca²⁺ mobilization, respectively. To understand the functions and regulatory mechanisms of Gq-signaling pathways, we first introduce the Gq-interacting proteins, which function as the effectors and the modulators of Gq. Next, we describe the Pasteurella multocida toxin and YM-254890, which are useful tools to investigate Gq signaling as activator and inhibitor, respectively. Finally, we discuss the physiological function of Gq in developmental brain, especially in neural progenitor cells.

Introduction

Many hormones, neurotransmitters and sensory stimuli elicit cellular response through the activation of seven transmembrane receptors coupled to heterotrimeric G proteins that consist of three subunits, α, β, and γ. The GTPase cycle of heterotrimeric G proteins is driven between inactive and active forms by binding to GTP and its hydrolysis [1, 2]. Activation of the G protein-coupled receptors (GPCRs) by extracellular stimuli induces the release of GDP and binding of GTP on G protein α subunit (Gα). This leads to the dissociation of Gαβγ into Gα-GTP and Gβγ. Gα-GTP and Gβγ then transmit the receptor-generated signals to downstream effector molecules until the intrinsic GTPase activity of Gα hydrolyzes GTP to GDP and the inactive Gα-GDP reassociates with Gβγ.

G proteins are divided according to structural and functional similarities of their Gα into four groups; Gs, Gi, Gq, and G12 [3]. Gq members including Gqα, Gq11, Gq14, and Gq15/16 (mouse/human orthologues, respectively) stimulate the β-isoforms of phospholipase C (PLC-β) that catalyzes the hydrolysis of phosphatidylinositol bisphosphate, resulting in the generation of inositol trisphosphate (IP₃) and diacylglycerol (DAG). These second messengers serve to propagate and amplify the Gq-mediated signal with intracellular calcium mobilization by Ca²⁺ release from IP₃-regulated intracellular stores, and DAG-dependent protein kinase C (PKC) activation. Ca²⁺ and PKC participate in diverse signaling to evoke different cellular events.

The Gα subunit is composed of a GTPase domain and an α helical domain (fig. 1) [4]. The GTPase domain is similar to that of other members of the GTP-binding protein superfamily, including monomeric small GTPases and translational elongation factors. The GTPase domain of Gα participates in the hydrolysis of GTP to GDP and...
provides the binding surfaces for the Gβγ dimer, GPCRs and effectors. The domain has three flexible loops, named switch regions I, II and III, whose conformations are dependent upon GDP or GTP binding. The helical domain contains six helices and is unique to G protein subunit, but the function of helical domain in G protein signaling remains to be fully clarified.

In this review, we first summarize the Gq-interacting proteins. The binding partners of Gq have a potential to be the effectors of Gq, or the modulators of Gq signaling. Second, the molecules targeted at Gq signaling are focused. To understand the regulatory mechanism of GTPase cycle and which G protein signaling is involved in certain cellular responses, these molecules should be useful tools. Gq family members, their effectors, interaction partners, and regulatory molecules are summarized in table 1. Third, we describe the physiological function of Gq in neural development. Many insights into the role of G protein signaling during development have been provided from studies on mouse lacking G protein α subunit. Moreover, to elucidate the cellular function of Gq signaling, we particularly focus on neural progenitor cells.

**Gq-Interacting Molecules**

**Phospholipase Cβ**

The mammalian PLC-β isoforms contain an N-terminal pleckstrin homology (PH) domain, four EF hand motifs, a catalytic X/Y domain, a C2 domain, and a long 400-residue C-terminal extension that is unique to the PLC-β family (fig. 1a). The four isoforms of PLC-β (β1–β4) are stimulated by Gq-GTP and Gβγ, and catalyze inositol phospholipid breakdown [5]. The detailed mechanism of activation by Gq is not yet revealed but the C-terminal extension of PLC-β is required for activation and interaction with Gq subunit. Activated Gq family members stimulate all four PLC-β isoforms. Purified recombinant Gq, Gα11 and Gα14 indistinguishably activate β1, β3, and β4 isoforms of PLC, and they are poor activators of PLC-β2 in vitro [6–9]. However, the physiological relevance between Gq, Gα11 and Gα14 in cells and tissues has not been fully clarified. In contrast, Gα15/16 which is expressed only in hematopoietic cells, activates PLC-β1, -β2, and -β3 in vitro [10]. Interestingly, PLC-β2 is also expressed specifically in hematopoietic cells. Therefore, it is suggested that Gα15/16 and PLC-β2 are functionally linked in these cells. Collectively, these...
findings suggest that some Gq family members may selectively couple to PLC-β isoforms in native cells to generate tissue- or cell-specific responses in vivo.

The PLC interaction sites on Gq are thought to be located in α-helix3 and its linker to β-sheet4, which are a part of a switch region whose conformation is changed upon activation [11]. Conversely, the activated Gq subunit appears to interact with the C-terminal region of PLC-β1 (residues 803–1216) that is unique to this subfamily of PLC isozymes. Deletion analysis suggested that the region downstream of residue 845 is required for binding of and stimulation by Gq [12, 13]. However, it has been also reported that the activated Gq subunit interacts with the C2 domains of PLC-β (residues 663–803) [14].

G protein signaling is turned off by hydrolysis of GTP bound to Gα, whose reaction is catalyzed by the intrinsic GTPase activity of the α subunit itself. This deactivation process of Gq was studied in detail by reconstitution of m1 muscarinic acetylcholine receptor, G protein, and PLC-β1 in lipid vesicles [15]. Although the intrinsic GTPase activity of purified Gq was low, the presence of PLC-β1 induced an increase in this activity. This result indicated that PLC-β1 is a GTPase-activating protein (GAP) for Gq. Kinetic analysis indicated that PLC-β acts directly on Gq to stimulate hydrolysis of bound GTP, and that the other components of the reconstituted system (the m1 muscarinic receptor, Gβγ, and phospholipids) promote only GDP-GTP exchange during steady-state hydrolysis [16]. A fragment of the C-terminal region of PLC-β1 (residues 903–1042) exhibited GAP activity [17]. These results indicate that the receptor and PLC-β1 coordinately regulate the amplitude of the PLC signal and the rate of signal termination.

### Regulator of G Protein Signaling

Recently, several proteins were identified to be involved in the regulation of GTPase cycle (fig. 2). GTPase cycle is regulated by guanine nucleotide exchange factors (GEFs) (fig. 2, green) and GTPase-activating proteins (GAPs) (fig. 2, purple). The regulator of G protein signaling (RGS) proteins act as GAP for Gα, leading to the termination of G protein cycle. RGS proteins preferentially interact with Gα in its transition state of GTP hydrolysis and accelerate endogenous GTPase activity of Gα. More than 20 members of RGS proteins have been identified [18]. The RGS family contains a conserved 120 amino acid ‘RGS box’, which binds Gα-GTP to confer GAP activity. RGS proteins are classified into 6–9 subfamilies (A/RZ, B/R4, C/R7, D/R12, E/RA, F/GEF, G/GRK, H/SNX, etc.) based on homology and functional conserved domains outside of the RGS domain [18, 19]. Members of

### Table 1. Mammalian Gq family members and their binding or regulatory molecules

<table>
<thead>
<tr>
<th>Gq family members</th>
<th>Gq</th>
<th>Gq11</th>
<th>Gq14</th>
<th>Gq15/16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identity with Gqq</td>
<td>100%</td>
<td>90%</td>
<td>80%</td>
<td>57%</td>
</tr>
<tr>
<td>Distribution</td>
<td>ubiquitus</td>
<td>ubiquitus</td>
<td>kidney, liver, lung</td>
<td>hematopoietic cells</td>
</tr>
<tr>
<td>Effectors</td>
<td>PLC-β, p63-RhoGEF</td>
<td>PLC-β, 3, 4, 6p63-RhoGEF</td>
<td>PLC-β1, 3, 4, 6p63-RhoGEF</td>
<td>PLC-β1, 2, 3</td>
</tr>
<tr>
<td>RGS</td>
<td>B/R4 RGS</td>
<td>B/R4 RGS</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Interaction partners</td>
<td>Ric-8</td>
<td>Ric-8A, -8B</td>
<td>Ric-8A, -8B</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Flotillin</td>
<td>flotillin-1, -2</td>
<td>flotillin-1, -2</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Caveolin</td>
<td>caveolin-1</td>
<td>caveolin-1</td>
<td>ND</td>
</tr>
<tr>
<td>Regulatory molecules</td>
<td>PMT1</td>
<td>sensitive</td>
<td>insensitive</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>YM-2548902</td>
<td>sensitive</td>
<td>sensitive</td>
<td>insensitive</td>
</tr>
</tbody>
</table>

Amino acid sequence identity was calculated using human Gq (Accession No. NP_002063), human Gα11 (NP_002058), human Gα14 (NP_004288), and human Gα16 (NP_002059). Gα15 is a mouse homologue of Gα16. Details of effectors, RGSs, interaction partners, and regulatory molecules are described in the text. ND = Not determined.

1 After the submission of this article, Orth et al. [129] reported that Gq as well as Gqα12/13 was activated by PMT.

2 Researchers may ask Dr. Taniguchi or Dr. Takasaki (Astellas Pharma Inc., Japan), who is a first author in [97, 102, respectively], for YM-254890.
the B/R4 subfamily, including RGS1, RGS2, RGS3, RGS4, RGS5, RGS8, RGS13, RGS16, and RGS18, are relatively small proteins (20–25 kDa). It has been reported that many of these members bind to Gq family and inhibit inositol lipid/Ca\textsuperscript{2+} signaling [20–29]. Although the measurement of Gq GTPase activity is somewhat difficult as compared with that of other G\alpha [30], limited RGS proteins (RGS2, RGS3, RGS4, and RGS18) have been shown to exhibit GAP activity for Gq. Non-receptor type GEFs are novel proteins, such as Ric-8. Group I of AGS family is most famous non-receptor type GEF for Gi. However, AGS for Gq has not been reported. Inactivation of Gq is promoted by GAP. RGSs are well-characterized molecule as GAP for heterotrimeric G proteins. 

PLC-\beta has also GAP activity. Gq interacts with scaffolding proteins in caveolae (orange) and lipid raft (pink). Flotillins mediate Gq-induced p38 MAPK activation through Src family tyrosine kinase (SFK) in lipid rafts. GTP bound form of Gq directly interacts with its effectors such as PLC-\beta and p63RhoGEF, and activates these molecules. Molecules that regulate Gq cycle are indicated with yellow. *P. multocida* toxin (PMT) activates Gq signal, while YM-254890 inhibits the guanine nucleotide exchange on Gq.

### Fig. 2.
Schematic representation of Gq cycle and its regulatory molecules. GTPase cycle is regulated by guanine nucleotide exchange factors (GEF; green) and GTPase activating proteins (GAP; purple). GEFs for Gq are classified into two types, receptor type and non-receptor type. GPCR stimulated by its ligand is a representative GEF for Gq. Non-receptor type GEFs are novel proteins, such as Ric-8. Group I of AGS family is most famous non-receptor type GEF for Gi. However, AGS for Gq has not been reported. Inactivation of Gq is promoted by GAP. RGSs are well-characterized molecule as GAP for heterotrimeric G proteins.

While the B/R4 class of RGS proteins is generally accepted as negative modulators of Gq signaling, evidence that Gq interacts with other RGS class proteins has been in conflict. Further studies are needed to clarify whether these RGS proteins directly regulate Gq family members.

**Rho Guanine Nucleotide Exchange Factor, p63RhoGEF**

Rho family small GTPases regulate essential cellular processes including cell morphological change, cell migration, and proliferation. RhoA, Rac1, and Cdc42 are the best-characterized members of this family, and they control the dynamics of the actin cytoskeleton and also stimulate gene transcription through several transcription factors, such as the serum response factor (SRF) or nuclear factor \( \kappa B \) (NF-\( \kappa B \)) [36, 37]. The activation step from an inactive GDP-bound state to an active GTP-bound state is accelerated by a large family of Rho guanine nucleotide exchange factors (RhoGEFs). More than 60 members of...
RhoGEFs contain a catalytic Dbl homology (DH) domain and a PH domain [38, 39].

The signaling from the stimulation of GPCR to the activation of RhoA is mediated by Dbl-family GEFs that are responsive to activated Gα subunits. Both Gα12/13 and Gαq/11 family members are upstream activators of RhoA. Moreover, members of the Gα12/13 and Gαq/11 families utilize distinct pathways to transmit the signal from RhoGEFs to RhoA. RhoA activation via Gα12/13 family is mediated by the p115 family members, which consists of p115-RhoGEF, PDZ-RhoGEF, and leukemia-associated RhoGEF (LARG) [40–43]. The p115 family members are activated by Gα12/13 via a protein-protein interaction mediated through RGS-dependent gene expression. Furthermore, co-immunoprecipitation studies postulated that activated Gαq associates with the C-terminal half of p63RhoGEF.

Recently, the Dbl-family member p63RhoGEF/GEFT has been identified as a novel effector of Gq [44]. Living cell assays demonstrated that Gq-coupled GPCR activation or activated mutants of Gqα promoted the ability of p63RhoGEF to stimulate RGS-dependent gene expression. Furthermore, co-immunoprecipitation studies postulated that activated Gαq associates with the C-terminal region of p63RhoGEF [45]. The interactions involve the effector-binding site and the C-terminal region of Gqα and appear to relieve autoinhibition of the catalytic DH domain by the PH domain. Using biochemical and biophysical approaches with purified protein components, it has been shown that p63RhoGEF directly and specifically associates with activated Gqα to enhance the guanine nucleotide exchange of RhoA, RhoB, and RhoC [46]. Furthermore, Trio, Duet, and p63RhoGEF constitute a family of Gqα effectors that appear to activate RhoA both in vitro and in intact cells. It was also reported that Trio’s Rho GEF domain is a major Gqα effector domain that mediates the Gqα signaling involved in the locomotion, egg laying, and growth of Caenorhabditis elegans [47].

Stimulation of Gq-coupled receptor and expression of activated Gqα family members can induce Rho-mediated responses including actin stress fiber formation, cell rounding, SRF-dependent gene expression, and increases in activated RhoA in various cells. Considerable evidence now indicates that Gq and its family members are also involved in the RhoA activation, but whether all members of Gq family induce Rho activation remains still elusive.

Gq Guanine Nucleotide Exchange Factor, Ric-8

Ric-8 was identified by a genetic study of C. elegans mutants, which are resistant to inhibitors of acetylcholinesterase [48]. Neurotransmitter release in C. elegans is controlled by the Gq- and Go-signaling pathways. EGL-30 (Gαq) pathway is negatively regulated by GOA-1 (Gαo), which stimulates DAG kinase to reduce the DAG level. Miller et al. have reported that ric-8 and egl-30 reduction-of-function mutants show similar phenotypes and Ric-8 functions upstream of or parallel with EGL-30. Additionally, it has been reported that Ric-8 is involved in the asymmetric division of C. elegans embryos [49–51] and Drosophila melanogaster neuroblasts [52–54].

There are two mammalian homologues of RIC-8. They are termed Ric-8A and Ric-8B. It has been demonstrated that Ric-8A has GEF activity for Gα11, Gαo, Gαq and Gα12 but not for Gαs in vitro [55]. Expression of Ric-8A gene in the neuronal system of the developing and adult mouse has been investigated using lacZ transgenic mice [56]. However, the role of Ric-8A on Gq-mediated signal transduction pathway in vertebrates has not been elucidated.

To identify a novel effector or regulator of Gqα, we performed a yeast two-hybrid screening using Gqα as bait and obtained mouse Ric-8A. We investigated the function of Ric-8A on the signal transduction in intact cells [57]. We showed that the N-terminal region of Ric-8A interacts with Gqα and the siRNA-mediated gene silencing of Ric-8A reduces Gqα-coupled receptor-mediated ERK activation and intercellular calcium mobilization. Ric-8A is located in the cytosol and partly translocates to the plasma membranes in response to Gq-coupled receptor stimulation. Additionally, the membrane-targeted Ric-8A mutant enhances Gqα-mediated ERK activation. Taken together, our results indicate that Ric-8A enhances the Gqα-mediated signal in vertebrates. Ric-8A is a novel non-receptor type GEF for Gqα, whereas GPCR stimulated by its ligand is well-known GEF for Gα (fig. 2, green).

Another mammalian RIC-8 homologue, Ric-8B, interacts with Gαq and Gαq but not with Gαi and Gα12 [55]. Von Dannecker et al. performed a yeast two-hybrid system to search for potential regulators for Gαolf, which links GPCRs to adenylyl cyclase in olfactory bulb [58]. They indicated that Ric-8B is predominantly expressed
in the mature olfactory sensory neurons and is able to potentiate Goα-dependent cAMP accumulation. Moreover, they showed that Ric-8B promotes functional expression of odorant receptors [59]. Although the interaction of Ric-8B with Goq was detected, the role of Ric-8B in Gq signaling has not yet been elucidated.

**Scaffold Proteins in Microdomain of Cell Membranes**

In cellular membranes, cholesterol, glycolipids and lipid-anchored proteins appear to organize the microdomains that are resistant to nonionic detergent solubilization. Two domains, named as caveolae and lipid rafts, are morphologically and biochemically distinguishable, but both domains contain many signaling molecules and are thought to be involved in compartmentalization and efficiency of cell surface signaling.

Lipid rafts are the microdomains of cell membranes enriched in a distinct set of lipids, such as cholesterol and sphingolipids [60]. Lipid rafts are distinguished as flat domain rich in glycosylphosphatidylinositol (GPI)-anchored proteins and lipid-modified proteins in which these molecules rely primarily on lipid-lipid interactions for their formation and detergent resistance. In contrast, caveolae are smooth, flask-shaped, cell-surface invaginations that appear to depend on caveolin oligomerization for their formation. Caveolin-1 is a scaffold protein enriched in caveolae. One report described the selective concentration of Goq in caveolae and the Goq binding to caveolin-1 [61]. While siRNA knockdown of caveolin-1 does not alter Goq subcellular localization [62], the signaling via Gq-coupled receptors is impaired, suggesting that the caveolin-1 scaffolding of Goq is important for efficient receptor coupling [63] (fig 2, orange). On the other hand, we identified lipid raft resident proteins, flotillin-1/reggie-2 and flotillin-2/reggie-1, as Goq-interacting proteins [64] (fig 2, pink). Flotillin-1/reggie-2 and its parologue, flotillin-2/reggie-1, were first identified as the proteins whose expression was increased during axon regeneration in gold fish [65]. Bickel et al. [66] independently identified flotillins as proteins that are highly enriched in the detergent-resistant low-density membrane; thereby, flotillins are used as lipid raft marker proteins. Flotillins harbor the evolutionary conserved stomatin/prohibitin/flotillin/HflK/C (SPFH) domain at their amino terminus and share about 50% amino acid identity between flotillin-1 and flotillin-2 [67]. The expression of flotillins is regulated in various cellular processes such as insulin signaling, T-cell activation, membrane trafficking, phagocytosis, cell motility, and transformation [68]. We showed that the interactions of Goq and flotillins were independent of the nucleotide-binding state of Goq, and the N-terminal region of flotillins was critical for the interaction. UTP, an agonist for the Gq-coupled P2Y receptor, activates mitogen-activated kinases (MAPKs) including p38 and ERK through Goq signal. Knockdown of flotillin-2 attenuated UTP-induced activation of p38 MAPK but not that of ERK1/2. The UTP-induced activation of p38 MAPK was inhibited by Src family kinase inhibitor PP2 and the cholesterol-depleting agent, methyl-β-cyclodextrin, which was used for disruption of lipid rafts [64]. Previously, we demonstrated that GPCR transduces the signal to p38 MAPK through Goq [69], and the GPCR-induced p38 MAPK activation depends on Src family kinase [70]. These results suggest that flotillins mediate Gq-p38 MAPK activation through Src family tyrosine kinase in lipid rafts [64]. Thus, the Gq-interacting proteins, caveolin and flotillins, may be scaffolding proteins in signal transduction of Gq (fig 2).

**Toxins as Pharmacological Tools**

The first bacterial toxins used for evaluating the molecular mechanisms of heterotrimeric G protein activation and adenyl cyclase-mediated signaling pathways were cholera toxin (CTX) and pertussis toxin (PTX). CTX acts on the α subunit of Gs and leads to constitutive activation of Goα [71, 72], whereas PTX acts on the heterotrimeric complex of Gi/o and leads to uncoupling with receptor [73, 74]. PTX were utilized for distinguishing a group of PTX-insensitive G protein [75]. One family of this group has been identified as the Gq family that stimulates PLC-β.

Until recently, there had been no specific modulating reagent available for studying the role of Gq proteins in hormonal communication and signal transduction. The toxin produced by *Pasteurella multocida* (PMT) and cyclic depsipeptide produced by *Chromobacterium* sp. QS3666 (YM-254890) can now be added to the list of bacterial-derived molecules that modulate Gq signaling (fig 2).

**P. multocida Toxin, PMT**

PMT is a bacterial protein toxin from the pathogen *P. multocida* [76–78]. In the field of cell biology, PMT is known to act on various types of cultured cells as a highly potent mitogen, causing anchorage-independent growth especially in some fibroblastic cells. PMT has been found to activate Gq-dependent PLC-β stimulation in Ca2+ and IP3 signaling [79–81]. Several investigators
have used PMT as a pharmacological tool for studying Gq-coupled PLC signaling [for review, see 82]. PMT seems to facilitate Gq coupling to PLC-β1, causing the same cellular responses elicited by Gq-coupled receptors. Wilson et al. [80] showed the intracellular target of PMT using the antibodies against the IP3-signaling proteins in Xenopus oocytes. The antibody against Gq/11 blocked PMT-induced Ca2+-dependent Cl− currents, whereas the antibodies against other Gs nor Gβ did not. They proposed that PMT might act on GDP-bound Gq/11, and convert it into an GTP-bound active form [82] (fig. 2). However, the molecular mechanism of the action of PMT is little known. Mouse knockout cell lines were used for confirming that PMT-induced formation of inositol phosphates was exclusively dependent on Gq, but not on Go11, Go12, or Go13 [83]. A recent study using a series of chimeras between Goq and Go11 in Goq/11-knockout cells identified a region in the helical domain of Goq, which is important for PMT-induced activation of PLC-β [84]. Substitution of Glu105 or Asn109 of Go11 in the helical domain with the corresponding His residues of Goq resulted in PMT-induced activation of PLC-β.

PMT has an ability to activate Rho [85] and MAP kinases, such as ERK and JNK [86], and induce tyrosine phosphorylation of focal adhesion kinases and paullin [87] in target cells. How PMT activates both PLC-IP3-signaling pathway and Rho-signaling pathway remained to be clarified.

One possibility was that PMT acts on the actin cytoskeleton through Rho-activation by Gq. Several laboratories have reported that RhoA is activated via signal pathway involving various types of G proteins, including G12/13 and Gq/11. Agonists that stimulate GPCRs coupled with both the Gq/11 and G12/13 families are able to activate RhoA even in the absence of Go12 and Go13 [88]. Moreover, the RhoGEF LARG has been shown to interact not only with G12/13 but also with Gq/11 [89]. As described previously, a novel guanine nucleotide exchange factor p63RhoGEF has been shown to be able to activate RhoA via Gq/11 [44]. Interestingly, this GEF protein appears to be specific for Gq/11 and not for G12/13. Now, it is possible that Gq activates RhoA via RhoGEFs.
PMT has been used for exploring the pathways that mediate interaction between endogenous Gq and Rho signaling. In COS-7 cells, Gq was co-immunoprecipitated with the Rho-guanine nucleotide exchange factor (Lbc), and Gq and Lbc synergistically activated serum response element (SRE)-dependent gene expression in a PLC- and Rho-dependent manner [90]. In this study, the ability of PMT to stimulate SRE-mediated gene expression synergistically with Lbc suggested that Gq is involved in Rho-GEF-regulated pathways. Although the stimulation of inositol phosphate signaling by PMT did not occur in Goq-deficient or Gq/Ga11-deficient cells, PMT could still induce other cellular responses, including Rho activation, Rho-dependent actin rearrangement and focal adhesion formation, in these knockout cells [91]. These results indicate that certain effects of PMT action may occur through other signaling pathways independent of Goq. Using a Gq/11-specific inhibitor YM-254890, and reconstitution of Go13 in Go12/13-deficient cells, it was demonstrated that PMT acts on RhoA through both Gq- and G12/13-signaling pathways [91].

PMT consists of a single polypeptide chain of 1,285 amino acids, and N-terminal region of the toxin binds to ganglioside-type receptors and enters cells via receptor-mediated endocytosis [92]. Recently the crystal structure of the C-terminal region (residues 569–1285) of PMT (C-PMT) has been determined [93]. C-PMT consists of three domains – C1, C2, and C3. Structural analysis indicated that C1 domain contributes to the membrane localization of C-PMT, and C3 domain organizes a cysteine protease-like catalytic triad that depends on redox state. Mutation analysis suggested that PMT may be an enzyme toxin with the Cys-His-Asp catalytic triad. More recently, the last 180 amino acids, which encompass the C3 domain, were defined as the minimum domain sufficient to stimulate both calcium-dependent and mitogenic signaling pathways [94].

Whether PMT directly or indirectly modulates Goq is unclear. It has been reported that PMT facilitates inositol phosphate formation by agonist stimulation through tyrosine phosphorylation of Goq [95]. However, Orth et al. [96] indicated that action of PMT is independent of phosphorylation of C-terminal tyrosine of Goq. PMT might affect Goq signal indirectly by modulation of GEF or RGS for Goq.

A Novel Type Gq-Specific Inhibitor, YM-254890
YM-254890 is a cyclic depsipeptide derived from culture broth of Chromobacterium sp. QS3666 and inhibits ADP-induced platelet aggregation with an IC50 value >0.4 μM [97, 98]. YM-254890 shows antithrombotic and thrombolytic effects [99–101]. This compound inhibits Ca2+ mobilization induced by ADP in platelet and in P2Y1-expressing cells. YM-254890 attenuates Ca2+ mobilization stimulated by several Gq/11-coupled receptors but not by Gi- and G15-coupled receptors [102], suggesting that YM-254890 is a specific modulator of Gq/11 activation. YM-254890 blocks the intracellular Ca2+ mobi-
lization and the SRF-mediated gene transcription in response to the Gq-coupled receptor. Moreover, the agonist-promoted GTPγS binding to heterotrimeric Gq/11 in crude cell membranes is inhibited by YM-254890 [102]. YM-254890 is an useful tool for analyzing G protein activation and Gq-mediated signaling events (fig. 2).

In the field of neurosciences, several investigators used YM-254890. Matsuo et al. [103] identified a novel Gq-coupled orphan GPCR, termed GPRg1, which is expressed preferentially in distinct regions of the central nervous system. Overexpression of GPRg1 in 293 cells activates SRF-mediated transcription, and the activation was completely inhibited by YM-254890. Morishita et al. [104] also used YM-254890 for investigating the involvement of Gq/11 signaling through endothelin-B receptor in neural progenitor proliferation. TWIK-related acid-sensitive K⁺ channel (TASK) subfamily of two-pore domain potassium channels contributes to the resting membrane potential in many neurons and regulates their excitability. Veale et al. [105] indicated that activation of M3 muscarinic receptors inhibits both TASK3 channels expressed in tsA-201 cells and standing outward potassium current (IK(SO)) in mouse cerebellar granule neurons through the activation of the Gq, because both effects were abolished by YM-254890.

The inhibitory mechanism of YM-254890 for Gqα at the molecular and atomic level is now being investigated. Crystal structure determination of Gqαβγ-YM-254890 complex should help for understanding of detailed molecular mechanism of G protein activation and deactivation.

**Physiological Function of Gq Signaling in Nerve System Development**

**Knockout Mice of Gqα Family Members**

Many studies have suggested the physiological significance of Gqα family members, especially Gqα and Ga11 that are widely expressed. Studies using knockout mice have contributed to elucidate the functions of G protein signaling in physiology and pathology.

Platelets express only Gqα among Gqα family members. Hence, platelets in Gqα knockout mice show the most pronounced phenotype. Gqα knockout mice have increased bleeding times and impaired platelet activation in response to thromboxane A₂ (TX2), ADP and thrombin, causing protection from thromboembolism [106]. Gqα and Ga11 are widely expressed in the central nervous system and induce the activation of PLC-β. The expression level of Gqα is 2- to 5-fold higher than that of Ga11 in most regions of the nervous system. Mice lacking Gqα develop an ataxia with clear signs of motor coordination deficits, and the defects could be observed in the cerebellar cortex [107]. These animals also exhibit slowed growth rates, likely due to impaired Gqα/11-dependent neurotransmitter signaling in the hypothalamic/pituitary axis, since mice with selective disruption of Gqα and Ga11 in neuronal and glial precursor cells display significant growth retardation in conjunction with somatotroph hypoplasia. On the other hand, mice homozygous for a null allele of the gene coding Gqα, Ga14, or Ga15 show no phenotypic abnormalities [108–110].

The first phase genetic studies of Gqα/11-dependent physiology were disturbed by embryonic lethality. Double homozygous null mutations for Gqα and Ga11 induce myocardial hypoplasia and embryonic lethality in engineered mice (embryonic day 10.5), and mice with one intact allele of either of the two genes die shortly after birth with cardiac malformation [110, 111].

Single gene knockout or cell-specific knockout approaches have provided the successful alternatives for studying Gqα and Ga11 in whole animals. Wettschureck et al. [112] generated the mice which lack the genes coding for the subunits of the two main members of the Gq/11 family, gnaq and gna11, selectively in neuronal and glial precursor cells using the Cre/loxP system under the nestin promotor. Interestingly, the Gqα/Ga11 deficiency in descendants of neuronal and glial precursors did not cause gross morphological abnormalities of the developing nervous system but was incompatible with postnatal survival.

**Function of Gq/G11-Mediated Signaling Pathways in Neural Progenitor Cells**

Many studies have established the physiological importance of the broadly expressed Gqα and Ga11. Many things regarding expression and signaling of these proteins under various conditions and in numerous cell types have been uncovered. However, this review cannot comprehensively summarize all of the contributing works. In the nervous system, Gqα and Ga11 are widely expressed, and are involved in multiple pathways that modulate neuronal function, such as modulation of synaptic transmission. In this review, we summarize the function of Gq especially in neural progenitor cells (NPCs). Pituitary adenylate cyclase-activating polypeptide (PACAP) is a pleiotropic neuropeptide that belongs to the...
Endothelin (ET) is known as vasoactive peptides that comprise three isoforms, ET-1, ET-2, and ET-3. These peptides bind to endothelin A (ETA) and endothelin B (ETB) receptors with different affinities [116]. In the peripheral nervous system, the ETB receptor and ET-1 are important for neural crest development [117]. Shinohara et al. [118] have shown that ETB receptor stimulation increases neural progenitor proliferation, partly in a Gi- and extracellular matrix-dependent manner. They investigated whether Gq/11 is involved in this effect [104]. An inhibitor of Gq/11, YM-254890, as well as pertussis toxin, partially inhibited ET-stimulated phosphorylation of Raf-1 and ERK. ET-induced PKC activation was partially inhibited by both YM-254890 and pertussis toxin. ET increased tyrosine phosphorylation of focal adhesion kinase (FAK) and paxillin in an integrin ligand-dependent manner. Both YM-254890 and PTX partially inhibited the phosphorylation of these proteins. In addition, ET-induced proliferation and DNA synthesis were also partially inhibited by YM-254890 and PTX. These results indicate that ET activates PKC via Gq/11 and Gi, and consequently stimulates the ERK cascade, resulting in induction of neural progenitor proliferation.

Neural stem cells are generated in the ventricular zone (VZ) in the developing brain. In the developing cerebral cortex, neural stem cells differentiate into more committed progenitor cells and migrate from VZ to superficial layers of the cortical plate (CP) along the fibers of radial glial cells [119]. This radial migration is an important process for the correct lamination of the cerebral cortex.

Some reports indicated that G protein signaling is involved in development of cerebral cortex by modulating cell migration. GABA<sub>B</sub> receptor, which is a member of GPCR, influences migration from the intermediate zone (IZ) to the CP during development [120, 121]. Stromal cell-derived factor-1 (SDF-1) and its receptor CXC chemokine receptor 4 (CXCR-4) regulate tangential migration of interneuron precursors in the developing neocortex. In the SDF-1- and CXCR-4-deficient mice, late-generated interneurons fail to integrate into the appropriate neocortical layer [122]. Whether Gq family members are involved in these GPCR-mediated signaling pathways remains to be clarified.

We previously found that the ETB receptor transduced the signal for inhibition of NPC migration through the Gq and JNK pathway in vitro and in vivo [123]. The physiological function of the stop signal in radial migration has been suggested recently. The new mode radial migration has been revealed by time-lapse imaging studies in slices of the developing neocortex [124, 125]. This new mode of the radial migration is that migrating cells do not move straight toward the CP, but change the migration rate and their morphology in the IZ. They become highly multipolar, and then become bipolar again as they progress through the IZ and enter the CP. It appears that the multipolar cells with the low migration rate search the signals that will determine whether the cells migrate radially or tangentially. Although the regulatory mechanism of the multipolar migration is obscure, the Gq- and JNK-mediated signals may be involved in the multipolar migration.

More recently, mutations of an orphan GPCR, GPR56, which is preferentially expressed in NPCs, were found in patients with brain cortex malformation [126], suggesting that human GPR56 is essential for proper lamination during brain development. Moreover, it is reported that GPR56 interacts with the transmembrane scaffolding proteins tetraspanins CD9 and CD81, and associates with Gq and G<sub>q11</sub> [127]. We found that GPR56 negatively
regulates the NPC migration through a G12/13 and Rho pathway [128]. Although an agonistic antibody for GPR56 induced Rho activation, but not intracellular Ca²⁺ mobilization, the involvement of Goq/11 in the GPR56 action on NPCs remains to be investigated.

The outcome of Gq/11-conditional knockout mouse suggests that Goq/Ga11-mediated signaling is crucial for the postnatal function of the nervous system but not for the prenatal development. The function of Goq seems to be not essential for proliferation, differentiation, and migration of NPCs. In our data, C-terminal peptide of Goq (Gq-ct), which inhibits Goq-GPCR coupling specifically, blocked the inhibitory effect of ET, but did not affect the migration. Furthermore, Gq-ct did not inhibit the radial migration in slice culture. Excess signaling of Gq, e.g. high concentration of ligand, or constitutively active Goq, inhibits the radial migration, but Gq signal may regulate the moderate speed of migration in vivo. Moreover, GPCRs couple with multiple members of G protein family. It has been reported that Gi signal also modulates differentiation, proliferation and migration of NPCs. The crosstalk of Gq signaling with other G protein family signaling or other signaling may be important in several functions of NPCs.

References


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