Review

Strike a pose: $G\alpha_q$ complexes at the membrane

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The heterotrimeric G protein $G\alpha_q$ is a central player in signal transduction, relaying signals from activated G-protein-coupled receptors (GPCRs) to effectors and other proteins to elicit changes in intracellular Ca^{2+}, the actin cytoskeleton, and gene transcription. $G\alpha_q$ functions at the intracellular surface of the plasma membrane, as do its best-characterized targets, phospholipase C-β, p63RhoGEF, and GPCR kinase 2 (GRK2). Recent insights into the structure and function of these signaling complexes reveal several recurring themes, including complex multivalent interactions between $G\alpha_q$, its protein target, and the membrane, that are likely essential for allosteric control and maximum efficiency in signal transduction. Thus, the plasma membrane is not only a source of substrates but also a key player in the scaffolding of $G\alpha_q$-dependent signaling pathways.

Signaling by heterotrimeric G protein α subunits

The proteins involved in GPCR signaling pathways are essential for vision, blood pressure regulation, cardiac contractility, and numerous other processes [1]. Activation of a GPCR by an extracellular signal, such as a photon, hormone, or drug, induces conformational changes that allow the GPCR to function as a guanine nucleotide exchange factor (GEF) for heterotrimeric G protein α subunits (Gα) [2], promoting the binding of GTP by Gα. Once activated, Gα·GTP dissociates from the heterodimeric Gβγ subunits and interacts with downstream effectors at the plasma membrane to elicit changes in the concentration of second messengers such as inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) (Figure 1) [3].

The Gα subunit consists of a Ras-like domain with a large α-helical domain insertion [3]. The guanine nucleotide binding pocket is formed at the interface of the Ras-like and α-helical domains, where three switch regions (SwI–III) adopt unique conformations dependent on the identity of the bound nucleotide. In the GTP-bound state, the switches help coordinate the catalytic Mg^{2+} ion, participate in catalysis, and contribute to the effector binding site (Figure 2A,B). In the GDP-bound state, SwII contributes to the Gβγ-binding site [3]. The N terminus of Gα is lipid-modified (e.g., myristoylated and/or palmitoylated) and can form an extended helix that interacts with GPCRs [2],Gβγ subunits [4,5], and some effectors [6].

The focus of this review is on the complexes formed by activated $G\alpha_q$, which represents one of the four canonical subfamilies of Gα subunits ($G\alpha_s$, $G\alpha_{i/o}$, $G\alpha_{q/11}$, and $G\alpha_{12/13}$). The other members of this subfamily include $G\alpha_{11}$, $G\alpha_{14}$, and $G\alpha_{15/16}$. $G\alpha_q$ subfamily members are activated by GPCRs that respond to hormones and neurotransmitters such as norepinephrine, endothelin, and glutamate [7], and consequently play important roles throughout the cardiovascular and nervous systems. Classically, $G\alpha_q$ activates phospholipase C-β (PLCβ), which hydrolyzes phosphatidylinositol-4,5-bisphosphate (PIP_2) to generate the second messengers inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG), leading to increases in intracellular Ca^{2+} and activation of protein kinase C [8,9]. More recently, it was discovered that $G\alpha_q$ also activates the GEF p63RhoGEF (ARHGEF25) and its homologs Trio and Duet [10–13], thereby linking GPCRs to the activation of RhoA and control of the actin cytoskeleton. In addition, $G\alpha_q$ binds to GPCR kinase 2 (GRK2) [14], an enzyme that initiates homologous desensitization by phosphorylating activated GPCRs [15]. However, GRK2 is not activated via binding to $G\alpha_q$. Instead, it seems to sequester $G\alpha_q$ from its other targets in a form of phosphorylation-independent desensitization (Figure 1) [16–19]. Not all members of the $G\alpha_q$ subfamily interact with these targets in the same manner. $G\alpha_{16}$ does not bind appreciably to GRK2 [20], and binds to but does not activate p63RhoGEF [21].

Structural and functional studies of activated $G\alpha_q$ in complex with PLCβ3 [6,22], p63RhoGEF [11], and GRK2 [14] reveal some general themes. PLCβ, p63RhoGEF, and GRK2 all must interact with the membrane and, in some cases, with other peripheral membrane proteins for optimal catalytic efficiency. A specific orientation of these enzymes with respect to the membrane plane is essential for their function. All three enzymes engage the effector-binding site of $G\alpha_q$ using exposed hydrophobic residues on helical elements. Unexpectedly, comparison of the likely membrane-bound orientation of $G\alpha_q$ in complex with PLCβ, p63RhoGEF, and GRK2 reveals that $G\alpha_q$ adopts a similar orientation with respect to the membrane plane in each assembly (Figure 3). It is also clear that the membrane itself plays one or more active roles in the function of these complexes: it can enhance the affinity

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Keywords: phospholipase Cβ; p63RhoGEF; ARHGEF25; GRK2; G-protein-coupled receptor; heterotrimeric G protein signaling; $G\alpha_q$; second messengers; phospholipids; membrane; protein structure; crystallography.

0165-6147/$ – see front matter
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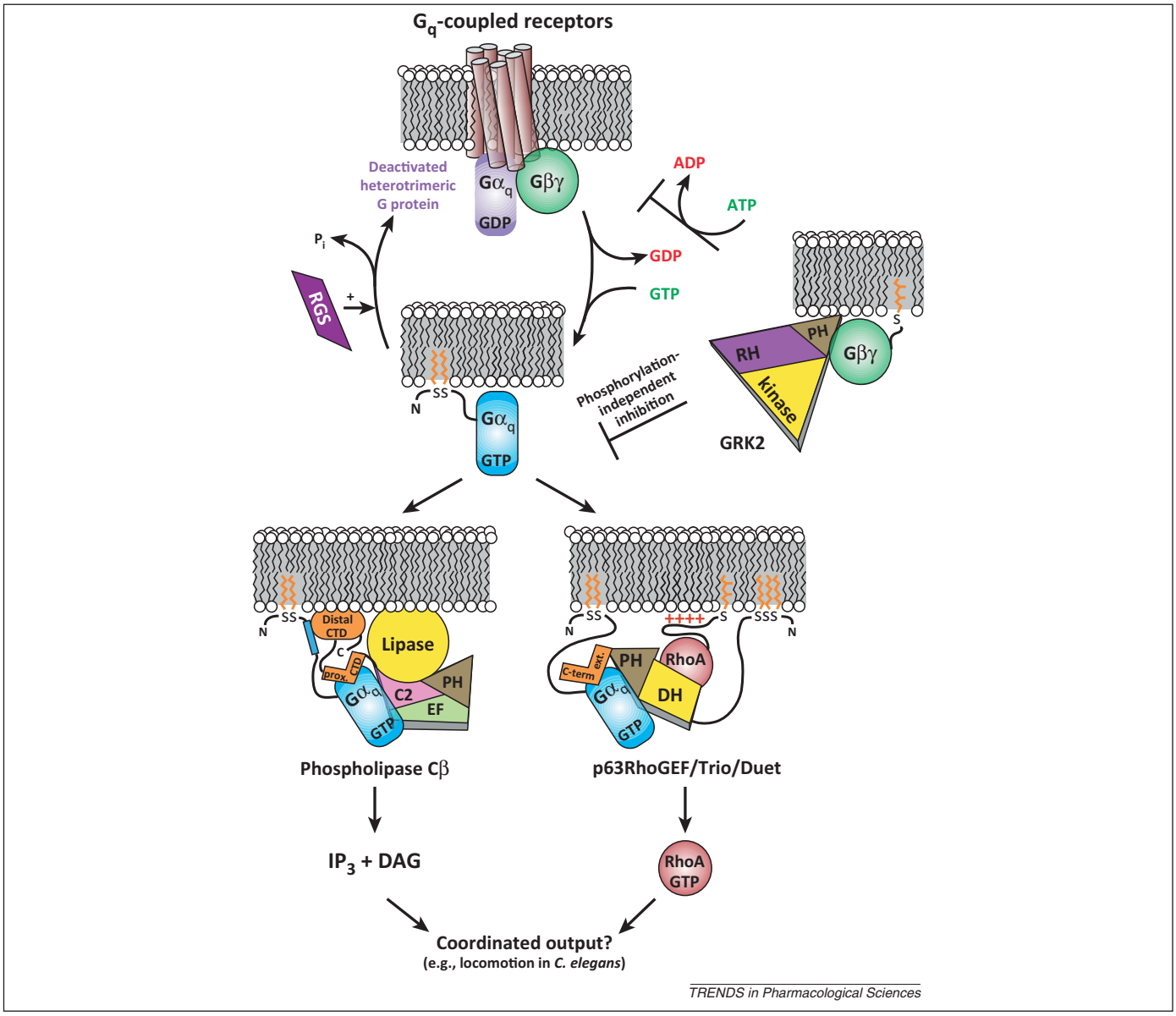

Figure 1. Interplay of $G\alpha_q$-mediated signaling processes. G-protein-coupled receptor (GPCR) activation catalyzes the binding of GTP to the $G\alpha_q$ subunit, leading to its activation and dissociation from the receptor and the $G\beta\gamma$ heterodimer. $G\alpha_q$·GTP then interacts with phospholipase C-β (PLCβ) or p63RhoGEF, leading to production of the second messengers inositol-1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) or activation of RhoA, respectively. GPCR kinase 2 (GRK2) mediated phosphorylation of G_q-coupled receptors initiates homologous desensitization, and GRK2 binding to $G\alpha_q$·GTP sequesters it from its downstream targets, resulting in phosphorylation-independent desensitization. Meanwhile, RGS proteins catalyze the hydrolysis of GTP on $G\alpha_q$, leading to its deactivation, and can act on $G\alpha_q$ while it is in complex with p63RhoGEF or GRK2 [50], or block its interactions with PLCβ. In addition to a helix–turn–helix motif (orange elbow), $G\alpha_q$ must interact with multiple domains of PLCβ and p63RhoGEF for full functionality. In the case of PLCβ, the N-terminal helix (blue rectangle) and the Ras-like domain of $G\alpha_q$ contact the distal C-terminal domain (CTD, orange), and the C2 and EF hand domains of the catalytic core, respectively. In the case of p63RhoGEF, the Ras-like domain and C-terminal helix of $G\alpha_q$ bridge the Dbl homology (DH) and pleckstrin homology (PH) domains of p63RhoGEF. $G\alpha_q$ must also simultaneously interact with the membrane via palmitoylation sites at its N terminus. Disordered regions within the proteins, primarily those bearing the lipid modifications of $G\alpha_q$, $G\beta\gamma$, p63RhoGEF, and RhoA to the membrane, are shown as black lines. Palmitoyl and geranylgeranyl groups are modeled as orange sticks embedded within the membrane.

of signaling components by co-localization, serve as a scaffold for the assembly of high-order assemblies, and/or optimize the orientation of these proteins for maximum signaling efficiency.

Scaffolding interactions in the activation of PLCβ by $G\alpha_q$

PLCβ enzymes comprise two major regions: a catalytic core responsible for PIP_2 hydrolysis and a ~400-residue C-terminal extension required for $G\alpha_q$ regulation and membrane association (Figure 3A,B). The catalytic core is conserved across all PLC isozymes [23] and contains an autoinhibitory element called the X–Y linker [24], which is immediately adjacent to the active site. This linker is poorly conserved but contains highly acidic stretches of amino acids in human PLCβ isoforms. In all PLCβ crystal structures reported to date, the C-terminal residues of the linker are ordered and occlude the active site [9]. It has been proposed that this linker is dislodged by electrostatic repulsion when the PLCβ catalytic core is brought in close proximity to the negatively charged inner leaflet of the plasma membrane [24]. Also adjacent to the active site is the so-called hydrophobic ridge, a series of loops with exposed hydrophobic

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