**Gαi protein subunit: A step toward understanding its non-canonical mechanisms**

Soraya Villaseca, Gabriel Romero, María J. Ruiz, Carlos Pérez, Juan I. Leal, Lina M. Tovar and Marcela Torrejón*

Laboratory of Signaling and Development, Group for the Study of Developmental Processes (GDeP), Department of Biochemistry and Molecular Biology, Faculty of Biological Sciences, University of Concepción, Concepción, Chile

The heterotrimeric G protein family plays essential roles during a varied array of cellular events; thus, its deregulation can seriously alter signaling events and the overall state of the cell. Heterotrimeric G-proteins have three subunits (α, β, γ) and are subdivided into four families, Gαi, Gα12/13, Gαq, and Gαs. These proteins cycle between an inactive Gα-GDP state and active Gα-GTP state, triggered canonically by the G-protein coupled receptor (GPCR) and by other accessory proteins receptors independent also known as AGS (Activators of G-protein Signaling). In this review, we summarize research data specific for the Gαi family. This family has the largest number of individual members, including Gαi1, Gαi2, Gαi3, Gαo, Gαt, Gαq, and Gαz, and constitutes the majority of G proteins α subunits expressed in a tissue or cell. Gαi was initially described by its inhibitory function on adenylyl cyclase activity, decreasing cAMP levels. Interestingly, today Gαi family G-protein have been reported to be importantly involved in the immune system function. Here, we discuss the impact of Gαi on non-canonical effector proteins, such as c-Src, ERK1/2, phospholipase-C (PLC), and proteins from the Rho GTPase family members, all of them essential signaling pathways regulating a wide range of physiological processes.

**KEYWORDS**
heterotrimeric G-protein, signaling, migration, asymmetric cell division, cell polarity

**Introduction**

Heterotrimeric G-proteins are the biggest signaling cores, acting as molecular switches that control the movement of information resulting from a variety of extracellular cues to the several intracellular effectors that control cell behavior (Gilman, 1987; Morris and Malbon, 1999). The heterotrimeric G-proteins are constituted by three subunits (Gα, Gβ, and Gγ) that cycle between a Gα GDP-bound form/Gβγ (OFF state) and a Gα GTP-bound form dissociated from the Gβγ dimer (ON state) (Birnbaumer, 2007). This change between the OFF and ON states is due to a conformational change in the intracellular portion of the protein after the binding of a specific ligand in the extracellular domain of the GPCR (G protein coupled receptor) (Goldsmith and Dhanasekaran, 2007). The active species (Gα-GTP and Gβγ dimer) can...
also interact with different effector proteins, regulating different signaling pathways (Sato et al., 2006). In addition, the activity of Ga subunits are regulated mainly through three classes of proteins: 1) GTPase activating proteins (GAPs) are negative regulators of G protein signaling, 2) guanine nucleotide-dissociation inhibitors (GDIs) inhibit dissociation of GDP from Ga subunits, and 3) guanine nucleotide-exchange factors (GEFs) that can induce guanine nucleotide exchange (GDP for GTP), activating the Ga subunit itself (Jaffe and Hall, 2005; Guilluy et al., 2011). Ga protein families are classified by their Ga subunits and are grouped into four families by their sequence and functional similarities: Gas/olf, Ga/o/z, Gaq/11, and Ga12/13 (Wilkie et al., 1992; Krishnan et al., 2015; Downes and Gautam, 1999).

Ga is the largest and most diverse family of Ga subunits and includes Ga1, Ga2, Ga3, Gao, Gat, Gag, and Gaz, all sensitive to pertussis toxin (PTX) (Malbon, 2005). Three Ga isoforms have been described in mammals, including Ga1, Ga2, Ga3, best known as “the inhibitory Ga subunits,” suppressing adenylyl cyclase activity, resulting in decreased intracellular cycle-AMP (cAMP) levels (Figures 1A,B) [Wong et al., 1991; Xiao et al., 1999; Pines et al., 1986]. As we shall see in this Review, despite that Ga subunits are generically classified by their ability to “inhibit adenylyl cyclase,” these three isoforms have other cAMP-independent functions. Ga proteins were first described localized exclusively at the plasma membrane, although cell fractionation and immunofluorescence studies, were key tools demonstrating that a fraction of Ga proteins were intracellularly identified and even free of Gβγ, suggesting the existence of non-canonical signaling pathways for Ga subunits (Stow et al., 1991; Schurmann et al., 1992; Pimplikar and Simons 1993; Wilson et al., 1993; Muller et al., 1994; Montmayeur and Borrelli 1994; Ogier-Denis et al., 1995; Maier et al., 1995; Denker et al., 1996; Lin et al., 1998; Marrari et al., 2007). Supporting this intracellular location for Ga subunits, we found studies by fluorescence and EPR (Electron Paramagnetic Resonance), showing that the

FIGURE 1
Ga canonical signaling network through receptor activation (A,B) and non-canonical activation (C). (A) The upper panel shows the G-protein canonical signaling activation by the GPCR GEF activity, generating two active species (Ga-GTP and Gβγ released). The intrinsic GTPase activity of the Ga subunit hydrolyze GTP to GDP, promoting its re-association with Gβγ. (B) Canonical signaling pathway of Ga, which is inhibitory of Adenylyl Cyclase (AC), and Gβγ activates MAPK signaling cascade. (C) The non-canonical activation signaling. During this process, Ga interacts with cytosolic GEFs such as Ric-8 or GIV which stimulate the GDP/GTP exchange, activating Ga in a receptor-independent manner potentiating the canonical signal or activating non-canonical effectors.
myristoylated amino terminus, a key lipid post-translational modification, sign for this Gαi family, present and intramolecular interaction with its surface in the GaGTP-bound state (Preininger et al., 2003). Gai membrane location is mediated by two lipid modification, myristoylation and palmitoylation at the amino terminus. Gai family is the only family that is myristoylated, in contrast with the other Ga (Gas, Gai12/13, and Gaq) families that are only palmitoylated, both lipid modifications are important to allow interaction with the membrane, in order to interact with their receptor and with their membrane effectors (Chen and Manning, 2001; Morales et al., 1998; Fishburn et al., 1999). Myristoylation has been also found important for palmitoylation of Gai (Dunphy et al., 1996). Thus, for Gai non-canonical functions that required an intracellular location, Gai may rearrange its structure hiding its lipid modification increasing its solubility, thus allowing interaction with other non-membrane effectors (Preininger et al., 2003).

Within the non-canonical function for Gai proteins, we found a critical role on Golgi structures and function (Jamora et al., 1999; Yamaguchi et al., 2000), as well as controlling steroids receptors and tyrosine kinase receptor signaling pathway (Kreuzer et al., 2004; Kumar et al., 2007), and regulating the mitotic spindle positioning during asymmetric cell division by interacting with GPR/GoLoco proteins (Yu et al., 2000; Gotta and Abririner 2001; Parmentier et al., 2000; Schaefer et al., 2001), the latest suggesting an important role during early development. Furthermore, studies using PTX to inhibit Gai mediated signaling and recently by gene targeted (Figure 1A), Group II has GPR motifs that stabilize the GDP-bound state (Preininger et al., 2003). Gai2 control macrophage and T lymphocyte migration in a GPCR-dependent and independent manner (Wiege et al., 2012; Hwang et al., 2017). The depletion of Gai2 contributes to inflammatory bowel disease, and Gai3 is needed to block the insulin antiautophagic action in mouse liver whereas deletion of both Gai2 and Gai3 in mice leads to death in utero (Gohla et al., 2007).

Although, the canonical activation of Ga subunits through ligand-GPCR complexes (canonical GEF activity) (Figure 1A) is well-established (Pierce et al., 2002), Ga subunit activity could also be regulated by several receptor-independent G protein activators (AGS: activators of G-protein signaling) classified into three groups: group I activates the Gαi/o subunit as a guanine exchange factor (Figure 1C), Group II has GPR motifs that stabilize the GDP-bound conformation of the Ga subunit (Cismowski et al., 2001), and group III bind Gβγ to dissociate it from the Ga subunit (Blumer et al., 2006a). Group I and II has been implicated in Gαi AMPc-independent signaling pathways controlling mitotic spindle dynamics during asymmetric cell division, polarity, growth, differentiation, and pathological processes, such as cancer (Feigin and Muthuswamy, 2009). Understanding how Gai controls cell polarity is essential to develop new strategies to impair cancer progression, treat developmental defects, and tissue regeneration. Therefore, in this review, we discuss and summarize the implications of non-canonical pathways of Gai proteins during cell polarity and several biological processes, such as proliferation, survival, tissue differentiation and cell migration.

**Gai regulates asymmetric cell division**

Several genetic studies in *Caenorhabditis elegans* embryos and *Drosophila neuroblasts* have reported that Gai and Gαo subunits regulate apicobasal cell polarity in a receptor-independent manner (Bellaiche and Gotta, 2005; Siderovski and Willard, 2005).

A variety of biological processes, such as asymmetric cell division and tissue morphogenesis require an apicobasal polarity, thus its alteration contributes to multiple diseases, including cancer (Feigin and Muthuswamy, 2009; Hirose et al., 2006; Knoblich, 2010). During asymmetric cell division, a non-canonical signaling pathway has been described for the Gai subunit in which it regulates microtubule function during mitotic spindle positioning (Figure 2A). Asymmetric cell division has been studied in *C. elegans* one-cell embryo (Figure 2B), *Drosophila* embryonic neuroblasts (Figure 2C), and *Drosophila* sensory organ precursors, where the correct placement and asymmetry of the spindle give rise to daughter cells of different sizes and cellular function (di Pietro et al., 2016). Specifically, in *C. elegans*, asymmetric cell division is controlled by a protein complex that associate with the astral microtubules generating an imbalance in cortical forces thus asymmetrically positioning the mitotic spindle (Grill et al., 2001). Indeed, evolutionary conservation of a molecular complex composed of Gai subunit from heterotrimeric G protein, leucine-glycine-asparagine (LGN), dynein/dynactin complex and nuclear and mitotic apparatus (NuMA) (respectively Gai, partner of inscutable (Pins), and mushroom body defect (Mud) in *Drosophila*, and guanine nucleotide-binding protein G (α) subunit (GOA-1)/G protein α subunit (GPA-16), G protein regulator 1/2 (GPR-1/2), and spindle apparatus lin-5 (LINS) in *C. elegans*) is localized in a specific subcortical domain leading the recruitment of dynein (motor protein), which also determines the movement along astral microtubules and generates pulling forces to orintate the spindle correctly (Figure 2A) (di Pietro et al., 2016; Kiyomitsu, 2019; Poon et al., 2019). The Gai subunit plays a crucial role during this process since inactivation of GOA-1 and GPA-16, as well as GPR-1/2 and Lin-5 result in a strongly reduced and symmetric pulling force (Figures 2A,B) (Kiyomitsu, 2019; Poon et al., 2019; Colombo et al., 2003; Gotta et al., 2003; Srinivasan et al., 2003).

In *Drosophila melanogaster* neuroblasts (NB), after delamination from the epithelium, the apically localized partitioning-defective 3 (Par3)/atypical protein kinase C (aPKC)/Par-6 complex recruits Inscutable (Insc) to drive
apicobasal polarity at the first asymmetric cell division (di Pietro et al., 2016). Gai and Pins (the homologue of GPR-1/2), both recruited by Insc are also required for apical basal orientation of the mitotic spindle; therefore, Insc is a critical protein for asymmetric localization of G\(\alpha\)i and its binding partner, Pins (Figure 2C) (Schaefer et al., 2001). In contrast, mammalian G\(\alpha\)i as well as LGN and NuMA are crucial for the interaction between astral microtubules and cell cortex (Figure 2D) (Du and Macara, 2004; Woodard et al., 2010). Interestingly, in addition to Gai/Pins, G\(\beta\)\(\gamma\) is also involved in the control of spindle asymmetry in Drosophila NB (Fuse et al., 2003; Yu et al., 2003), and the overexpression of Gai or G\(\beta\)\(\gamma\) in NB leads to large or small symmetrical spindle formation, respectively (Fuse et al., 2003). However, considering that Pins acts as GDI for Gai, by dissociating Gai from G\(\beta\)\(\gamma\), the mechanism by which G\(\beta\)\(\gamma\) acts upstream of Gai/Pins is not yet clear. Some studies have shown that LGN interacts with GDP-bound Gai, and the interaction stability is regulated by resistance to inhibitor of cholinesterase 8 (Ric-8) (Figure 1C), a chaperon and non-canonical GEF for the Gai subunits (David et al., 2005). Accordingly, during asymmetric cell division, in order to form the Gai/GPR-1/2 complex, Ric-8, by its GEF activity, is needed to allow Gai subunit go through one round of GTP hydrolysis, therefore, Ric-8 mutants display several defects leading to a loss in the asymmetry of the spindle mitotic positioning (Hampoelz et al., 2005). Interestingly, Ric-8 has a crucial role during this process as its absence also disrupts the localization of Gai, Lin-5, GPR-1/2, and dynein (Woodard et al., 2010), suggesting a probably scaffold protein function (Toro-Tapia et al., 2018; Gabay et al., 2011; Klattenhoff et al., 2003). Correspondingly, in C. elegans, Ric-8 is essential to localize GPA-16 at the cell membrane by directly interacting with it (Afshar et al., 2005). As well as, in Drosophila, Ric-8, in addition to its GEF function on Gai, is also required for Gai plasma membrane localization, probably acting as a scaffold protein (Wang et al., 2005; Hampoelz et al., 2005; David et al., 2005).

On another hand, we found several examples supporting the essential role for Ric-8 and Gai controlling cell division. Gai
isomers (Gai1-3) and regulator of G protein signaling 14 (RGS14) (a GAP for Gai proteins) have been described to be localized at the centrosome in non-polarized HeLa cells (Figure 2D) (Cho and Kehrl, 2007), as well as, RGS14-Gaii-GDP-Ric-8A complex in mouse brain (Cho and Kehrl, 2007; Velanno et al., 2011). Another GEF for Gai, Girdin (GIV) (Figure 1C), has been also found regulating other polarity processes. Specifically, GIV and Gaii are regulated by Par-3, which interacts with GIV, inducing tight junction formation and apical domain development, thus, promoting apicobasal polarity (Sasaki et al., 2015).

Together all these results raise the question, which is the specific role that Gai plays during asymmetric cell division. For one side, as was mention above Gai by its myristylation is localized at the plasma membrane (Chen and Manning, 2001; Morales et al., 1998), thus localizing the Gai, LGN, dynemin/dynactin complex and NuMA at the membrane, where together with Ric-8A, this latter acting as a GEF and scaffold protein, allowing the cycle between GDP-GTP that later by RGS and Gaii intrinsic GTPase activity hydrolyzes GTP to GDP, prompting the interaction between astral microtubules and cell cortex (di Pietro et al., 2016) (Figure 2A). Indeed, the association of Gai with the spindle microtubules suggests that the G-protein subunit may regulate the assembly and disassembly of mitotic spindles by controlling microtubule assembly/dynamics. This insight, although not yet fully understood, provides a rational basis to understand the mechanism by which Gai contributes to other biological processes through the control of microtubule dynamics and polarity establishment.

During development, symmetric cell division allows the cells to be cloned, whilst during asymmetric cell division different cells are originated in other to accomplish different functions. The last process, also contributes during adult life, specifically in physiological events like wound healing and tissue regeneration, cell differentiation, immune response and diseases, such cancer (Mascré et al., 2012; Aragona et al., 2017). Therefore, Gai family and fate determinants are crucial to induce asymmetric cell divisions in order to create multiple type of cells contributing to tissue and organism diversity.

**Gai regulates growth factor signaling pathways**

As mentioned above, Gai protein subunits were originally characterized by their ability to inhibit adenyl cyclase activity, and epinephrine, acetylcholine, dopamine, and serotonin have been used to stimulate physiological responses through Gai protein subunits (Kindt et al., 2002; Heubach et al., 2004; Watts et al., 1998; Gou et al., 2010). Upon GPCR-ligand binding, Gai proteins are activated and released from Gβγ subunits, which can now indirectly interact with PI3K, leading to the activation of several downstream effectors (Bondeva et al., 1998; Schwindinger and Robishaw, 2001; Zhang et al., 2015; Nůsková et al., 2021) (Figures 3A,B). Several studies have described Gβγ-PI3K signaling, specifically in the chemotaxis context, (Haastert, and Devreotes, 2004), although, few described Gai association with PI3K, as we will describe below (Zhang et al., 2015; Nůsková et al., 2021). PTX ADP-ribosylates Gai protein subunits at their C-terminus preventing their interaction with GPCRs and has been used as a molecular tool to determine the multiple cellular processes where Gai proteins are involved, acting as signal transducer for GPCRs at the plasma membrane (Marrari et al., 2007). However, Gai proteins are also found intracellularly, suggesting the possibility that these proteins perform cytoplasmic functions (Preininger et al., 2003; Marrari et al., 2007, Koelle, 2006). It has been described in mammals, the Src family tyrosine kinase members, acting as targets of Gai proteins upon activation of GPCR, RTK, and non-RTK proteins (Ma et al., 2000), suggesting a crosstalk between Gi proteins and tyrosine kinase signaling pathways (Natarajan and Berk 2006). Accordingly, in vitro and in vivo assay demonstrated that c-Src tyrosine kinase interacts and is activated by the active conformation state of both Gai and Gas (Ma et al., 2000). Likewise, c-Src phosphorylates several Ga proteins included Gai and Gas on tyrosine residues enhancing G-protein function (Hausdorff et al., 1992). On another hand, Gai proteins control cell proliferation, growth, migration, and survival by interacting with downstream effectors from receptor tyrosine kinases (RTKs), such as epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), fibroblast growth factor receptor (FGFR), and keratinocyte growth factor receptor (KGF), activating the phosphatidylinositol 3-kinase- protein kinase B-mammalian target of rapamycin complex 1 (PI3K-AKT-mTORC1) pathway (Figure 3A) (Cao et al., 2009; Liu et al., 2018; Zhang et al., 2015; Wang et al., 2014; Sun et al., 2018; Marshall et al., 2018). Indeed, Cao et al. (2009) showed that in response to epidermal growth factor (EGF), loss of Gai1 and Gai3 proteins inhibited cell proliferation and survival by decreasing the levels of cyclin D and by its mean decreasing the phosphorylation of AKT and mTORC1, thus impairing the interaction with downstream targets, such as glycogen synthase kinase 3 (GSK-3), forkhead box O (FoxO) transcription factor, eukaryotic translation initiation factor 4E (eIF4E)-binding protein (4E4E-BP1), and ribosomal protein S6 (S6).

In the canonical heterotrimeric G-protein signaling pathway, Gi heterotrimers are activated by the GPCR, Gai and Gβγ subunits are released, acting synergistically or in opposition towards tyrosine kinase signaling yields. On another hand, non-receptor GEFs, including GIV (Girdin), for Gai have been discovered (Garcia-Marcos et al., 2009) (Figure 1C). In the cell migration during wound healing, macrophage chemotaxis, and tumor cell metastasis, all cellular processes triggered by growth factors (EGF and insulin), required the activation of Gai by GIV, showing the need of Gai-GTP state.
in order to activate downstream processes (Ghosh et al., 2008). In addition, GIV overexpression promotes colon and breast cancer metastasis (Jiang et al., 2008; Ling et al., 2011; Garcia-Marcos et al., 2011). Indeed, GIV directly interacts with both Gαi and EGFR, increasing EGFR autophosphorylation and extending its association to the membrane, thus activating PI3-kinase-AKT and PLCγ1 signaling pathways (Garcia-Marcos et al., 2011). Accordingly, in GEF-deficient-GIV mutant cells, Gai–GIV–EGFR complex cannot be assembled, EGFR autophosphorylation is reduced, and mitogenic signals, such as ERK1/2 and Src, are amplified, triggering cell proliferation (Ghosh et al., 2010). Together these evidences, show the crucial role for Gai-GTP state, indeed Gβγ free, triggered either by receptor-GEF activation or GIV-activation in order to trigger these processes.

As we have been discussing here, Gai plays a crucial role in growth factor signaling. Indeed, brain-derived neurotrophic factor (BDNF)-TrkB (tropomyosin-related kinase) receptor

FIGURE 3
Gai non-canonical signaling network models that show regulation of cell proliferation (A), cell migration in immune system (B) and cancer (C). There are multiple integrated pathways and crosstalk between Gi and tyrosine kinases signaling pathways regulating cell proliferation, growth, migration, and survival. All processes induced upon activation of receptor tyrosine kinases (RTKs), including epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), vascular endothelial growth factor receptor (VEGFR), and keratinocyte growth factor receptor (KGFR), activating PI3K-AKT pathway. RTK upon activation by its ligand is coupled to Gai by an unknown mechanism, promoting the activation of PI3K-AKT signaling by Gai/Gab interaction (A). On another hand, CXCR4 (C-X-C chemokine receptor type 4) activated by SDF-1 is coupled to Gai, promoting the activation of PI3K-AKT signaling accumulating D3-phosphoinositol lipids (B). Finally, during invasive migration in breast cancer cells, Ga2 in response to the same ligand, SDF-1, regulates the activation of Rac proteins through Elmo1/Dock180 interaction, contributing on the actin polymerization and migration (C).
signaling, that mediate activity-dependent dendrite formation, via PI3K and mitogen-activated protein kinase (MAPK) (Dijkhuizen and Ghosh, 2005), is also regulated by Gai proteins (McAllister et al., 1996; O’Neill et al., 2017; Marshall et al., 2018). In hippocampal neurons, Gai1 and Gai3 knockdown significantly reduces BDNF signaling and disrupts dendrite morphology, producing larger depressive behavior effects, demonstrating that both G-proteins are essential for TrkB receptor signaling and brain function (Marshall et al., 2018).

Finally, Gai proteins have been also involved in the activation of PI3K-AKT-mTORC1 signaling by Gab1 (growth-factor receptor binding 2 [Grb2]-associated binding protein 1) in response to EGF (Cao et al., 2009; Liu et al., 2018; Zhang et al., 2015; Li et al., 2016; Nůsková et al., 2021) (Figure 3A). Therefore, Gai proteins can control cellular growth, proliferation, and migration by modulating signaling through RTKs. For instance, cell proliferation and migration induced by keratinocyte growth factor- (KGF) and EGF are impaired upon Gai1 and Gai3 knockdown (Cao et al., 2009; Zhang et al., 2015). Accordingly, cells that are proliferating, such as wounded human skin or cancer cells, display an increase in the expression of Gai1/3 (Zhang et al., 2015; Liu et al., 2018; Nůsková et al., 2021). In summary, the Gai proteins are critical to activate oncogenic signaling downstream of growth factor receptors that control cell proliferation and migration, although the detailed mechanism is yet to be fully elucidated.

**Gai controls cell migration**

Cell migration is a fundamental process involved in multicellular organisms to establish and maintain the proper organization of cells and tissues under variable physiological conditions. In adults, cell migration is essential to perform a proper tissue homeostasis, immune response, and wound repair, while changes in cellular motility are involved in the etiology of severe pathologies including cancer, atherosclerosis, defective immune response, and birth defects. Cell migration during tissue morphogenesis, requires changes in cell polarity by modifications at the cytoskeleton organization, upon mechanical and chemical cues from the surrounding environment (Schwarz and Gardel, 2012; Abercrombie et al., 1971; Roubinet et al., 2012). For instance, during an immune response, the chemical signals generated by the interaction between chemotaxtractants with their receptors direct the localization of leukocytes toward several tissues and peripheral organs to mediate inflammation. In this context, most chemotaxtractants and chemokines signal through GPCRs that coupled with Gi, dissociating Gai subunit from its associated Gβγ heterodimers (Hepler and Gilman, 1992; Neer, 1995) to activate downstream effectors in order to control immune cell migration (Arai et al., 1997; Neptune et al., 1997; Neptune et al., 1999). Gai2 and Gai3 are highly expressed in the immune system (Han et al., 2005), thus, Gai signaling is involved during many leukocyte biological functions, among these we found, macrophage phagocytosis and migration (Lee et al., 2009; Weiss-Hajiti et al., 2004), T and B cell migration towards the lymph nodes (Han et al., 2005; Hwang et al., 2007), eosinophils migration to sites of allergic tissue injury (Pero et al., 2007), and neutrophils migration during acute inflammation (Pero et al., 2007; Zarbock et al., 2007).

Several molecular tools together with *in vivo* analysis have allowed to understand Gai functions during immune cells migration. For example, using knockout animals, cell knockdown and rescue experiments, together with microscopy tools revealed the essential role of Gai2 during homeostatic and inflammation-induced migration by controlling actin cytoskeleton remodeling and chemotactic migration of macrophages (Wiege et al., 2012). Gai2−/− mice displays defects in the signaling upon B cell chemokine receptor, causing depressed B cell chemotaxis and poor B cell adherence to lymph node high endothelial venules (HEVs), defects that were not rescued by Gai1 and Gai3 (Han et al., 2005). The use of knockdown and knockout cellular models has also revealed that different Gai subtypes may play distinct roles. For example, Gai2 and Gai3 expressed at T lymphocytes act differently to regulate cell migration. Indeed, the lack of Gai2 function in T cells, impairs migration mediated upon stimulation of C-X-C chemokine receptor type 3 (CXCR3) by chemokine C-X-C motif ligand 9, 10, 11 (CXCL9, CXCL10, or CXCL11) (Thompson et al., 2007). For the contrary, the lack of Gai3 function in T cells increases migration upon CXCR3 stimulation, suggesting that this Gai isofrom may be a negative regulator of migration (Thompson et al., 2007).

It is well-established that C-X-C chemokine receptor type 4 (CXCR4) is coupled to Gai, promoting the activation of PI3K (Figure 3B) (Dutt et al., 1998; Curnock et al., 2003). Specifically, stromal derived factor 1 (SDF) activate migration by a molecular mechanism mediated by Gai-proteins, the tyrosine kinases, Src and IL2-inducible T cell kinase (ITK), as well as PI3K (Figure 2B) (Fischer et al., 2004). In Jurkat T cells upon SDF-1-CXCR4 stimulation, Gβγ is released from Gai, triggering molecular downstream effector to promote cell migration (Tan et al., 2006). Likewise, it has been described a similar mechanism for lysophosphatic acid receptor 2 (LPA2 receptors). Specifically, LPA (lysophosphatic acid)-induced migration of CAOV-3 ovarian cancer cells involved activation of the Gai/SRC/EGFR/ERK signaling axis (Jeong et al., 2008). In the same context, Ward and Dhanasekaran, 2012 demonstrated a critical role for Gai2 in LPA-stimulated cell migration, by regulating the tyrosine phosphorylation of the scaffolding protein, p130 Crk associated substrate (p130Cas), to induce metastasis in ovarian cancer cells. In breast cancer cells, Gai2 regulates the activation of Rac proteins through the GEF activity of Elmo1/Dock180 (Engulfment and cell motility 1/dedicator of cytokinesis) interaction, contributing on the actin polymerization and migration (Figure 3C) (Li et al., 2013).
Activated Gαi also directly impacts neutrophil migration (Surve et al., 2016) by regulating adhesion through a cAMP-independent mechanism. Gipγ activation using the small molecule 12,155 inhibited cell migration, altered the cell polarity, and increased the adhesion of neutrophils (Surve et al., 2016). In the same context, a Gai1 constitutively active mutant, Gai1 (Q204L), rescued the loss of migration phenotypes caused by Gipγ activation (Surve et al., 2016). Specifically, under Rap1a-Radil (Ras related protein 1a-Ras associating and dilute domain-containing protein) signaling pathway, Gai1Q204L but not the wild type Gai1 is sufficient to rescue the neutrophil morphology, from an elongated phenotype to circularly cell shape, suggesting that active Gai can regulate cell rear retraction, critical process during cell migration (To and Smrcka, 2018). Therefore, Gai has critical and varied functions regulating cell migration depending on the physiological context, through releasing Gipγ, tyrosine kinase regulation and also by regulating small G protein family, such Rac, in order to control cytoskeleton organization, although the detailed mechanism still an exciting topic to understand.

**Gai function during development**

By 1993, the first in vivo knockdown of Gai2 in mice revealed further insights into the possible roles of G proteins in early mammalian development (Moxham et al., 1993). Indeed, in totipotent mouse F9 teratocarcinoma cells, Gai2 reduction by retinoic acid promotes formation of a primitive endoderm, whereas Gai2 activation blocks the formation of a primitive endoderm (Watkins et al., 1992). Also, wingless-related integration site (Wnt)-mediated signaling through the GPCR, Frizzled, is PTX-sensitive in mouse F9 teratocarcinoma cells (Liu et al., 2001), zebrafish and Xenopus embryos (Slusarski et al., 1997), and Drosophila melanogaster (Katanaev et al., 2005), suggesting that Gai has crucial roles during early development. In 2016, our group demonstrated that Gai2 transcript is expressed at early neurula stages in Xenopus embryos within neural and neural crest tissue. Specifically, at embryo stages 23 and 24, Gai2 transcript is displayed at the presomitic mesoderm and at the front of the embryos, region which later differentiate into the brain and neural tube. From stage 27, Gai2 transcript is expressed in neural crest routes and placodes, and in vascular tissue derivatives including the posterior cardinal and intersomitic (Fuentealba et al., 2016). Interestingly, Gai2 transcript was also expressed at the dorsal marginal zone, a region critical to neural crest induction (Fuentealba et al., 2016).

In mice, loss of Gai2 function inhibits the insulin receptor tyrosine kinase signaling by reducing the levels of phosphotyrosine phosphatase (Moxham and Malbon, 1996). Also, in mice, retinal pigment epithelium formation is controlled by Gai family proteins, specifically Gai3 (Young et al., 2013). In addition, Gai influences osteoblast differentiation, which is induced by CXCR4 (Zhu et al., 2007), as well as osteoblast proliferation and survival induced by LPA (Grey et al., 2001). Interestingly, Gai3 is also involved during normal patterning of the axial skeleton, thus its expression is critical in sclerotomal derivatives and Gai1 and Gai2 are able to rescue partially the loss of Gai3, although depending on genetic background (Plummer et al., 2012).

As we mentioned above, three isoforms from the Gai family have been described, including Gai1, Gai2 and Gai3. However, Gai another member of the PTX-sensitive Gai/o family, have been reported to be involved in the central nervous system (CNS) development. Gai is the most abundant isoform in the CNS, although, its role remains to be elucidated. Nevertheless, recent studies have found that global deletion of Gai impairs cerebellar cortical development in mice (Cha et al., 2019). Specifically, depletion of Gai induced cerebellar hypoplasia and reduced arborization and dendritic spines of Purkinje cell dendrites from the inferior olivary nucleus in mice (Cha et al., 2019).

In C. elegans, Gai has been involved during neuronal migration in early development. Indeed, the neurotransmitter, serotonin, induce neuronal migration by stimulation of GPCR/ Gao signaling (Kindt et al., 2002). Gai also regulates the migration of neurons in moth (Horgan et al., 1994), as well as during growth cones of developing neurites in cultured human pheochromocytoma PC12 cells (Strittmatter et al., 1990). In Drosophila, Gao is asymmetrically localized in cells, and together with PINS, regulates Frizzled-mediated asymmetric cell division and planar cell polarity (Katanaev et al., 2005; Katanaev and Tomlinson, 2006). Although, these findings support the crucial and pleiotropic function that Gai signaling pathways have during development, the detailed mechanism(s) and role of other signaling cascades remain to be elucidated.

**Conclusion and future perspectives**

In this review, we have briefly summarized the activation, signaling, and physiological functions of the Gai subunit from heterotrimeric G proteins. Gai proteins are well known to transduce signals between GPCRs and their downstream effectors in response to extracellular ligands (Birnbaumer, 2007). Nevertheless, several studies indicate that G protein function during establishment of cell polarity and asymmetric cell division may not involve any extracellular signal (di Pietro et al., 2016). Although, Gai acts with several other proteins (e.g., AGS and GEFs) to regulate these processes, many questions remain to be answered regarding the detailed mechanism of Gai-protein regulation of astral microtubules pulling forces during cell division. Several evidences showed that tubulin could be a direct downstream target of G-proteins in the context of cell division (Willard et al., 2004), and possible in other biological processes such as cell migration and wound healing. This is
supported by evidence that both Ga and Gi subunits can bind directly and regulate microtubule dynamics in vivo and in vitro (Chen et al., 2003; Popova and Rasenick, 2003; Roychowdhury et al., 1999; Roychowdhury and Rasenick, 1997; Wang et al., 1990; Sarma et al., 2003). Indeed, activated state GTP-bound Gi1 can directly interact with tubulin, transactivating its intrinsic GTPase activity and modulating microtubule dynamics (Chen et al., 2003; Roychowdhury et al., 1999). In addition, Lin-5 and GPR-1 interact with dynein, a motor protein that moves along microtubules transporting various cellular cargos and providing forces during mitosis, to control spindle positioning, suggesting that Gai could regulate the spindle pulling forces through interaction with Lin-5/GPR-1 and dynein (Couwenbergs et al., 2007).

In cancer, embryonic development, and cell growth, tyrosine kinases play an important role in cell division and proliferation. As was mentioned here, c-Src, a non-receptor tyrosine kinase phosphorylates specific tyrosine residues in other downstream tyrosine kinases to regulate these processes. Several studies described here also indicate possible direct interactions between Ga subunits and tyrosine kinases (Luttrell and Luttrell, 2004), supporting the idea that Gai is able to activate tyrosine kinases signaling in a direct manner. Furthermore, c-Src is also involved in cell adhesion during migration. Inhibition of c-Src induces loss of cell adhesion and membrane blebbing, affecting cell migration (Logue et al., 2018). There is also substantial evidence supporting that Src family kinases can regulate the activity of Ga subunits through tyrosine phosphorylation as it phosphorylates Gas, Gai1, Gai2, and Go in vitro, suggesting that Ga subunits are potentially involved in cell adhesion and migration (Hausdorff et al., 1992). As we mentioned above, it has been described a novel role for Gai1-GTP regulating cell adhesion during neutrophils migration in a Rap1a-Radil manner, which likely functions through c-Src activity (To and Smrcka, 2018).

Although the Gai family was first classified as inhibitors of adenylyl cyclase, new data have revealed non-canonical functions that impact cell behavior. However, several interesting questions remain to be answered. For example, could Gai control cell polarity during cell migration in development using the same mechanism used in cell division (i.e., controlling the interaction between microtubules to induces morphology changes). Given that Gai subunits are highly similar (e.g., Gai1 and Gai3) with around 94% sequence identity, they clearly perform different functions, demonstrating the complex mechanisms underlying Gai signaling pathways. Thus, further studies are required to systematically dissect the multiple signaling mechanisms that regulate cell behavior.

**Author contributions**

SV and MT wrote the first draft and the final manuscript after edition. SV, MT, and JL designed figures for the manuscript. GR, MR, CP, JL, and LT, all read and edited the first draft. SV, GR, MR, CP, JL, LT, and MT read and approved the final draft.

**Funding**

Work in MT laboratory is supported by a grant from the Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) #1180926. We also acknowledge the Agencia Nacional de Investigación y Desarrollo de Chile (CONICYT) for supporting the graduate studies of SV and JL and to the Dirección de Postgrado UdeC for supporting the graduate studies of GR, MR, CP, and LT.

**Acknowledgments**

We acknowledge Vicerrectoría de Investigación y Desarrollo (VRID-UdeC) for publishing support. We also acknowledge and thank Gustavo Minder for the artwork. Finally, we would like to thank worldwide researchers who have contributed to the understanding of Gi-protein function.

**Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher’s note**

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

**References**

Abercrombie, M., Heaysman, J. E., and Pegrum, S. M. (1971). The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. Exp. Cell Res. 67, 359–367. doi:10.1016/0014-4827(71)90420-4

Afshar, K., Willard, F. S., Colombo, K., Siderovski, D. P., and Gonczy, P. (2005). Cortical localization of the Galpha protein GPA-16 requires RIC-8 function during C. elegans asymmetric cell division. Development 132, 4449–4459. doi:10.1242/dev.02039
Villaseca et al. 10.3389/fcell.2022.941870

Dutt, P., Wang, J. F., and Groopman, J. E. (1998). Stromal cell-derived factor-1 alpha and stem cell factor/kit ligand sharing signaling pathways in hemopoietic progenitors: A potential mechanism for cooperative induction of chemotaxis. J. Immunol. 161, 3652–3658.

Feigen, M. E., and Muthuswamy, S. K. (2009). Polarity proteins regulate mammalian cell-cell junctions and cancer pathogenesis. Curr. Opin. Cell Biol. 21, 694–700. doi:10.1016/j.cgb.2009.07.003

Fischer, A. M., Mercer, J. C., Iyer, A., Ragin, M. J., and August, A. (2004). Regulation of CXCR4 chemokine receptor 4-mediated migration by the Tec family tyrosine kinase ITK. J. Biol. Chem. 279, 29816–29820. doi:10.1074/jbc.M312848200

Fishturb, C. S., Herzmark, P., Morales, J., and Bourne, H. R. (1999). Betagamma and palmitate target newly synthesized Galphazto the plasma membrane. J. Biol. Chem. 274, 18793–18800. doi:10.1074/jbc.274.26.18793

Fuentelba, J., Toro-Tapia, G., Rodriguez, M., Arriagada, C., Maureira, A., Beyer, A., et al. (2016). Expression profiles of the Ga subunits during Xenopus tropicalis embryonic development. Gene Expr. Patterns. 22, 15–25. doi:10.1016/j.gep.2016.09.001

Fuse, N., Husta, K., Katzen, A. L., and Matuszaki, F. (2003). Heterotrimeric G proteins regulate daughter cell size asymmetry in Drosophila neuroblast divisions.Drosophila neuroblast divisions. Curr. Opin. Cell Biol. 13, 947–954. doi:10.1065/oc0020882

Gabay, M., Pinter, M. E., Wright, F. A., Chan, P., Murphy, A. J., Valenzuela, D. M., et al. (2013). Ric-8 proteins are molecular chaperones that direct nascent G protein asubunit membrane association. F061 subunit membrane association. Sci. Signal. 4, ra79. doi:10.1126/scisignal.2002223

Garcia-Marcos, M., Ghosh, P., and Farquhar, M. G. (2009). GIV is a nonreceptor GEF for Galphaz with a unique motif that regulates Akt signaling. Proc. Natl. Acad. Sci. U. S. A. 106, 3178–3183. doi:10.1073/pnas.0900249106

Garcia-Marcos, M., Jung, B. H., Ear, J., Cabrera, B., Carethers, J. M., and Ghosh, P. (2011). Expression of GIV/Girdin, a metastasis-related protein, predicts patient survival in colon cancer. FASEB J. 25, 590–599. doi:10.1096/fj.10-191530

Ghosh, P., Peas, A. O., Bornheimer, S. I., Garcia-Marcos, M., Forry, E. P., Johansson, C., et al. (2010). A Galphaz-GIV molecular complex binds epidermal growth factor receptor and determines whether cells migrate or proliferate. Mol. Biol. Cell 21, 2338–2354. doi:10.1091/mbc.e10-01-0028

Ghosh, P., Garcia-Marcos, M., Bornheimer, S. J., and Farquhar, M. G. (2008). Activations of Galphaz triggers cell migration via regulation of GIV. J. Biol. Chem. 182, 381–393. doi:10.1038/jbc.200712066

Gilman, A. G. (1987). G proteins: Transducers of receptor-generated signals. Annu. Rev. Biochem. 56, 615–649. doi:10.1146/annurev.bi.56.070187.003151

Gohla, A., Klement, K., Pickroz, R. P., Peka, K., vom Dahl, S., Spicher, K., et al. (2007). An obligatory requirement for the heterotrimeric G protein Goα in the antiautophagic action of insulin in the liver. Proc. Natl. Acad. Sci. U. S. A. 104, 3003–3008. doi:10.1073/pnas.0611443104

Goldsmith, Z. G., and Danasekaran, D. N. (2007). G protein regulation of MAPK networks. Oncogene 26, 3112–3142. doi:10.1038/sj.onc.1210407

Gotta, M., and Ahringer, J. (2001). Distinct roles for Galphaz and Gbetagamma in regulating spindle position and orientation in caenorhabditis elegans embryos. Nat. Cell Biol. 3, 297–300. doi:10.1038/35060092

Gotta, M., Dong, Y., Peterson, Y. K., Lanier, S. M., and Ahringer, J. (2003). Asymmetrically distributed C. elegans homologs of AG53/PINS control spindle position in the early embryo. Curr. Biol. 13, 1029–1037. doi:10.1016/s0960-9822(03)00731-3

Gou, M. L., Liu, Z., Chu, X. P., Mao, L. M., and Wang, J. Q. (2010). CaMKIIa, a modulator of M4 muscarinic acetylcholine receptors. Commun. Integr. Biol. 3, 465–467.

Grey, A., Banovic, T., Naot, D., Hill, B., Callon, K., Reid, I., et al. (2001). Lysophosphatidic acid is an osteoblast mitogen whose proliferative actions involve G (i) proteins and protein kinase C, but not P42/44 mitogen-activated protein kinases. Endocrinology 142, 1098–1106. doi:10.1210/endo.142.3.8011

Grill, S. W., Goycz, P., Stelzer, E. H., and Hayman, A. A. (2001). Polarity controls forces governing asymmetric spindle positioning in the Caenorhabditis elegans embryo. Nature 409, 630–635. doi:10.1038/35054572

Guillay, C., Garcia-Mata, R., and Burridge, K. (2011). Rho protein crosstalk: Another social network? Trends Cell Biol. 21, 718–726. doi:10.1016/j.tcb.2011.08.002

Haaster, P. J. M. V., and Devreotes, P. N. (2004). Chemotaxis: Signalling the way forward. Nat. Rev. Mol. Cell Biol. 5, 626–634. doi:10.1038/nrm1435
phagocytosis in alveolar macrophages via combinatorial effects on cyclic AMP. J. Immunol. 182, 530–537. doi:10.4049/jimmunol.182.1.530
Li, H., Yang, L., Fu, H., Yan, J., Wng, Y., Guo, H., et al. (2013). Association between Gα2 and ELMO1/Dock180 connects chemokine signalling with Rac activation and adhesiveness. Nat. Commun. 4, 1709. doi:10.1038/ncomms2608
Li, Z., Cai, S., Liu, Y., Yang, C.-L., Tian, Y., Chen, G., et al. (2016). Over-expression of Gα3 in human glioma is required for Akt-mTOR activation and cell growth. Oncotarget 7, 1. doi:10.18632/oncotarget.10995
Lin, H. C., Duncan, J. A., Kosza, T., and Gilman, A. G. (1998). Sequestration of the G protein beta gamma subunit complex inhibits receptor-mediated endocytosis. Proc. Natl. Acad. Sci. U. S. A. 95, 5057–5060. doi:10.1073/pnas.95.5057
Ling, Y., Jiang, P., Cai, S.-P., Ren, Y.-L., Zhu, S.-N., Yang, J.-P., et al. (2011). Clinical implications for Girdin protein expression in breast cancer. Cancer Invest. 29, 405–410. doi:10.1080/07357907.2011.585688
Liu, T., DeCostanzo, A. J., Liu, X., Wang, H.-Y., Hallagan, S., Randall, T., et al. (2001). Gi protein signaling from activated rat frizzled-1 to the beta-catenin-Lef-Tcf pathway. Science 297, 1712–1718. doi:10.1126/science.1061010
Liu, Y., Chen, M. B., Cheng, L., Zhang, Z.-G., Yu, Z.-Q., Jiang, Q., et al. (2018). microRNA-200a downregulation in human glioma leads to Gαi1 over-expression, Akt activation, and cell proliferation. Oncogene 37, 2890–2902. doi:10.1038/s41388-018-0184-5
Logue, J. S., Cartagena-Rivera, A. X., and Chadwick, R. S. (2018). c-Src activity is differentially required by cancer cell motility modes. Oncogene 37, 2104–2121. doi:10.1038/s41388-017-0071-5
Luttrel, D. K., and Luttrel, L. M. (2004). Not so strange bedfellows: G protein-coupled receptors and src family kinases. Oncogene 23, 7969–7978. doi:10.1038/sj/onc.1206182
Ma, Y. C., Huang, J., Ali, S., Lowry, W., and Huang, X. Y. (2000). Src tyrosine kinase is a novel direct effector of G proteins. Cell 102, 635–646. doi:10.1016/s0092-8674(00)00866-6
Maer, O., Ehmsen, E., and Westermann, P. (1995). Trimeric G protein alpha subunits of the Gα and Gi families localized at the Golgi membrane. Biochim. Biophys. Acta. 1283, 135–143. doi:10.1016/0167-4889(95)03138-M
Malab, C. M. (2005). G proteins in development. Nat. Rev. Mol. Cell Biol. 6, 898–701. doi:10.1038/nm1716
Marrari, Y., Crouthamel, M., Irnejad, R., and Wedegaertner, P. B. (2007). Assembly and trafficking of heterotrimeric G proteins. Biochemistry 46, 7665–7677. doi:10.1021/bi700338m
Marshall, J., Zhou, X.-Z., Chen, G., Yang, S.-Q., Li, Y., Wang, Y., et al. (2018). Antidepressive action of BDNF requires and is mimicked by Gαi3 expression in the hippocampus. Proc. Natl. Acad. Sci. U. S. A. 115, E5349–E5355. doi:10.1073/pnas.1722493115
Mascri, G., Dekoninck, S., Drogat, B., Yousef, K. K., Brohée, S., Sototopoula, P., et al. (2012). Distinct contribution of stem and progenitor cells to epidermal maintenance. Nature 489, 257–262. doi:10.1038/nature11393
McAllister, A. K., Katz, L. C., and Lo, D. C. (1996). Neurotrophin regulation of cortical dendritic growth requires activity. Neuron 17, 1057–1064. doi:10.1016/s0896-6273(00)80239-1
Montmayeur, J. P., and Borrelli, E. (1994). Targeting of G alpha 12 to the Golgi by alternative spliced carboxyl-terminal region. Science 263, 95–98. doi:10.1126/science.8272874
Morris, A. J., and Malab, C. M. (1999). Physiological regulation of G protein-linked signaling. Physiol. Rev. 79, 1373–1430. doi:10.1152/physrev.1999.79.4.1373
Moxham, C. M., Hod, Y., and Malab, C. C. (1993). Induction of Gα2-specific antisense RNA in vivo inhibits neonatal growth. Science 260, 991–995. doi:10.1126/science.8493537
Moxham, C. M., and Malab, C. C. (1996). Insulin action impaired by deficiency of the G-protein subunit Gαi2. Nature 379, 840–844. doi:10.1038/379840a0
Müller, L., Picart, R., Barret, A., Bockart, J., Homburger, V., and Tougaard, C. (1994). Identification of multiple subunits of heterotrimeric G proteins on the membrane of secretory granules in rat proactin anterior pituitary cells. Mol. Cell. Endocrinol. 9, 556–566. doi:10.1016/0303-7207(94)91068-7
Natarajan, K., and Berk, B. C. (2006). Crosstalk regulation mechanisms of G protein-coupled receptors and receptor tyrosine kinases. Methods Mol. Biol. 332, 51–77. doi:10.1007/978-1-59745-048-4_51
Neer, E. J. (1995). Heterotrimeric G proteins: Organizers of transmembrane signals. Cell 80, 249–257. doi:10.1016/0092-8674(95)90487-7

Villaseca et al.
10.3389/fcell.2022.941870
Neptune, E. R., and Bourne, H. R. (1997). Receptors induce chemotaxis by releasing the beta subunit of Gi, not by activating G or Go. Proc. Natl. Acad. Sci. U. S. A. 94, 14489–14494. doi: 10.1073/pnas.94.14.14489

Neptune, E. R., Iti, T., and Bourne, H. R. (1999). Galphai is not required for chemotaxis mediated by Go-coupled receptors. J. Biol. Chem. 274, 2824–2828. doi: 10.1074/jbc.274.3.2824

Nasková, H., Serebyraková, M. V., Ferrer-Caçes, A., Sachsenheimer, T., Luchtenborg, C., Miller, A. K., et al. (2021). Stearic acid blunts growth-factor signaling via oleylation of GNAI proteins. Nat. Commun. 12, 4590. doi: 10.1038/s41467-021-24844-9

Oger-Denis, E., Coustoune, A., Maeret, J. J., Hour, J., Bany, C., De Stefanis, D., et al. (1995). A heterotrimeric Gi3-protein controls autophagic sequestration in the human colon cancer cell line HT-29. J. Biol. Chem. 270, 13–16. doi: 10.1074/jbc.270.13.1073

O’Neill, K. M., Kwon, M., Donohue, K. E., and Firestein, B. L. (2017). Distinct effects on the dendritic arbor by microbe versus bath administration of brain-derived neurotrophic factor. Cell. Mol. Life Sci. 74, 4369–4385. doi: 10.1007/s00018-017-2589-7

Parmentier, M. L., Woods, D., Greig, S., Phan, P. G., Radovic, A., Bryant, P., et al. (2000). Rapynoid/partner of inactive controls asymmetric division of larval neuroblasts in Drosophila. J. Neurosci. 20, RC84. doi: 10.1523/JNEUROSCI.20-14-0003.2000

Pero, R. S., Borchers, M. T., Speicher, K., Ochlik, S. I., Sikora, L., Rao, S. P., et al. (2007). Galphai-mediated signaling events in the endothelium are involved in controlling leukocyte extravasation. Proc. Natl. Acad. Sci. U. S. A. 104, 4371–4376. doi: 10.1073/pnas.0700185104

Pierce, K. L., Fremont, R. T., and Leftowitz, R. J. (2002). Seven-transmembrane receptors. Nat. Rev. Mol. Cell Biol. 3, 639–650. doi: 10.1038/nrm908

Pimplikar, S. W., and Simons, K. (1993). Regulation of apical transport in epithelial cells by a Go class of heterotrimeric G protein. Nature 362, 456–458. doi: 10.1038/362456a0

Pines, M., Santora, A., and Spiegel, A. (1986). Effects of phorbol ester and pertussis toxin on agonist-stimulated cyclic AMP production in rat osteosarcoma cells. Biochem. Pharmacol. 35, 3639–3641

Plummer, N. W., Speicher, K., Malphurs, J., Akiyama, H., Abramowitz, J., Thompson, B. D., Jin, Y., Wu, K. K., Colvin, R. A., Luster, A. D., Birmbaum, L., et al. (2007). Inhibition of G alpha i32 activation by G alpha i3 in CCR3-mediated signaling. J. Biol. Chem. 282, 9547–9555. doi: 10.1074/jbc.M610931200

Rai, J. Y., and Smrcka, A. V. (2018). Dynamic regulation of neutrophil polarity and migration by the heterotrimeric G protein Gai1-GTP and Gai2. Sci. Signal. 9, ra22. doi: 10.1126/scisignal.aas8163

Roycroft, A., Marcellini, S., Mayor, R., Toro-Tapia, G., Villaseca, S., Beyer, A., et al. (2016). The Ric-8A/Gα13/FAK signaling cascade controls focal adhesion formation during neural crest cell migration in X. J. Biol. Chem. 291, 1570–1578. doi: 10.1074/jbc.RA117.000646

Ric-8 controls Drosophila neural progenitor asymmetric division by inhibiting Rap-dependent cell adhesion and promoting cell migration. J. Biol. Chem. 293, 1570–1578. doi: 10.1074/jbc.RA117.000646

Roycroft, A., Marcellini, S., Mayor, R., Toro-Tapia, G., Villaseca, S., Beyer, A., et al. (2016). The Ric-8A/Gα13/FAK signaling cascade controls focal adhesion formation during neural crest cell migration in X. J. Biol. Chem. 293, 1570–1578. doi: 10.1074/jbc.RA117.000646

Ric-8 controls Drosophila neural progenitor asymmetric division by inhibiting Rap-dependent cell adhesion and promoting cell migration. J. Biol. Chem. 293, 1570–1578. doi: 10.1074/jbc.RA117.000646

Roycroft, A., Marcellini, S., Mayor, R., Toro-Tapia, G., Villaseca, S., Beyer, A., et al. (2016). The Ric-8A/Gα13/FAK signaling cascade controls focal adhesion formation during neural crest cell migration in X. J. Biol. Chem. 293, 1570–1578. doi: 10.1074/jbc.RA117.000646

Ric-8 controls Drosophila neural progenitor asymmetric division by inhibiting Rap-dependent cell adhesion and promoting cell migration. J. Biol. Chem. 293, 1570–1578. doi: 10.1074/jbc.RA117.000646

Ric-8 controls Drosophila neural progenitor asymmetric division by inhibiting Rap-dependent cell adhesion and promoting cell migration. J. Biol. Chem. 293, 1570–1578. doi: 10.1074/jbc.RA117.000646

Ric-8 controls Drosophila neural progenitor asymmetric division by inhibiting Rap-dependent cell adhesion and promoting cell migration. J. Biol. Chem. 293, 1570–1578. doi: 10.1074/jbc.RA117.000646
Wilkie, T. M., Gilbert, D. J., Olsen, A. S., Chen, X. N., Amatruda, T. T., Korenberg, J. R., et al. (1992). Evolution of the mammalian G protein alpha subunit multigene family. Nat. Genet. 1, 85–91. doi:10.1038/ng0592-85

Willard, F. S., Kimple, R. J., and Siderovski, D. P. (2004). Return of the GDI: The GoLoco motif in cell division. Annu. Rev. Biochem. 73, 925–951. doi:10.1146/annurev.biochem.73.011303.073756

Wilson, B. S., Palade, G. E., and Farquhar, M. G. (1993). Endoplasmic reticulum-through-Golgi transport assay based on O-glycosylation of native glycophorin in permeabilized erythroleukemia cells: Role for Gi3. Proc. Natl. Acad. Sci. U. S. A. 90, 1681–1685. doi:10.1073/pnas.90.5.1681

Wong, Y. H., Federman, A., Pace, A. M., Zachary, I., Evans, T., Pouyssegur, J., et al. (1991). Mutant alpha subunits of Gi2 inhibit cyclic AMP accumulation. Nature 351, 63–65. doi:10.1038/351063a0

Woodard, G. E., Huang, N.-N., Cho, H., Miki, T., Tall, G. G., and Kehrl, J. H. (2010). Ral-8A and Gi alpha recruit LGN, NuMA, and dynein to the cell cortex to help orient the mitotic spindle. Mol. Cell. Biol. 30, 3519–3530. doi:10.1128/MCB.00394-10

Xiao, R. P., Avdonin, P., Zhou, Y. Y., Cheng, H., Alder, S. A., Eschenthaler, T., et al. (1998). Coupling of β2-adrenoceptor to G proteins and its physiological relevance in murine cardiac myocytes. Circ. Res. 84, 43–52. doi:10.1161/01.res.84.1.43

Yamaguchi, T., Nagahama, M., Itoh, H., Hatsuzawa, K., Tani, K., and Tagaya, M. (2000). Regulation of the golgi structure by the alpha subunits of heterotrimeric G proteins. FERS Lett. 470, 25–28. doi:10.1016/s0014-5793(00)01284-9

Young, A., Wang, Y., Ahmed, N. B., Jiang, M., and Farber, D. B. (2013). A constitutively active Gai3 protein corrects the abnormal retinal pigment epithelium phenotype of Oa1−/− mice. PLoS One 8, e62480. doi:10.1371/journal.pone.0076240

Yu, F., Cai, Y., Kaushik, R., Yang, X., and Chia, W. (2003). Distinct roles of Galphai and Gbeta13F subunits of the heterotrimeric G protein complex in the mediation of Drosophila neuroblast asymmetric divisions. J. Cell Biol. 162, 623–633. doi:10.1083/jcb.200303174

Yu, F., Morin, X., Cai, Y., Yang, X., and Chia, W. (2000). Analysis of partner of inscuteable, a novel player of Drosophila asymmetric divisions, reveals two distinct steps in inscutable apical localization. Cell 100, 399–409. doi:10.1016/s0092-8674(00)80676-5

Zarbock, A., Deem, T. L., Burcin, T. L., and Ley, K. (2007). Galphai2 is required for chemokine-induced neutrophil arrest. Blood 110, 3773–3779. doi:10.1182/blood-2007-06-094565

Zhang, Y. M., Zhang, Z. Q., Liu, Y. Y., Zhou, X., Shi, X. H., Jiang, Q., et al. (2015). Requirement of Gai1/3-Gab1 signaling complex for keratinocyte growth factor-induced PI3K-AKT-mTORC1 activation. J. Invest. Dermatol. 135, 181–191. doi:10.1038/jid.2014.326

Zhu, W., Bouchie-Adjei, O., Rawlins, B. A., Frenkel, B., Boskey, A. L., Ivashkov, L. B., et al. (2007). A novel regulatory role for stromal-derived factor-1 signaling in bone morphogenic protein-2 osteogenic differentiation of mesenchymal C2C12 cells. J. Biol. Chem. 282, 18676–18685. doi:10.1074/jbc.M610232200