Chapter 1

Introduction

Function and Regulation of Heterotrimeric G Proteins during Chemotaxis

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Function and Regulation of Heterotrimeric G Proteins during Chemotaxis

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Abstract: Chemotaxis, or directional movement towards an extracellular gradient of chemicals, is necessary for processes as diverse as finding nutrients, the immune response, metastasis and wound healing. Activation of G-protein coupled receptors (GPCRs) is at the very base of the chemotactic signaling pathway. Chemotaxis starts with binding of the chemoattractant to GPCRs at the cell-surface, which finally leads to major changes in the cytoskeleton and directional cell movement towards the chemoattractant. Many chemotaxis pathways that are directly regulated by $G_{\beta\gamma}$ have been identified and studied extensively; however, whether $G_{\alpha}$ is just a handle that regulates the release of $G_{\beta\gamma}$ or whether $G_{\alpha}$ has its own set of distinct chemotactic effectors, is only beginning to be understood. In this review, we will discuss the different levels of regulation in GPCR signaling and the downstream pathways that are essential for proper chemotaxis.

Keywords: chemotaxis; G-protein coupled receptors; heterotrimeric G proteins; adaptation; non-canonical regulators; $G_{\alpha}$ effectors

1. Introduction

Chemotaxis, the process of directed cell movement towards a chemical gradient, plays an important role in both prokaryotes and eukaryotes. Prokaryotic chemotaxis is essential for food scavenging, while in mammals, chemotaxis plays, for example, a role in wound healing and embryogenesis [1]. Defects in chemotaxis are critically linked to the progression of many diseases including cancer, asthma, atherosclerosis and other chronic inflammatory diseases [2]. Although cells can detect chemoattractant gradients of highly diverse chemical compounds produced by many different sources, the main signaling pathways regulating chemotaxis are highly conserved among eukaryotes [3].

The most commonly used model systems for studying chemotaxis are the slime mold Dictyostelium discoideum and mammalian neutrophils [4]. Although having clearly distinct physiological roles, Dictyostelium and neutrophils have a highly similar chemotactic behavior. They display strong chemotactic responses, their stimuli are well-defined and their chemotaxis is characterized by amoeboïd migration, creating actin-rich pseudopods at the front and retraction of the back of the cell using myosin filaments [3,5]. Chemotaxis is essential for the Dictyostelium life cycle: during the vegetative phase of their life cycle, Dictyostelium scavenges the soil for bacteria by chemotaxing towards folic acid released by bacteria; however, if food is scarce, Dictyostelium cells secrete cyclic AMP (cAMP), which is used as a chemoattractant by neighboring cells to form a multicellular structure with spores that can resist harsh conditions.

During its lifecycle, Dictyostelium, as well as neutrophils, have to cope with a wide range of chemoattractant concentrations, e.g., during development, Dictyostelium encounters cAMP gradients.
ranging from 3 nM to 10 μM [6,7]. Activation of G-protein coupled receptors (GPCRs) is at the very base of the signaling pathways that enable this very sensitive and broad chemotaxis response. Chemotaxis starts with binding of the chemoattractant to GPCRs at the cell surface. The receptors transmit these signals into the interior of the cell by activation and dissociation of the heterotrimeric G protein complex. This subsequently results in the activation of a complex network of signaling molecules and the coordinated remodelling of the cytoskeleton. The final outcome is cellular movement up the chemoattractant gradient [8,9].

In this review, we highlight the crucial role of regulators of GPCR and heterotrimeric G-protein signaling and discuss the heterotrimeric pathways regulating chemotaxis.

2. Regulation of GPCRs and Heterotrimeric G Proteins during Chemotaxis

2.1. Chemotaxis Receptors and Their Regulation

Cells are able to detect and respond to a wide variety of chemoattractants and repellents, including peptides, lipids, and small proteins of several classes [2]. Although the structure of these compounds is highly diverse, most of them are detected by receptors of the GPCR family. The human GPCR family consists of nearly 800 genes divided into three main families; β2 adrenergic–like receptors, glucagon-like receptors, and metabotropic neurotransmitter-like receptors [10]. Chemotaxis receptors belong to the family of β2 adrenergic-like receptors. An overview of the chemotaxis receptors discussed in this review, their respective ligands and their expression is provided in Table 1. GPCRs consist of seven transmembrane α-helices, with an intracellular C-terminus and an extracellular N-terminus [11]. The extracellular domain regulates accessibility of the receptor, the transmembrane is the main binding surface for the ligand and, through conformational changes, the signal is transduced to the intracellular domain, which interacts with and activates the heterotrimeric G protein signaling cascade (see below) [8]. To be able to detect both very low and high concentrations of chemoattractant and migrate in a complex environment of competing chemotaxis cues, GPCR activation is highly regulated.

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand(s)</th>
<th>Cellular Expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5</td>
<td>CCL2/3/4/5/15</td>
<td>T cell, NK cell, monocyte, macrophage, dendritic cell</td>
</tr>
<tr>
<td>CCR6</td>
<td>CCL19, β-defensin</td>
<td>B cell, T cell, NK cell, dendritic cell</td>
</tr>
<tr>
<td>CXCR2</td>
<td>CCL28, CXCL1/2/5/6/7/8</td>
<td>T cell, NK cell, neutrophil, monocyte, dendritic cell, granulocyte</td>
</tr>
<tr>
<td>CXCR4</td>
<td>CXCL12 (SDF-1)</td>
<td>B cell, T cell, NK cell neutrophil, monocyte, macrophage, dendritic cell, granulocyte, neurons</td>
</tr>
<tr>
<td>CXCR5</td>
<td>CXCL13</td>
<td>B cell, T cell</td>
</tr>
<tr>
<td>BLT1/2</td>
<td>LTB4</td>
<td>B cell, T cell, neutrophil, monocyte, macrophage, dendritic cell, granulocyte</td>
</tr>
<tr>
<td>LPA1</td>
<td>LPA</td>
<td>NK cell, macrophage</td>
</tr>
<tr>
<td>PAPR</td>
<td>PAP</td>
<td>B cell, neutrophil, monocyte</td>
</tr>
<tr>
<td>FPRL1/2</td>
<td>Formyl peptides</td>
<td>T cell, neutrophil, monocyte, macrophage, dendritic cell</td>
</tr>
<tr>
<td>A1 receptor</td>
<td>Adenosine</td>
<td>Neutrophil, monocyte, macrophage, dendritic cell</td>
</tr>
<tr>
<td>A2A receptor</td>
<td>Adenosine</td>
<td>B cell, NK cell, neutrophil, monocyte, macrophage, dendritic cell</td>
</tr>
<tr>
<td>A2B receptor</td>
<td>Adenosine</td>
<td>B cell, NK cell, neutrophil, monocyte, macrophage, dendritic cell</td>
</tr>
<tr>
<td>A3 receptor</td>
<td>Adenosine</td>
<td>B cell, NK cell, neutrophil, monocyte, macrophage, dendritic cell</td>
</tr>
<tr>
<td>cAR1</td>
<td>cAMP</td>
<td>Dictyostelium. Peaks at 4 h of development, then drops dramatically, early aggregation</td>
</tr>
<tr>
<td>cAR2</td>
<td>cAMP</td>
<td>Dictyostelium. Peaks at 16 h of development, mound formation</td>
</tr>
<tr>
<td>cAR3</td>
<td>cAMP</td>
<td>Dictyostelium. Peaks at 4 h of development, then slowly decreases, late aggregation stage</td>
</tr>
<tr>
<td>cAR4</td>
<td>cAMP</td>
<td>Dictyostelium. Peaks at 20 h of development, culmination</td>
</tr>
<tr>
<td>To be identified</td>
<td>Folic acid</td>
<td>Dictyostelium. Vegetative cells</td>
</tr>
</tbody>
</table>
2.1.1. Ligand Binding Properties and Expression

In Dictyostelium, four cAMP receptors (cAR) have been identified that are involved in chemotaxis (Table 1). To cope with an increase in extracellular cAMP concentration during the aggregation stage [12,13], cells express the cAR1-4 receptors sequentially and with decreasing affinities. cAR2-4 have a relatively low affinity for cAMP and are important during the multicellular stage, whereas cAR1 has a high affinity for cAMP and is essential for signal transduction during early development and chemotaxis. At the onset of Dictyostelium aggregation the very shallow (starting from 3 nM) cAMP gradient is detected by the high affinity ($K_d$ of 30 nM) cAR1 receptor. At late aggregation stages, the cAMP concentrations increase, thereby saturating cAR1 receptors [14]. The cAR1 receptors become phosphorylated at this stage resulting in a five-fold lower affinity [15]. Subsequently, cAR1 expression is down-regulated, while expression of the low affinity cAR3 receptor ($K_d$ of 100 nM), cAR2 and cAR4 (both $K_d$ in the mM range) increases, thereby enabling the cell to respond to the higher concentrations of cAMP [16].

Neutrophils use a highly similar mechanism to sense adenosine released by tissue cells. Inflammation or injury of tissue cells results in a more than 100-fold increase in adenosine release [17]. The cells use a combination of low and high affinity receptors to cope with these different levels of adenosine: where $A_1$ and $A_3$ show an EC$_{50}$ between 0.2–0.5 µM, $A_{2A}$ an EC$_{50}$ between 0.6–0.9 µM, and $A_{2B}$ an EC$_{50}$ between 16–64 µM for adenosine (Table 1) [18]. At low concentrations, both high affinity receptors $A_1$ and $A_3$ promote chemotaxis, while, at higher concentrations, the low affinity $A_2$ receptors are activated and neutrophil recruitment is diminished [17].

2.1.2. Receptor Adaptation and Internalization

Upon ligand binding, all GPCRs transiently induce their own phosphorylation (homologous desensitization) while, as exemplified below, several receptors in addition induce phosphorylation of other receptors (heterologous desensitization) [19]. The desensitization of the GPCRs is achieved by the uncoupling of heterotrimeric G proteins, making it impossible for the receptor to transduce the signal via G$\alpha$ or G$\beta\gamma$ [20], whereas, upon removal of the ligand, resensitization is accomplished by fast recycling of the receptors and digestion of the ligand.

The first step in desensitization is activation of G protein-coupled receptor kinases (GRKs), which subsequently phosphorylate the C-terminal domain of the receptors (Figure 1). Phosphorylated receptors not only have a decreased affinity for heterotrimeric G proteins, but more importantly, also bind with higher affinity to $\beta$-arrestins [21]. $\beta$-arrestin binding uncouples heterotrimeric G proteins from the receptor and can induce receptor internalization. Internalization can result in either recycling to the surface and resensitization, or degradation and persistent desensitization of receptors (Figure 1, [22,23]). Homologous receptor desensitization with associated changes in ligand affinities allows sensitivity to a broader concentration range of chemoattractant, which has been extensively studied for CXCR4, a chemokine receptor that responds to stromal derived factor 1 (SDF-1 or CXCL12). Upon CXCR4 phosphorylation, the E3 ubiquitin ligase IAP4 is recruited and ubiquinates the C-terminal tail of CXCR4 (step 5 in Figure 1) [24,25]. The ubiquitin tags the receptor for lysosomal degradation through the endosomal sorting complex required for transport (ESCRT) pathway. The ubiquitin tag is detected by the ubiquitin binding domain (UBD) on the ESCRT proteins and transported to lysosomes where both the receptor and ligand are degraded (steps 11–12 in Figure 1) [26,27]. The receptor degradation reduces signaling in high concentration gradients, and stops cells from moving when they reach the source. Although the mechanism is not completely understood, homologous desensitization also seems to play a role in Dictyostelium, since a mutant strain expressing a non-phosphorylatable cAR1 showed impaired chemotaxis [28].
Neutrophils operate under very complex conditions of competing chemotaxis cues and opposing directions. Heterologous internalization allows classification of these signals, e.g., because of GRK-mediated receptor phosphorylation and degradation formyl peptides of bacterial or mitochondrial origin are dominant attractants over CXCL8 and LTB4 for neutrophil chemotaxis [29]. These properties are essential for neutrophil chemotaxis towards a necrotic core; they initially use the CXCL2 receptor (CXCR2) to migrate up an intravascular gradient of CXCL2, which they subsequently ignore and instead use FPR1 to migrate up a gradient of mitochondrion-derived formyl peptides.

Interestingly, recent studies have shown that β-arrestins not only regulate receptor adaptation, but also directly bind and activate downstream chemotaxis pathways. At the leading edge, β-arrestins can function as scaffold proteins for coflin, which regulates actin polymerization at the front of the cell [30]. Furthermore, when the chemotaxis receptor CCR5 is activated by MIP1β (CCL4) a scaffold is made consisting of β-arrestin 2, PI3K and some non-receptor kinases. This β-arrestin 2 dependent scaffold is essential for MIP1β induced chemotaxis of human macrophages [31].

2.2. Kinetics and Regulation of Heterotrimeric G Proteins during Chemotaxis

The general paradigm of GPCR activation is that ligand binding induces a conformational change in intracellular receptor domains resulting in the release of GDP from the Ga subunit (Figure 2). The GDP is quickly replaced by GTP from the cytosol, which promotes dissociation of the three subunits as Ga-GTP and a Gβγ dimer, both of which can regulate a diverse set of downstream effectors. Due to the intrinsic Ga-associated GTPase activity, GTP is hydrolysed to GDP, and the inactive heterotrimeric complex is re-associated [32]. The reassembled heterotrimeric G protein complex can form a complex with the GPCR again. However, it is not yet clear whether heterotrimeric G proteins only bind to activated receptors encountered upon lateral diffusion (collision coupling model) [8,33], or whether the G proteins are able to bind to GPCRs prior to activation (pre-coupled model) [34].
Dictyostelium cells stop migrating, however, surprisingly, under these conditions, the heterotrimeric G protein signaling during chemotaxis (Figure 2, [41]).

The kinetics of heterotrimeric G protein dissociation in response to chemoattractant have been extensively studied in both mammalian and Dictyostelium cells. Activation of the receptor occurs in the time frame of ms [35,36], with maximum dissociation of the heterotrimeric G protein complex within 3–6 s after uniform stimulation with chemoattractant [37]. The amount of dissociated Gα and Gβγ at the front and back of Dictyostelium cells corresponds to the relative amount of cAMP at the front and back of the cell, indicating that signal amplification occurs downstream of Gα and Gβγ proteins [38]. The rate limiting step in the heterotrimeric G protein activation cycle is re-association of Gα-GDP and Gβγ with the receptor, which can take up to 15–30 s in mammalian cells [37,39] and several minutes in Dictyostelium cells [40]. Because of the fast intracellular response upon receptor activation and slow re-association rate, the signaling rate of chemotaxis receptors is limited. Under continuous uniform stimulation, the downstream chemotaxis pathways, such as PIP3 production, adapt and Dictyostelium cells stop migrating, however, surprisingly, under these conditions, the heterotrimeric G proteins remain dissociated from the receptor. This strongly suggests that ligand-bound active receptors continuously activate Gα and Gβγ subunits, and that adaptation to the signal at least partly occurs downstream of heterotrimeric G proteins [40].

Based on the conventional heterotrimeric G protein cycle, the duration of downstream signaling is controlled by the lifetime of the Gα subunit in its GTP-bound state. In the last couple of years, several guanine nucleotide exchange factors (GEFs), GTPase-activating proteins (GAPs), guanine nucleotide dissociation inhibitor (GDIs) and regulators of Gβγ signaling have been identified that regulate and fine-tune the heterotrimeric G protein signaling during chemotaxis (Figure 2, [41]).

**Figure 2.** A schematic representation of mammalian Gα regulation. Upon binding of extracellular chemoattractant, GPCRs undergo conformational changes to act as guanine nucleotide exchange factors (GEFs) for Gα subunits, facilitating GDP release and subsequent binding of GTP, and release from Gβγ dimers (A) Non-receptor GEFs can bind to Gα-GDP and extend Gα subunit activation by stimulating the exchange of Gα-GDP to the active GTP-bound state. Regulator of G protein signaling (RGS) proteins stimulate the exchange of Gα-GTP back to Gα-GDP, serving as GTPase-accelerating proteins (GAPs) for Gα, thereby dramatically enhancing their intrinsic rate of GTP hydrolysis; (B) Upon GTP hydrolysis of Gα, the heterotrimer of Gα-GDP and Gβγ can reform, restoring the coupled GPCR/G protein complex; (C) However, in the presence of guanine nucleotide dissociation inhibitors (GDIs), Gα can become trapped in a Gα·GDP/GDI complex, preventing Gβγ from reassociation and re-coupling to GPCRs (D).
2.2.1. Regulation of Gα Signaling by GEFs

In the conventional model of G protein signaling, GPCRs are the GEFs for Gα proteins, stimulating the exchange of G protein bound GDP to GTP and inducing dissociation of free Gα-GTP and Gβγ. However, in the last decades, several non-receptor GEFs have been identified that stabilize a nucleotide-free transition state of Gα, thereby reducing the high nucleotide affinity by many orders and promoting nucleotide release. This subsequently facilitates binding of GTP, which is present in excess over GDP in the cytosol of the cell. So far, several non-receptor GEFs have been identified that play an important role in regulating Gα activation during chemotaxis (Table 2). For instance, GIV (Gα-interacting vesicle-associated protein, also known as Girdin) has been described as a GEF for mammalian Gαi3 [42]. Through the GEF motif located in the C-terminus, GIV binds and exchanges Gαi3-GDP to Gαi3-GTP that is available for the activation of downstream effectors [42]. It has been revealed that GIV is required to stimulate the Gβγ-dependent PI3K/Akt pathway via GIV/Gαi3 activation, which remodels the actin cytoskeleton and regulates cell migration during cancer cell invasion [42–45].

Table 2. Regulation of Gα subunit signaling in chemotaxis.

<table>
<thead>
<tr>
<th>Classification</th>
<th>G Protein Selectivity</th>
<th>Chemotactic Downstream Pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GEF</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GIV</td>
<td>Gαi3</td>
<td>PI3K/Akt pathway</td>
</tr>
<tr>
<td>Mammalian Ric-8A</td>
<td>Gαi3, Gαq, and Gα12</td>
<td>Gαq-linked ERK activation</td>
</tr>
<tr>
<td>Mammalian Ric-8B</td>
<td>Gαq and Gαq</td>
<td>Not defined</td>
</tr>
<tr>
<td><em>D. discoideum</em> Ric8</td>
<td>Gα2 and Gα4</td>
<td>Ras, small G proteins</td>
</tr>
<tr>
<td><strong>RGS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammalian RGS1</td>
<td>Gαi3</td>
<td>Down-regulation of Gβγ</td>
</tr>
<tr>
<td>Mammalian RGS3</td>
<td>Gαq</td>
<td>Blocking binding of Gα to adenylyl cyclase</td>
</tr>
<tr>
<td>Mammalian RGS4</td>
<td>Gαq</td>
<td>MAPK pathways: ERK1/2 and p38MAPKs</td>
</tr>
<tr>
<td>Mammalian RGS13</td>
<td>Gαq and Gαq</td>
<td>Intracellular calcium production and pERK1/2 induction</td>
</tr>
<tr>
<td><em>D. discoideum</em> RCK1</td>
<td>Gα2</td>
<td>Not defined</td>
</tr>
<tr>
<td><strong>GDI</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mammalian AGS3/LGN</td>
<td>Gαi3</td>
<td>Binding to Gxi-GDP and mlnsc</td>
</tr>
<tr>
<td>Mammalian Rap1GAP</td>
<td>Gαi2</td>
<td>Rap1/B-Raf/ERK pathway</td>
</tr>
</tbody>
</table>

Resistance to Inhibitors of Cholinesterase (Ric8A and Ric8B) are regulators of heterotrimeric G protein signaling that can act both as non-receptor GEFs and as chaperone for Gα proteins [46]. Ric-8A only interacts with GDP-bound Gα in the absence of Gβγ, resulting in release of GDP and formation of a stable, nucleotide-free Gα-Ric-8A complex. GTP then binds to Gα and disrupts the complex, releasing Ric-8A and the activated Gα protein [47]. Ric-8A is crucial for cranial neural crest cell migration; it localizes to the plasma membrane of the leading edge, where it amplifies Gα signaling to downstream effectors [48]. Furthermore, silencing of Ric-8A in embryonic fibroblasts inhibited PDGF-induced cell migration and prevented the translocation of Gα13 to the cell cortex [49]. Our work has shown that *Dictyostelium* Ric8 also serves as a non-receptor GEF that is important for development and chemotaxis to cAMP and folate [50]. *Dictyostelium* Ric8 is not important for the initial activation of Gα but competes with Gβγ to bind free Gα-GDP, and converts it back to the active Gα-GTP form. It thereby amplifies and extends the G protein signal. In contrast to mammalian Ric8, there is so far no evidence that *Dictyostelium* Ric8 in addition has a role as a chaperone for Gα proteins [51]. Both in mammals and in *Dictyostelium*, the regulation of Ric8 is still not completely understood. However, it has been shown that, in humans, RGS14 integrates conventional Gαi1 and Ric8A signaling, suggesting the presence of a heterotrimeric G protein regulator complex that contains both GAP and GEF activity [52].
2.2.2. Regulation of $G_\alpha$ Signaling by RGS

The heterotrimeric G protein signal is terminated by hydrolysis of $G_\alpha$-bound GTP by the intrinsic GAP activity of $G_\alpha$ subunits assisted by RGS proteins (Regulators of G protein Signaling) [53–55]. So far, more than 30 family members have been recognized that all contain a conserved RGS domain of approximately 130 amino acid residues in length which interact with active $G_\alpha$ subunits [56]. RGS proteins can regulate $G_\alpha$ signaling pathways in three ways: (i) they act as GAPs for $G_\alpha$ by stimulating the low intrinsic GTPase activity [57]; (ii) they act as effector antagonists that inhibit G proteins from binding to their effectors [58]; and (iii) they enhance the affinity of $G_\alpha$ subunits for $G_\beta\gamma$ subunits after GTP hydrolysis, thereby accelerating reformation of the inactive heterotrimeric complex [57].

Several RGS proteins have been identified that play an important role in the regulation of chemotaxis (Table 2). The Dictyostelium RGS domain-containing protein kinase 1 (RCK1) has been described as a negative regulator of Dictyostelium chemotaxis, however the mechanism remains to be determined [59]. Human RGS1 and RGS3 are important for chemotaxis of germinal center B cells towards lymphoid tissue chemokines [37,39]. Overexpression of RGS1 and RGS3 in B cells impaired the recruitment of B cells to inflammatory sites initiated by the two chemoattractants, lysophosphatidic acid (LPA) and platelet-activating factor (PAF). RGS1 accelerates heterotrimeric complex formation [60], while RGS3 probably acts as an antagonist, blocking the binding of $G_\alpha$ to its effector adenylyl cyclase [39]. Human RGS4 acts as GAP for $G_\alpha_i$ which is important for the migration of Mv1Lu cells to fibronectin [61]. The constitutive expression of RGS13, a GAP for $G_\alpha_q$, reduces B cells chemotaxis to a variety of chemoattractants, including CXC chemokine ligand 12 (CXCL12), CXCL13, and CC chemokine ligand 19 (CCL19) [62]. Reversely, the reduction of RGS13 expression enhances chemotactant signaling [62]. Consistently, RGS1/RGS13 double knock-out cells have a more polarized cellular morphology and improved chemotaxis towards CXCL12 [62]. Interestingly, $G_\beta\gamma$ is also important in $G_\alpha$ signal termination by recruitment of the R7 family of RGS proteins (RGS6, 7, 9 and 11), and subsequent R7-$G_\beta_5$ dimerization which increases the GTPase activity of $G_\alpha$ subunits of the $G_\alpha_i$ family [63].

2.2.3. Regulation of $G_\alpha$ Signaling by GDIs

Guanine nucleotide dissociation inhibitors (GDIs) are the third class of regulators of the heterotrimeric G protein cycle. $G_\alpha$ specific GDIs possess one or more highly conserved 19-amino acid polypeptide GoLoco (“$G_\alpha_i/o$-Loco” interaction) motifs that specifically interact and inhibit the nucleotide exchange of $G_\alpha$ proteins [64,65]. The GoLoco motif has been identified in several diverse proteins, including mammalian RGS12 and RGS14, Purkinje cell protein-2, Rap1GAP and GPSM2/LGN (Table 2, [65]).

Mammalian Rap1GAP possesses both a Rap1-specific GTPase-activating protein domain and a GoLoco motif. Previously it was shown that Rap1GAP binds $G_\alpha_z$ in its GTP-bound state [66]. $G_\alpha_z$-mediated recruitment of Rap1GAP attenuated the Rap1-mediated Rap1/B-Raf/ERK signal transduction cascade, which stimulates cell migration [67].

Activator of G protein signaling 3 (AGS3) is another well characterized GDI for $G_\alpha_i$ [68]. AGS3 together with its homolog LGN translocates to the leading edge to form a $G_\alpha_i$-GDP-AGS3/LGN complex. This complex simultaneously binds to mlnsc, which results in mlnsc-mediated targeting of the Par3-Par6-aPKC complex to pseudopods at the leading edge to regulate directionality during neutrophil chemotaxis [69].

2.2.4. Regulation of $G_\beta\gamma$ Signaling

In addition, signaling of $G_\beta\gamma$ is tightly regulated by various mechanisms. Phosducin has both been reported as an inhibitor and activator of $G_\beta\gamma$ signaling [70]. Initial reports showed that phosducin competes with downstream effectors and $G_\alpha$ for binding to free $G_\beta\gamma$ and thereby thus down regulates the chemotaxis response [71]. In contrast, recently phosducin-like protein 1 (PhLP1) was shown
to enhance Gβγ signaling by acting as a cochaperone assisting in the assembly of Gβ and Gy into a functional Gβγ complex [72]. Consistently, Dictyostelium cells lacking phlp1 have strongly impaired heterotrimeric G protein signaling and are unable to chemotax [73,74].

Another regulator of Gβγ is a receptor of activated C kinase 1 (RACK1), which inhibits interaction between Gβγ and the downstream effectors PLCβ and PI3Kγ, therefore overexpression of RACK1 or RACK1 fragments leads to decreased leukocyte chemotaxis [75]. Conversely, WD40-repeat containing protein 26 (WDR26) might act as a positive regulator of Gβγ signaling functioning as a scaffold that recruits and translocates Gβγ effectors, suppression of WDR26 in HL60 cells resulted in loss of directionality and cell migration speed. Moreover, WDR26 suppression blocks RACK1 interaction with Gβγ, indicating that RACK1 functions downstream of WDR26 [76].

2.3. Heterotrimeric G Protein Activated Chemotaxis Pathways

As described above, amplification and cell polarization during chemotaxis is established downstream of receptor binding and heterotrimeric G protein activation. Recent studies have identified a complex network of GPCR regulated interconnecting signaling pathways that lead to the intracellular amplification of the extracellular chemoattractant gradient that includes the preferential activation of monomeric G proteins at the leading edge, major changes in the cytoskeleton with actin polymerization at the leading edge, and actin-myosin filament assembly at the rear and sides of the cell [3]. The new actin filaments induce the formation of local pseudopodia, while the acto-myosin filaments inhibit pseudopod formation in the rear and retract the uropod; in this way, coordinated cell movement is achieved [9]. Many chemotaxis pathways that are directly regulated by Gβγ have been identified (Figure 3, [77,78]); however, we are only beginning to understand whether Gα-GDP/GTP exchange mediates downstream signaling mainly through the release of Gβγ and/or whether distinct signaling pathways are regulated through Gα-GTP and/or Gα-GDP subunits.

![Figure 3](image-url)
2.3.1. Gβγ Mediated Chemotaxis Pathways

Many studies have suggested that the Gβγ dimer functions as the main transducer of chemotactic signals [79]. Deletion of the single Gβ gene in Dictyostelium completely impairs the chemotactic response [80]. In Dictyostelium, the released Gβγ interacts with PI3K in a Ras-dependent manner [81]. The product of PI3K, PtdIns(3,4,5)P3 (PIP3), then accumulates asymmetrically at the leading edge of chemotaxing cells and provides a binding site for a subset of PH-domain-containing proteins, including cytosolic regulator of adenyl cyclase (CRAC) protein and PKB/Akt (protein kinase B). CRAC acts as a stimulator for the adenyl cyclase (ACA), which leads to the production of cAMP that is rapidly secreted to stimulate neighboring cells, a process that is essential for Dictyostelium development [82,83]. PKB/Akt contributes to the regulation of cell polarity and chemotaxis through the activation of PAKα, which is required for myosin II assembly at the rear of chemotaxing cells [84]. In addition PIP3 activates Rac small G proteins through Rac specific GEFs, resulting in the regulation of Wiskott–Aldrich syndrome protein (WASP) and the SCAR/WAVE complex [85], which stimulate the Arp2/3 complex that is required for the production of branched filaments and pseudopod extension [86]. Moreover, a PH domain-containing protein A (PhdA) is another PI3K interacting PH-domain-containing protein that regulates actin polymerization and cell polarization [87].

Despite many efforts, so far, only ElmoE has been identified as direct Gβγ effector in Dictyostelium [88]. Gβγ mediated activation of ElmoE promotes actin polymerization at the leading edge of chemotaxing Dictyostelium cells via the association of Dock-like proteins that function as activator for small G proteins of the Rac family [88]. In mammalian neutrophils, in the presence of PIP3, Gβγ can directly bind and activate P-Rex1 [89] and PI3Kα [90], which are GEFs for Rac and Cdc42, respectively. This results in a gradient of activated Rac and Cdc42 to activate the WASP and SCAR/WAVE complex at the leading edge, which is important for the regulation of cell motility and migration of neutrophils [59,63]. Additionally, Dock2, another PIP3-dependent GEF for Rac, has been described to play a predominant role in regulating leading edge formation [91,92]. Activated Rac and Cdc42 together with polymerized actin act as a positive feedback loop that recruits more PIP3 in the leading edge of the cell; however the mechanism of how PIP3 is recruited still remains unclear [93]. A recent study reveals that Gβγ can directly activate phospholipase PLCβ and its effector PKC (protein kinase C) [94]. Further work demonstrates that both PLCβ-PKC and PI3Kγ-Akt signaling pathways can phosphorylate and deactivate Glycogen synthase kinase 3 (GSK3) that attenuates GSK3-mediated inhibition of the cofolin phosphatase SSH2 (slingshot2) and dephosphorylation of cofolin, leading to fMLP-induced neutrophil polarization and actin cytoskeleton remodeling [94].

2.3.2. Gα Mediated Chemotaxis Pathways

For a long time, Gα subunits were only considered as a “timer” to govern Gβγ signaling by regulating the release and reassociation of the Gβγ dimer. However, recently the first mammalian Gα effectors important for chemotaxis have been reported (Figure 3, [69]), including Homer3 that we identified as a novel Gαi2-binding protein that spatially organizes actin assembly to support polarity and motility during neutrophil chemotaxis [95]. Gαi2 also interacts with the Elmo1/Dock180 complex functioning as a RacGEF to activate Rac1 and Rac2 proteins, thereby inducing actin polymerization and cell migration [96]. Additionally, mInsc indirectly binds Gαi2-GDP via LGN/AGS3 and helps maintain directionality in neutrophils through its downstream effector of Par3-Par6-aPKC complex [69]. Furthermore, the Gα12-mediated mTORC2 (mammalian TORC2)-PKC signaling pathway is critically important for LPA induced fibroblast migration [97]. Interestingly, via the Rho specific GEF, p115RhoGEF, Gα12/13 is able to activate Rho and its effector kinase ROCK, leading to myosin II assembly at the rear of chemotaxing cells [98,99]. Gα-mediated pathways thus seem to be important for both the signaling at the leading edge and rear of the cell. Surprisingly, the ELMO1/Dock180 complex interacts specifically with Gα12-GTP [99], while LGN/AGS3 specifically interacts with Gα12-GDP at the leading edge of migrating neutrophils [69]. This might suggest that free Gα can activate downstream pathways independent of their nucleotide bound state.
3. Conclusions

Tight regulation of GPCR and heterotrimeric G protein signaling on several levels is essential for proper chemotaxis. Receptor affinity and expression is strictly regulated, thereby increasing the range of concentrations to which cells can respond. Recent studies have shown the importance of non-canonical regulators for chemotaxis: RGS, GDI and GEF proteins either extend or shorten the lifetime of Gα-GTP, thereby fine-tuning the chemotactic response. Accumulating evidence suggests that both Gα and Gβγ have their separate downstream pathways; however, which nucleotide bound state of Gα transduces the signals and the exact link between heterotrimeric G protein activation and cytoskeletal rearrangements remains to be determined. It is of high interest to fully understand the regulations and intracellular targets of chemotaxis receptors, which could help in finding new drug targets for several diseases, including cancer, asthma and rheumatoid arthritis.

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References


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Aim of this thesis

Rap1, a member of the Ras superfamily and has been implicated in the regulation of various important processes, including cytokinesis and chemotaxis (Jeon et al., 2007a, 2007b; Plak et al., 2014). Despite extensive investigations conducted on Rap1 signaling, only a few Rap1 regulators and downstream effectors have been unveiled in chemotaxis. Potentially there are many unknown proteins to be identified, serving as activators, inhibitors, or effectors in a Rap1-participated chemotactic singling network. The aim of this thesis therefore is to discover and characterize more regulators implicated in *Dictyostelium discoideum* Rap1 signaling pathways. In Chapter 2 of the thesis we identified GflB as a novel Ga2 stimulated Rap1 specific GEF in a proteomic screen by using purified *Dictyostelium* Ga2 protein as a bait (Kataria et al., 2013). Further investigation revealed that GflB contributes to efficient directional sensing and cell movement during chemotaxis. More importantly, GflB plays an important role in regulating the balance between Ras- and Rap1 signaling. It therefore serves a direct connection between heterotrimeric G protein and monomeric G protein signaling. Subsequently in Chapter 3, we identified two additional GEFs for Rap1, GefQ and GefL, that together with GflB and the previously described GbpD regulate Rap1 activation during *Dictyostelium* development and life cycle. Interestingly, Rap1 activation is not only regulated by specific proteins, but also the cytoskeleton as described in Chapter 4. Conclusively, our data contributes to a better understanding of Rap1 regulation and function in *Dictyostelium* chemotaxis.

References


