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Chemotaxis in the Model Organism *Dictyostelium discoideum* and Human Neutrophils

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Chemotaxis is referred as directional cell migration guided by chemoattractant gradients and plays critical roles in many physiological processes, including neuron patterning [1], the recruitment of neutrophils to sites of inflammation [2], metastasis of cancer cells [3], and development of model organism *Dictyostelium discoideum* [4]. All eukaryotic cells detect chemoattractants by G protein-coupled receptors (GPCRs) and share remarkable similarities in the signaling pathways which control chemotaxis [5]. *D. discoideum* has been proven as a powerful model system to identify new components essential for chemotaxis. During postdoc training, I developed and applied the state-of-the-art live cell/single molecule imaging techniques to visualize spatiotemporal dynamics of GPCR-mediated signaling network that leads to the chemotaxis in *D. discoideum* [6,7]. The interplay between computational simulation and experimental verification, my studies have revealed a locally-controlled inhibitory mechanism in the GPCR signaling network upstream of PI_3K [8]. Ras is a key component of the chemosensing machinery upstream of PI3K. My long-term research interests is to investigate molecular mechanisms underlining chemotaxis in multiple systems: *first,* identify novel components and signaling pathways essential for chemotaxis using model organism *D. discoideum;* next, understand the roles of their mammalian counter partners in mammalian systems to identify new therapeutic strategies for inflammatory diseases and metastasis of breast cancer.

Introduction

All eukaryotic cells detect chemo attractants by G protein-coupled receptors (GPCRs) and share remarkable similarities in the signaling pathways which control chemotaxis. In mammals, chemoattractants bind to their receptors (GPCRs) to trigger the dissociation and activation of heterotrimeric G-proteins consisting of Gαi and Gβγ, which, in turn, regulate a variety of signaling pathways involved in chemotaxis. GPCR-mediated pathways leading to chemotaxis are best known in *D. discodieum*. Binding of chemoattractant cAMP to its receptor cAR1 induces the dissociation of hetro-trimeric G-proteins into Gα2 and Gβγ subunits [9]. Free Gβγ activates the small G-protein Ras, leading to the activation of PI3K by which phosphorylates membrane phospholipid PIP_2 to PIP_3 [10]. Once generated, PIP_3 mediates intracellular polarization by recruiting proteins with Pleckstrin Homology (PH) domains to the plasma membrane [11]. Among these proteins are cytosolic regulator of adenylyl (CRAC), protein kinase B (PKB) and myosin I proteins (actin motors), which play roles in the regulation of actin polymerization during chemotaxis. PIP_3-indepdedent pathways involving PLA2 and cGC have also been implicated in chemotaxis in *D. discoideum* [12]. Active Ras proteins control the TorC2-PKB pathway, which signals to the actin cytoskeleton [13]. Reverently, we revealed an evolutionary conserved pathway in which G-protein subunits directly associate with an Elmo/dock complex, which serves as GEF (guanine exchange factor) to activate Rac, thereby promoting actin polymerization first in chemotaxis of *D. discoideum* [14,15] and then in chemokine-mediated metastasis of breast cancer cells [16] (Figure 1).

Recent Major Findings

Interplay of computation simulation and experimental verification reveals novel components and new signaling events in GPCR-mediated chemotaxis in *D. discoideum*

During postdoc training in NIH, I have developed and applied the state-of-art live cell imaging techniques to monitor spatiotemporal dynamics of many steps of signaling events in live single cells in real time. I measured cAMP binding to the cAR1 receptor, cAR1-induced G-protein dissociation using Fluorescence Resonance Energy Transfer (FRET) imaging, dynamic translocation of GFP tagged PI3K and PTEN and their net enzyme activity by monitoring the dynamic membrane production of PIP_3 [17]. These spatiotemporal dynamics of signaling components has provided perimeters for computational modeling. Combining these dynamics with computational simulation led to a better understanding of GPCR-signaling network at a system level [18,19]. My research suggests that inhibitory mechanisms that shut-down signaling from free Gβγ, Ras and then PI3K are essential for our observed dynamics [8]. Two major inhibitory signaling pathways for gradient sensing are adaptation of Ras signaling and redistribution of PTEN on the plasma membrane. Recently, I focus on understanding the molecular mechanism of Ras adaption in combining with computation simulation Ras signaling (Figure 2).

A computational simulation of GPCR-mediated Ras signaling during chemosensing: It is not clear how GPCR/G-protein machinery regulates spatiotemporal dynamics of Ras activation to achieve these cellular responses. We first measured spatiotemporal dynamics of Ras activation in *D. discoideum* cells in response to various cAMP stimuli using live cell imaging methods. Our quantitative measurements demonstrate that different signaling events downstream of GPCR have distinct kinetic patterns, and provide a foundation for modeling to understand how these events are linked to each other to produce chemotactic responses. We then constructed a spatiotemporally resolved model of cAR1-mediated Ras signaling network based on detailed molecular interactions, using the computer interfaces of SIMMUNE, a software package that allows biologists to construct computational models of a signaling network without dealing with mathematical equations. We then carried out computer simulations that test performance of a model in response to various stimuli, using SIMMUNE. These analyses allowed us to evaluate molecular mechanisms of Ras regulators,

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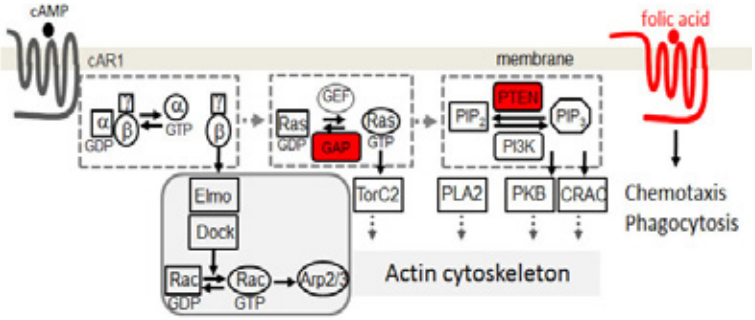

Figure 1: GPCR-mediated signaling pathways leading to chemotaxis.

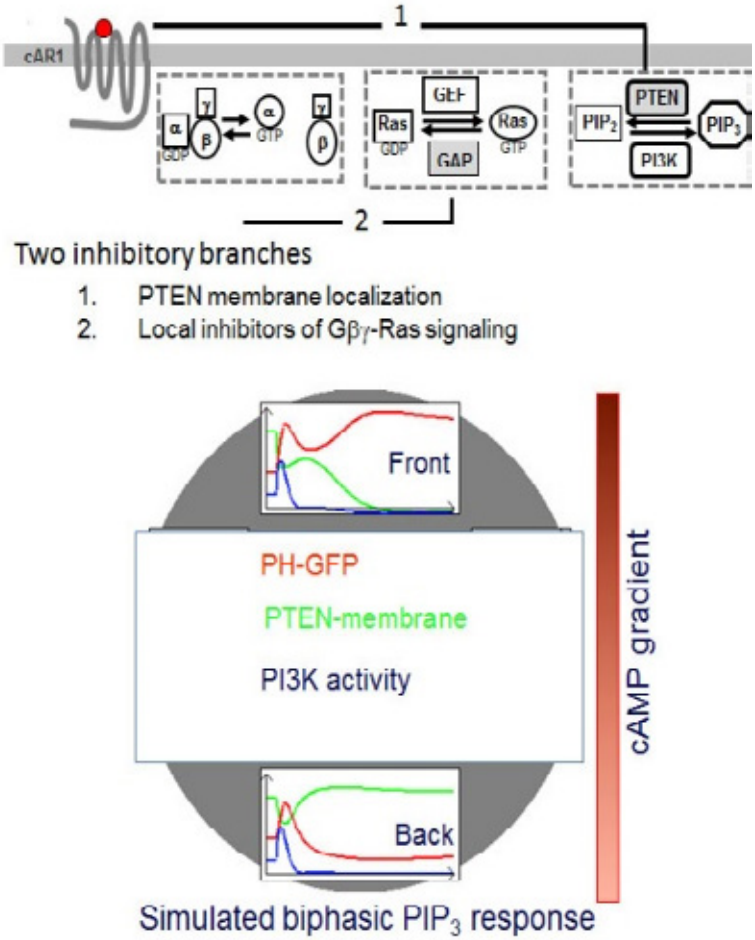

Figure 2: Two major inhibitory signaling pathways for gradient sensing.

RasGEF and RasGAP, by incorporating different molecular mechanisms into the models. We examined dynamic behaviors of potential RasGAP regulatory mechanisms in a GPCR-mediated Ras signaling network in different models *in silico*. Our detailed computational models allow us to reveal how a GPCR-mediated signaling network organizes at a molecular level, dynamically encodes information at each signaling steps, and systematically produces outputs to achieve temporal adaptation and spatial amplification in chemo-attractant gradient sensing (Figure 3).

GPCR-Mediated PLC/PKC/PKD Signaling Pathway Regulates the Cofilin Phosphatase Slingshot2 in Neutrophils Chemotaxis

Directional cell migration requires precisely coordinated polymerization and de-polymerization of the actin cytoskeleton at leading fronts of cells. Cofilin is one well-known F-actin depolymerization factor (ADF). The activity of cofilin is regulated mainly through phosphorylation and dephosphorylation: phosphorylation at Ser-3 by LIM kinases and testicular protein kinases

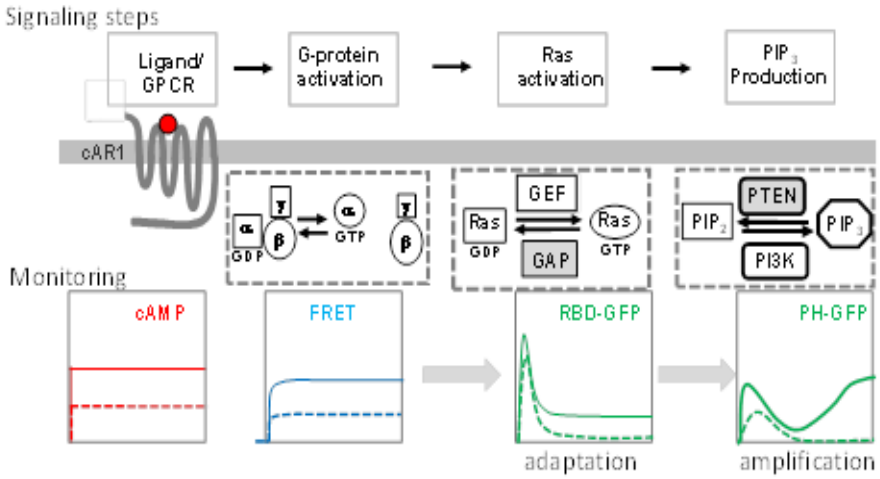

Figure 3: GPCR-mediated signaling pathway to regulate Ras activation and PIP_3 production during gradient sensing.

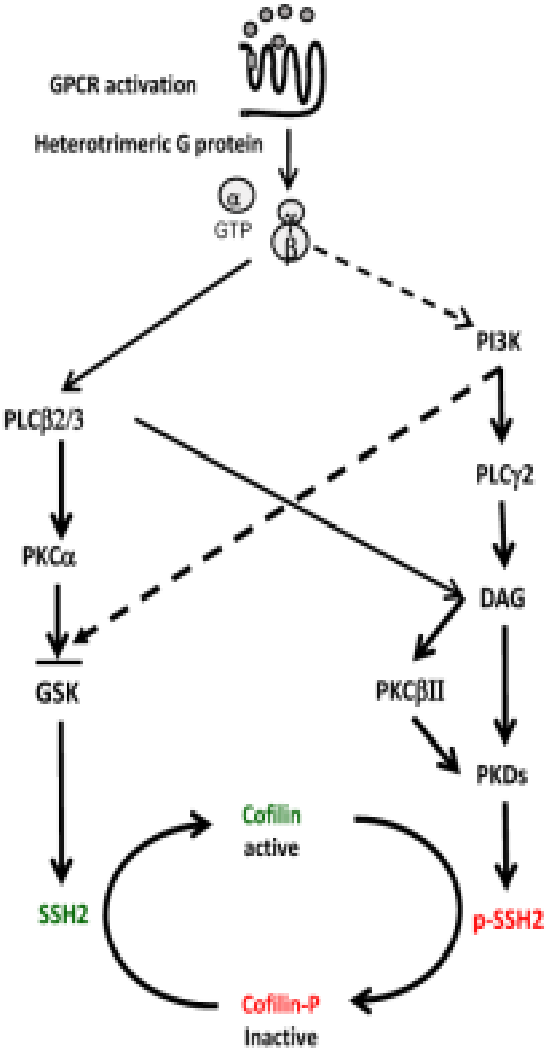

Figure 4: GPCR-mediated PLC/PKC/PKD signaling pathway regulates the cofilin phosphatase slingshot2 in neutrophils chemotaxis.

(TESKs) inhibits its actin binding, severing, and depolymerizing, and dephosphorylation at Ser-3 by slingshot proteins (SSHs) and chronopin (CNP) reactivates cofilin. In neutrophils, chemoattractants induce rapid dephosphorylation of cofilin. However, how chemokine GPCR controls F-actin de-polymerization remains largely elusive [20,21]. We revealed a novel signaling pathway, consisting of Gαi, PLC, PKCβ, PKD and SSH2, in control of cofilin phosphorylation and actin cytoskeletal reorganization, which is essential for neutrophils chemotaxis [22]. We showed that PKD is required for neutrophil chemotaxis and the chemokine GPCR-mediated PKD activation depends on PLC/PKC singling. We discover that activation of chemokine GPCRs recruits and activates PLCγ2 in a PI3K-dependent manner. We verify that PKCβ interacts with PKD1 and is requires for chemotaxis. Furthermore, we identify slingshot 2 (SSH2) as a target of PKD1 that regulates cofilin phosphorylation and remodeling of the actin cytoskeleton during neutrophil chemotaxis. Taken together, we discover a new pathway that transduces signals from chemokine GPCRs to control de-polymerization of the actin cytoskeleton for neutrophil chemotaxis (Figure 4).

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