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Editorial
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G Protein Coupled Receptors-Mediated Chemotaxis in the Model Organism *Dictyostelium Discoideum* and Neutrophils

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Editorial

Chemotaxis, a directional cell migration guided by extracellular chemoattractant gradients, plays critical roles in many physiological processes, recruitment of neutrophils to sites of inflammation, metastasis of cancer cells, and development of model organism *Dictyostelium discoideum* [1-4]. Both *D. discoideum* and mammalian neutrophils sense chemoattractants using G protein-coupled receptors (GPCRs) and share remarkable similarities in the signaling pathways by which regulate cell migration Jin et al. [5]. It has been proven that *D. discoideum* is a powerful model organism to establish new concepts and identify new components essential for chemotaxis [6,7]. We developed and applied state-of-the-art live cell/single molecule imaging technologies to visualize spatiotemporal dynamics of GPCR-mediated signaling network in *D. discoideum* [8,9]. We also built up chemosensing signaling network for computational modeling [10]. By interplaying computational simulation and experimental verification (Figure1), we revealed locally-controlled inhibitory mechanism upstream of PI_3K that is essential for chemosensing [9,11,12]. Recently, we are focusing on identifying the inhibitors, such as negative regulators of Ras, for chemosensing in both *D. discoideum* and mammalian neutrophils. Our long-term goal is to identify novel components and signaling pathways essential for chemotaxis to provide new therapeutic targets and strategies for inflammatory diseases and metastasis of cancer.

Introduction

All eukaryotic cells sense chemoattractants by G protein-coupled receptors (GPCRs) and share remarkable similarities in the signaling pathways which mediate chemotaxis. The knowledge of GPCR-mediated signaling pathways leading to chemotaxis are mostly from *D. discoideum* and neutrophils. In both system, the binding of chemoattractants to their receptors induces the dissociation of heterotrimeric G-proteins into Gα and Gβγ subunits, which, in turn, activate multiple pivotal effectors, such as small GTPase Ras, phosphatidylinositol (PtdIn)-3 kinases (PI3K), and phospholipase C (PLC) [13-21]. PI3K phosphorylates membrane phospholipid PIP_2 to PIP_3. The generated PIP_3 mediates intracellularly polarized localization and activation of the proteins with Pleckstrin Homology (PH) domains, such as cytosolic regulator of adenylyl (CRAC), protein kinase B (PKB), and myosin I proteins (actin motors) [13,14,17,18,20,22]. PIP_3-indepdedent pathways involving PLA2 and cGC have also been implicated in *D. discoideum* chemotaxis [23,24]. All together, they control the reorganization of the actin-myosin cytoskeleton for a directed cell migration [25-30]. The key feature of chemotaxis in both *D. discoideum* and neutrophils is that cells sense a large range of chemoattractant concentrations (about 10^{-9} to 10^{-5} M) by applying a mechanism called adaptation [31,32]. Adaptive cell no longer responds to the present stimuli but still remains sensitive for higher concentration stimuli Hoeller et al. [31]. GPCR-mediated adaptive behavior occurs in many steps of GPCR-mediated signaling pathway of chemotaxis, such as activation of PLCβ2/3 and Ras [18,21,33-37] PI3K/PTEN-mediated transient PIP_3 production [15,17,18,37,38] Rho/Rac activation and actin polymerization [39-41] indicating that adaptation is an fundamental strategy cell apply to chemotax across huge range of chemoattractant concentrations. To explain adaptation (Figure 2), different models agree upon the temporal dynamics of adaptation: an increase in receptor occupancy activates two antagonistic signaling processes: a rapid "excitation" that triggers cellular responses and a temporally delayed "inhibition" that terminates the responses to reach adaptation [2,6,12,26,42,31]. Many excitatory components has been identified, however, the inhibitors and their function in chemosensing and directed cell migration are still largely elusive.

We developed and applied the state-of-art live cell imaging techniques to monitor spatiotemporal dynamics of GPCR-mediated signaling events in live single cells. In both *D. discoideum* and neutrophils, gradient sensing is able to be uncoupled with initial polarization and cell migration

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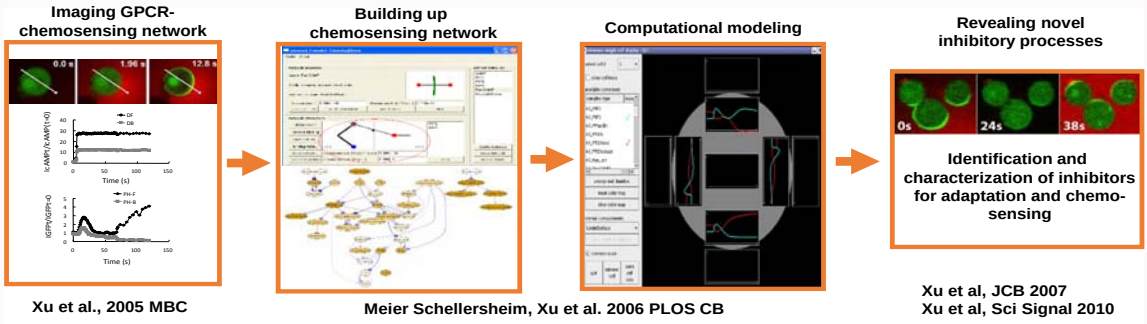

Figure1: The interplay between computational stimulation and experimental vitrification proposes a locally regulated inhibitory process, which is required for GPCR-mediated chemosensing.

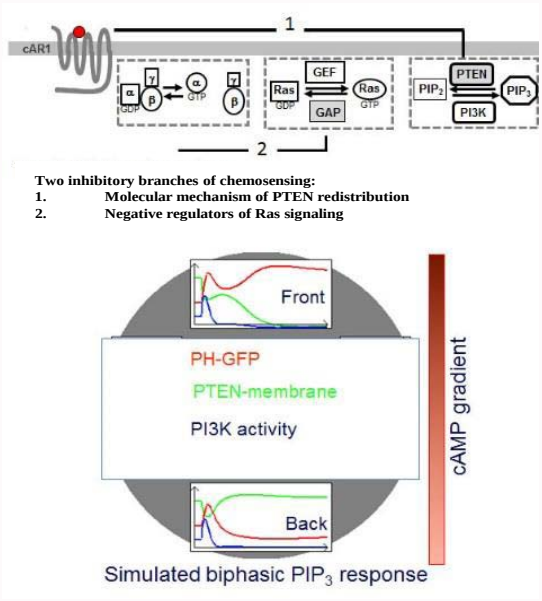

Figure 2: Two major inhibitory branches of chemosensing.

by actin polymerization inhibitor such as latrunculin [2,28]. Non-polarized immobile cells, hence, provides a simplified cell system for simultaneous monitoring multiple signaling events at subcellular level in real time [8]. We systematically measured the binding of cAMP binding to its receptor cAR1 by visualizing single receptor using total internal reflection (TIRF) microscopy [11], cAR1-induced dissociation of heterotrimeric G protein using Fluorescence Resonance Energy Transfer (FRET) technique [9], the dynamic membrane translocation of PI3K, PTEN, and PHcrac, a biosensor for PIP_3 [11,9,12]. The spatiotemporal dynamics of signaling components have provided perimeters for computational simulation. Combining these dynamics with computational simulation led to a better understanding of GPCR-signaling network at a system level. Our findings suggest that a locally-regulated inhibitory mechanism, downstream of heterotrimeric G protein but upstream or at PI3K/PTEN, is essential for gradient sensing. We postulate two types of inhibitors for chemosensing: 1) negative regulators for Ras signaling; 2) components regulates the redistribution of PTEN dynamics. Recently, we focus on understanding the molecular mechanism of Ras adaption and PTEN membrane localization.

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