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# The PLCβγ/PKCβ/PKD Signaling Axis in GPCR-Mediated Neutrophil Chemotaxis

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**Abstract**

Chemotaxis, the directional cell migration guided by chemoattractant gradients, plays essential roles in many physiological processes, such as recruitment of neutrophils to sites of inflammation. Neutrophils detect chemoattractants by G protein-coupled receptors (GPCRs). Chemoattractant stimuli activate multiple signaling pathways to regulate directional migration of neutrophils. Recently, we identified a novel GPCR-mediated PLCβγ/PKCβ/PKD1 signaling axis that regulates cofilin activity through cofilin phosphatase slingshot 2 (SSH2) and remodels actin cytoskeleton during neutrophil chemotaxis. In the future, it will be important to understand how multiple signaling pathways are spatiotemporally regulated to precisely control the rapid remodeling of actin cytoskeleton in the leading front of chemotaxing neutrophils.

**Keywords:** Chemotaxis; G protein-coupled receptor; Phospholipase C; Protein kinase C; Protein kinase D; Cofilin; Slingshot 2

## Abbrevieations

GPCR: G Protein-Coupled Receptor; PLC: Phospholipase C; PKC: Protein Kinase C; PKD: Protein Kinase D; SSH2: Slingshot 2

## Introduction

Many eukaryotic cells detect and migrate toward chemoattractants, a process known as chemotaxis. Chemotaxis plays important roles in many physiological processes, including recruitment of neutrophils to sites of inflammation, neuron pattering, and metastasis of cancer cells. Chemotaxing neutrophils display polarized morphology. The migrating cells extend their leading edges by assembling a force-generating actin network beneath the plasma membrane. Actin also collaborates with myosin to retract the rear of migrating cells and prevent errant pseudopod extension. Over the last decade, multiple signaling pathways have been identified that control GPCR-mediated reorganization of actin cytoskeleton in directional cell migration. At the leading edge, signaling pathways activate the Arp2/3 complexes that initiate the formation of new branches of actin filaments. In neutrophils, chemokines detected by GPCRs regulate multiple signaling pathways to activate the Rho family of small GTPases (cdc42 and Rac1/2) to promote the growth of actin filaments (F-actin) [1-7]. Depolymerization of F-actin is also essential for controlling F-actin dynamics during cell migration. The family of actin-depolymerizing factor (ADF)/cofilin proteins is comprised of cofilin-1 (a non-muscle type of cofilin), cofilin-2 (a muscle type of cofilin), and ADF (also known as destrin) in mammals [8]. They bind to both monomeric G-actin (globular actin) and F-actin and mediate the dynamic reorganization of the actin cytoskeleton by stimulating the severance and depolymerization of actin filaments [9]. Cofilin also contributes to F-actin assembly by increasing the actin monomer concentration for polymerization and consequently increasing the turnover rate of actin filaments in cells [10]. Active cofilin severs actin filaments and creates new barbed ends for actin polymerization [11]. Cofilin might also increase new barbed ends by its intrinsic nucleation activity [12]. However, phosphorylation is the most important and best studied mechanism of regulating cofilin activity. LIM kinases (LIMKs) and testicular protein kinases (TESKs) phosphorylate cofilin to deactivate it while slingshot proteins (SSHs) and chronophin dephosphorylate p-cofilin to activate it [8]. In neutrophils, however, the mechanism by which GPCR signaling regulates cofilin activities and cofilin-mediated actin polymerization/de-polymerization has only recently begun to be revealed.

## PLC Activation in Response to Chemoattractant Stimuli in Neutrophils

Phospholipase C (PLC) activation is an early event in the response to numerous extracellular stimuli. Upon activation, PLC produces two important second messengers: diacylglycerol (DAG) and $IP_3$. Both DAG and $IP_3$ play important roles in many signaling pathways, including the induction of calcium influx [13] and the activation of downstream effectors, such as protein kinase C (PKC) and protein kinase D (PKD) [14]. Alterations of PLC isozymes are associated with several diseases, such as dysfunction in innate and adaptive immunity [15,16], brain disorder [17], and cancers [18,19]. Mammalian neutrophils express PLCβ2, -β3, and -γ2 [20]. In murine neutrophils, chemoattractant stimulation robustly activates both PLCβ2 and PLCβ3 [4]. However, the evidence for the roles of PLC signaling in neutrophil chemotaxis are contradictory. Murine neutrophils lacking both PLCβ2 and PLCβ3 still chemotax fairly well [4]. Some leukocytes with a single PLCβ2 deficiency actually have enhanced chemotaxis ability [21]. These results led to the assumption that PLC signaling might not be required for neutrophil chemotaxis. However, a PLCβ/PI3Kγ/GSK3 signaling pathway has been reported to regulate the activity of cofilin phosphatase SSH2 and neutrophil chemotaxis [22]. We recently showed that inhibition of PLC activity significantly reduces chemotaxis of human neutrophils, suggesting an essential role of PLC signaling in neutrophil chemotaxis [23]. In addition to PLCβ2/3, GPCRs mediate the membrane targeting and subsequent activation of PLCγ2 in a

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PI3K-dependent manner [23], providing an explanation for the normal chemotaxing behaviors observed in murine neutrophils with a single or double PLCβ2/3 deficiency. In neutrophils, PLCβ/γ2 might also be activated by other mechanisms. It has been reported that PLCβ2/3 and PLCγ2 are specifically activated by small GTPase Rac1 [24] and Rac2 [25,26], respectively. In neutrophils, chemoattractant stimulation triggers robust activation of Rac1 and Rac2 [6,27]. Rac1 specifically regulates the chemotaxis compass, while Rac2 mainly controls actin polymerization. The Rac1-mediated PLCβ2/3 and Rac2-mediated PLCγ2 activation adds another layer of complexity to the existing signaling networks of PLC and Rac signaling (Figure 1).

## PKC Isoforms Involved in Neutrophil Chemotaxis

PKC is an important effector of PLC signaling. Neutrophils express PKCα, -βI, -βII, and -δ [28]. PKC isoforms share a similar overall structure, consisting of an $NH_2$-terminal regulatory domain joined through a flexible linker to a conserved COOH-terminal catalytic domain that binds ATP and substrates [14]. Various stimuli activate all four PKC isoforms, and the activation of PKC is required for the assembly and activation of NADPH oxidase and oxidative burst [29-31]. PKC isoform-specific functions in neutrophil chemotaxis have been revealed only in recent years. Both PKC-α and -β are conventional PKCs that translocate and are subsequently activated in a PLC-dependent manner in neutrophils [22,23]. However, PKCα and -β interact with and activate different effectors to regulate neutrophil chemotaxis. GSK3, a substrate of PKCα, phosphorylates SSH2 and decreases its cofilin-phosphatase activity. In resting neutrophils, GSK is active and suppresses SSH2 activity to maintain cofilin in an inactive, phosphorylated state. Upon fMLP stimulation, PKCα phosphorylates GSK3 and inhibits its activity, consequently increasing SSH2 activity [22]. Recently, we showed that PKCβ plays an essential role in neutrophil chemotaxis [22]. We identified PKD1 as a PKCβ substrate that phosphorylates SSH2 and inhibits its phosphatase activity. PKCβII has also been found to specifically phosphorylate and activate AC9 to mediate the trailing edge contraction in chemotaxing neutrophils [31]. The membrane translocation and activation of PKCβII was decreased by inhibiting mTORC2 activity through Rictor knockdown. It is not clear whether Rictor knockdown affects PLC activation. However, mTOR-mediated membrane translocation and activation provides a DAG-independent mechanism of activating PKCβII. PKCδ is a novel PKC isoform that lacks the C2 domain [29]. PKCδ translocates to the plasma membrane through the binding of its C1a domain with DAG or phorbol esters [32] and is involved in the oxidative burst in neutrophils [33,34]. Recently, it has been reported that PKCδ is required for neutrophil transmigration mediated by IL-1β and fMLP (integrin-dependent), but not IL-8 (integrin-independent), by regulating adherence of neutrophils [35]. However, the molecular mechanism of PKCδ's function still remains unclear. The PKC isoform-specific function in neutrophil chemotaxis is still not fully understood.

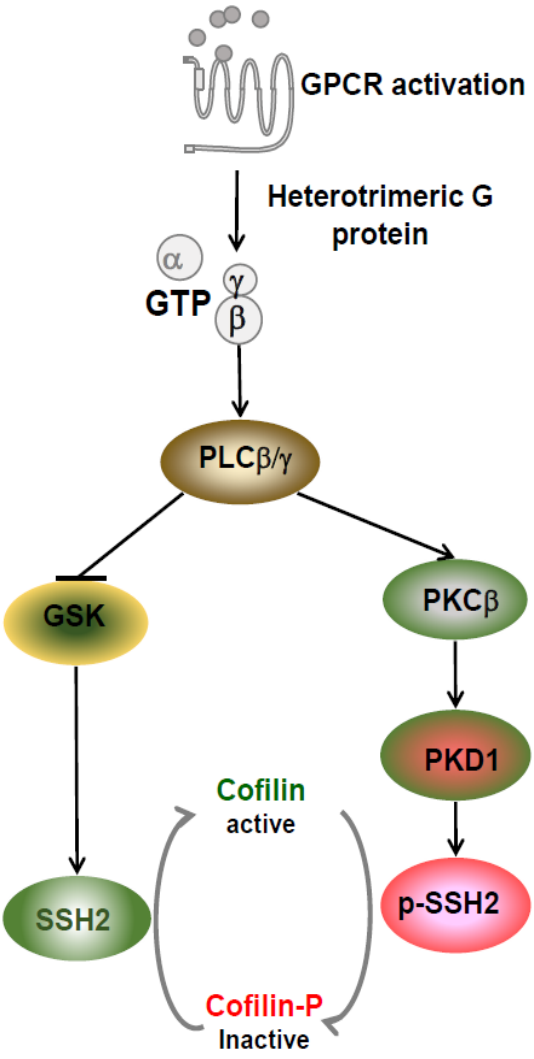


**Figure 1:** Scheme shows the signaling pathways in which PKD1 phosphorylates cofilin phosphatase SSH2 to regulate cofilin activity in GPCR-mediated chemotaxis of neutrophils."

## Cofilin Activation and its Regulation in Neutrophils

Cofilin activity is regulated mainly through phosphorylation: phosphorylation at Ser-3 by LIMKs and TESKs deactivates cofilin, and dephosphorylation at this site by SSHs and chronophin reactivates it [8]. In neutrophils, chemoattractants induce a rapid and transient dephosphorylation of cofilin [36]. Chemoattractant-mediated dephosphorylation of cofilin at Ser3 is required to initiate actin-mediated chemotaxis in leukocytes [6,37]. Recently, it has been shown that the chemoattractant-mediated PLCβ/PI3Kγ-GSK3 pathway relieves phosphorylation and inhibition of cofilin phosphatase SSH2, and hence reduces the level of p-cofilin in neutrophils (Tang et al., 2011). Compared to the ubiquitous expression of SSH1 in many tissues, SSH2 is the major isoform expressed in mammalian neutrophils [22,23]. SSH2 efficiently dephosphorylates p-cofilin [22,23,38]. Hirayama and his coworkers used HL60 cells to study the cofilin activation cycle and demonstrated a clear activation cycle [39]. The activation cycle of cofilin is especially important at the leading front, where rapid polymerization and depolymerization of F-actin cytoskeleton are required. We discovered a GPCR-mediated PLCβγ/PKCβ/PKD signaling pathway that increases the level of p-cofilin to complete the cycle of cofilin activation in neutrophils [23]. PKD is a family of serine/threonine kinases that is highly expressed in neutrophils [28]. We found that chemo attractant stimuli trigger robust membrane translocation and activation of all three PKDs [23]. More importantly, PKD is essential for neutrophil chemotaxis. The membrane targeting of PKD1 requires DAG, the product of PLC activation. DAG also recruits PKCβ which phosphorylates Ser-744/Ser-748 in the PKD1 activation loop to activate it. Lastly, we discovered that active PKD interacts with and phosphorylates SSH2 to decrease its activity, leading to an increase in the level of p-cofilin to complete the cofilin activation cycle. In

conclusion, GPCR activation triggers two pathways to control the cycle of cofilin activity, which is essential for a rapid and coordinated cycling of F-actin polymerization and depolymerization at the leading edge of chemotaxing cells (Figure 1).

## Future Perspectives

It will be important to dissect the spatiotemporal activation mechanism of PLC isoforms in neutrophils and its subsequent effects on neutrophil chemotaxis. Moreover, the signaling pathways and kinases that phosphorylate cofilin are still not fully understood in neutrophils. Above all, it will be particularly important to understand how tempo-spatially distinctive signaling pathways control the rapid and precisely coordinated remodeling of actin cytoskeleton in the leading front of chemotaxing neutrophils. A live probe to visualize cofilin activity in migrating cells is urgently needed.

## Conclusion

Chemoattractants trigger diverse signaling pathways to mediate cell migration. It is particularly important to understand how spatiotemporally distinctive activations of signaling pathways control the rapid and precisely coordinated remodeling of actin cytoskeleton in chemotaxing neutrophils. GPCR-mediated PLC activation induces two distinctive signaling pathways to achieve fast cycling between active and inactive cofilin in the leading front of chemotaxing neutrophils.

## Competing Interests

The authors state that they have no competing interests.

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