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Role of G protein-coupled receptor kinases in cell migration

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ABSTRACT

G protein-coupled receptor kinases (GRKs) are emerging as important integrative nodes in cell migration processes. Recent evidence links GRKs (particularly the GRK2 isoform) to the complex modulation of diverse aspects of cell motility. In addition to its well-established role in the desensitization of G protein-coupled receptors involved in chemotaxis, GRK2 can play a effector role in the organization of actin and microtubule networks and in adhesion dynamics, by means of novel substrates and transient interacting partners, such as the GIT-1 scaffold or the cytoplasmic α-tubulin deacetylase histone deacetylase 6 (HDAC6). The overall effect of altering GRK levels or activity on chemotaxis would depend on how such different roles are integrated in a given cell type and physiological context, and may have relevant implications in inflammatory diseases or cancer progression.

Introduction

Cell migration plays a central role in physiological processes as embryogenesis, wound healing or immunity, whereas aberrant cell motility underlies pathological conditions such as inflammation or cancer progression [1,2]. Chemotaxis integrates complex steps coordinated by transiently activated signalling networks, leading to cell polarization, membrane protrusion and generation of dynamic adhesion and traction forces [3, 4, 5].

Many G protein-coupled receptors (GPCR), such as those for chemokines, are involved in gradient sensing and cell migration [5,6]. Upon stimulation, GPCR initiate G protein-dependent signalling pathways and also become phosphorylated by serine/threonine kinases termed G protein-coupled receptor kinases (GRKs), of which GRK2 is the most ubiquitous and well-characterized [7,8]. This event promotes the association of arrestins, leading to GPCR desensitization and internalization. GRKs are emerging as complex regulators of cell migration processes, since their involvement in cell motility not only relies in their canonical role as "negative" modulators of GPCR signalling, but also involves novel substrates and interacting partners [8-11].

We discuss herein recent evidence linking GRKs (particularly GRK2) to the different functional facets of cell migration, how such diverse roles might be orchestrated in response to stimuli, and the implications of these findings in physio-pathological contexts.

The conundrum of GRK2 in migration: how does GRK2 actually modulate the chemotactic response?

Numerous reports have ascribed the effects of GRK2 on chemotaxis to its ability to trigger desensitization of particular chemokine receptors, thus controlling the intensity and duration of agonist stimulation [6,7,12]. Down-modulation of GRK2 levels increases chemotactic responses to different agonists in immune cell types, whereas its enhanced expression attenuates chemotaxis, consistent with its canonical negative role in GPCR signalling (Table 1).

However, the overall impact of GRK2 levels on cell migration is not straightforward and varies dependent on the stimuli and/or cell type considered [Table 1, refs. 8-11]. GRK2 down-modulation can lead to decreased migration of immune cell types towards certain stimuli [13], and this kinase plays a positive role in epithelial cell migration [14].

These heterogeneous results raise questions about the functional role of GPCR desensitization during directed migration, and suggests that the global effect of GRK2 on chemotaxis would depend on the integrated modulation of different steps of the chemotactic process (receptor sensing, cell polarization, membrane protrusion, adhesion/de-adhesion cycles) in given cell types and in response to specific stimuli.

GRK2 in gradient sensing: a role for receptor phosphorylation?

Directional sensing involves the conversion of graded receptor activation by chemoattractants into a sharp asymmetry of key downstream signalling components. PI3K and PTEN (besides other redundant pathways), are redistributed towards and away the gradient, respectively, allowing for local accumulation of $PI(3,4,5)P_3$,, what serves to specify pseudopodia formation and to guide cell movement [15,16].

This response is extremely sensitive and localized $PI(3,4,5)P_3$ production depends only on the gradient steepness [15, 16]. In steep gradients, cells generate a single, well-defined pseudopodium and move straight towards the signal source. Conversely, in shallow gradients, chemotaxis is mediated by biased choices between randomly projected pseudopodia, resulting in a tortuous and time-consuming tracking [3,5,16]. In steep gradients, high local fraction of receptor occupancy versus lower global occupancy would favour the generation of internal asymmetry [15]. In this context, it is tempting to suggest that GRK2-triggered receptor desensitization/internalization might influence how receptors sense the strength and steepness of chemotactic gradients (less GRK2, steeper, more GRK2, weaker gradients, see detailed model in Figure 1).

Consistently, GRK2 levels are important for defining the stop signal of neutrophil migration [17]. These cells adopt a random walk and cease directed locomotion when exposed to saturating concentrations of fMLP, which triggers receptor desensitization/internalization in a GRK2-dependent manner. Therefore, upon GRK2 silencing cessation of migration is not achieved properly, resulting in sustained motility.

Interestingly, dependence on steeper chemotactic gradients diminishes in polarized cells. Therefore, GRK2-mediated receptor phosphorylation/desensitization may become more relevant in immune cells, in contrast to intrinsically polarized epithelial cells and fibroblasts, leading to striking differences in how GRK2 impacts migration [9, 11, 14]. Receptor desensitization also might be more critical when cells migrate between opposed chemoattractant gradients. Notably, GRK2-mediated

desensitization of S1P1 receptors allows T cells to “ignore” high vascular S1P concentrations in order to follow lymph node guiding cues [13, 18].

GRK2 in cell polarization and membrane protrusion: shaping cells for motion

Cell polarization and the formation of a leading edge is a key step in chemotaxis. GRKs can also regulate these processes by modulating novel substrates and effectors (Figure 2), what would underpin the positive contribution of GRKs to chemotaxis in a stimuli- and cellular context-dependent manner.

GRK-mediated GPCR phosphorylation promotes the recruitment of arrestins, which, in addition to their uncoupling/desensitizing function, have very important roles in cell migration by scaffolding key actin assembly proteins and protein kinase cascades in discrete cellular locations [5]. The precise sets of receptor residues phosphorylated by one or another GRK or by the same GRK in response to different stimuli [19-21] can engage distinct β-arrestin-conformation-dependent signalosomes with different functional competences. Therefore, the relative cellular GRK dosage or activation could modulate chemotaxis at this level.

More importantly, agonist-dependent recruitment of GRKs to the plasma membrane would also promote their direct modulation of specific effectors. The association of GRK2 to PI3Kγ in response to β2AR activation aided to recruit the lipid kinase to the receptor complex [22]. A similar interaction with PI3Ks upon chemokine or integrin receptor activation has not been reported, but this mechanism could reinforce gradient sensing.

GRK2 also interacts with and phosphorylates the ERM proteins ezrin and radixin in response to serum or muscarinic receptor activation [23,24]. By bridging the plasma membrane and actin filaments at the leading edge in a phosphorylation-dependent manner, ERMs contribute to local F-actin polymerization-dependent membrane protrusion. Consistently, GRK2 stimulated cortical actin reorganization and migration in an ERM-mediated manner [23,24]. Interestingly, EGF induces ERM phosphorylation/translocation to lamellipodia via S1P receptor activation [25]. Since GRK2 positively modulates the chemotactic responses to EGF and S1P and plays effector roles in S1P signalling [14, 26], it is feasible that the GRK2/ERM module could also be triggered by additional chemotactic cues.

Besides, GRK2 can engage in pathways linked to polarity persistence by scaffolding GIT1 complexes [14]. GIT1 plays a central role in cell motility by acting as an adaptor protein that promotes Rac/PAK activation both at focal adhesions and at the cell leading edge [9,14]. The challenge of epithelial cells or fibroblasts by fibronectin or S1P causes Gβγ-dependent GRK2 translocation to the plasma membrane and GRK2-mediated recruitment of GIT1. The transient GRK2/GIT1 interaction at the leading edge, controlled by the sequential phosphorylation of GRK2 by c-Src and MAPK, enhances Rac1 activation and results in F-actin cortical remodelling and increased migration [14].

GRK2 also plays an important regulatory role in microtubule (MT) dynamics in these cell types [10,11,26]. MTs exert an overriding influence on the actin cytoskeleton and on the balance of the activity of Rho-family GTPases. During cell migration, the MT cytoskeleton is polarized, and the protruding and retracting cell regions display different MT dynamics, associated proteins and posttranslational modifications. MTs become acetylated in the stable subset arranged in the lamella region, while highly dynamic, "pioneer" MTs facing the lamellipodium are de-acetylated [26,27]. GRK2 directly interacts with and phosphorylates HDAC6 [26], a cytoplasmic histone deacetylase responsible for the de-acetylation of tubulin and other substrates involved in motility such as cortactin [28]. This phosphorylation event enhances HDAC6-mediated α-tubulin (but not cortactin) de-acetylation, and it is required for the positive effect of HDAC6 in the migration of epithelial cells and fibroblasts challenged by fibronectin or EGF [10,11, 26].

The role of MT in cell polarity and motility varies with cell type [27], and might be engaged preferentially in the anterior or the posterior cell region. In migrating neutrophils and T cells, the MTOC is positioned behind the nucleus and MTs are grouped in the uropod, exerting a negative reciprocal feedback loop with RhoA that is mandatory for migratory persistence [29]. Both excessive or defective dynamics of MTs due to altered levels of GRK2 could compromise uropod specification and motility. Conversely, in most other cells the MTOC face the leading edge and more dynamic MTs increase protrusive activity. These differences might help to explain the prevalence of positive effects of GRK2 in the chemotaxis of epithelial and fibroblastoid cells compared to its overall impact in immune cell migration.

GRK2 in cell adhesion and tension: targeting focal adhesions

Highly spread epithelial cells and fibroblasts display large adhesions in the central lamella during migration, which mature in response to increased actomyosin contractility. Simultaneously, nascent and very dynamic focal complexes are formed at the lamellipodium by ligand-induced integrin clustering and actin-polymerization in order to stabilize membrane protrusion. As traction forces move the cell forward, focal adhesions (FA) at the rear must be disassembled. Conversely, round cells seem to migrate (either in amoeboid or blebby modes) using weak adhesions around the cell [4]. Therefore, cells adopting a mesenchymal mode of migration are more dependent on activities that enhance adhesion turnover.

GRK2 could stimulate chemotactic migration (and random motility as well) by modulating the formation/disassembly of integrin-based cell-extracellular matrix contacts (Figure 2). GRK2 expression might favour the nucleation of nascent contacts by either facilitating FN- and S1P-directed activation of Rac1 and actin reorganization [14] or the transition of integrins to high-affinity conformations important for clustering (our unpublished observations). On the other hand, GRK2 weakens FA of epithelial cells by increasing the extent and duration of the GIT/PAK/MEK/ERK1/2 signalling module upon engagement of integrin receptors, what promotes FA turnover [14]. Interestingly, GRK2 can serve as a RhoA-activated scaffold protein for MAPK activation in response to EGF stimulation [30]. In a motile cell, GRK2/RhoA interaction would be expected to occur predominantly at the rear edge, wherein RhoA activation peaks, and localized MAPK could increase FA turnover.

Besides, GRK2 might influence the disassembly of FAs by means of the control of tubulin deacetylation. Targeting of FAs by dynamic MTs exerts a negative influence on focal adhesion maintenance [27]. Upon GRK2 downregulation, hyper-acetylated MTs would be less efficient in disassembling FAs, enhancing cell spreading and impairing motility [11,24].

Role of GRKs other than GRK2 in cell migration

Other GRKs have been reported to modulate cell migration, although information about the molecular mechanisms involved is scarce. Comparison of GRKs knockout mice in an acute inflammatory arthritis model indicated a role for GRK2, GRK3 and GRK6, but not GRK5, in granulocyte migration in such conditions [31, 32]. Interestingly, lymphocytes from beta-arrestin-2 and GRK6-deficient animals displayed

defective chemotaxis towards CXCL12, suggesting a positive role in cell motility in such context [33].

GRK5 attenuates atherosclerosis by desensitizing CCR2 in monocytes and inhibiting their migration [34]. In epithelial cells, a functional screening identified GRK6 as a critical mediator in integrin-mediated cell adhesion and migration of tumor cells [35], and GRK6 deficiency reportedly promotes CXCR2 receptor-mediated tumor progression and metastasis in a lung carcinoma model [36]. It would be interesting to investigate whether the modulation of cell migration by these GRKs is mostly related to their canonical role as specific or more active regulators of given GPCRs in different cell types or novel substrates and interactors as those identified for GRK2 are also targeted.

Physiological and pathological implications

Altered activity/expression of GRKs might critically contribute to deregulate cell motility in development or wound healing or in pathological situations related to inflammation or tumor progression [7, 8, 11,12]. Several GRK2-interacting signalling modules play well-known roles in invasive motility, including integrins, GPCRs (such as S1P, chemokine or PAR receptors), the EGFR family, RhoA, Rac1, or ERMs [37-39]. Increased GRK2 levels could strengthen features of tumor cell motility by increasing integrin and S1P-mediated signaling, which are amplified in breast cancer patients and are critical for invasive migration in 3D-collagen matrices [39-40]. In addition, GRK2 could also collaborate with HDAC6 in promoting cytoskeletal and adhesion dynamics required for tumoural migration and invasion, as well as in HDAC6-promoted invadopodial matrix degradation and 3D invasion [11, 26, 41, 42] Interestingly, altered GRK2 levels have been found in human granulosa cell tumors, thyroid and prostate cancer or some breast tumors (reviewed in [8, 11]), and are up-regulated in different malignant mammary cell lines displaying aberrant migration [43]. Concurrent HDAC6 and GRK2 up-regulation in human tumor malignancies may favor migration and invasion, and point these proteins as new potential therapeutic targets for suppressing metastasis.

On the other hand, GRKs are highly expressed in different cellular types of the immune system and are important regulators of inflammation. Decreased GRK2 activity/levels were found in peripheral blood mononuclear cells of patients and in animal models of rheumatoid arthritis and multiple sclerosis [reviewed in 12].

Activation of the Toll-like receptor (TLR)-4 pathway promotes GRK2 and GRK5 transcriptional down-regulation, leading to decreased chemokine receptor desensitization and increased migration of polymorphonuclear leukocytes [44]. Interestingly, several reports have related the inability of neutrophils from severe sepsis patients to migrate towards CXCL2 or CXCL8 chemoattractants, and thus to promote bacterial clearance, to increased levels of GRK2 and GRK5 [31]. Notably, sepsis attenuation by Interleukin-33 may involve down-modulation of GRK2 expression in neutrophils, leading to enhanced CXCR2 signaling and neutrophil chemotaxis [45]. Higher up-regulation of GRK2 in diabetic mice exacerbated CXCR2 downregulation and reduction of neutrophil migration in sepsis [46]**.** High GRK2 expression in neutrophils from malaria patients suggests a possible mechanism for an enhanced susceptibility to secondary bacterial infection during malaria [47].

Overall, these data encourage future studies to investigate the role of changes in the expression/functionality of GRKs in the triggering or development of physio-pathological processes related to cell motility.

Perspectives and conclusions

GRKs appear as physiologically relevant integrative nodes in cell migration,. Given the distinct signalling and cytoskeleton modules related to chemotaxis potentially modulated by GRKs, one key issue is to understand how are orchestrated in response to specific stimuli in distinct cell types. Moreover, an adequate combination of in vitro and in vivo experimental approaches is needed to fully decipher the functional role of GRKs in motility.

GRK2 phosphorylation by other kinases is emerging as a key "signaling switch" governing substrate specificity and interaction with cellular partners during cell migration (Figure 3). Tyrosine-phosphorylation of GRK2 in response to chemotactic signals enhances interaction with GIT1, whereas S670 phosphorylation by MAPK displays an opposite effect [14]. In turn, dynamic S670 GRK2 phosphorylation specifically allows GRK2 to phosphorylate HDAC6 and thus trigger transient local tubulin de-acetylation [26]. Notably, S670 GRK2 phosphorylation by ERK or p38 Mapk inhibits its interaction with Gβγ and prevents GPCR desensitization. [7,8,48]. Alternatively, phosphorylation of the FPR1 receptor by p38MAPK has been reported to prevent GRK2 recruitment and so facilitate neutrophil migration [17]. Therefore, the subcellular localization and phosphorylation status of GRK2 would be a key factor

underlying the dynamic and stimuli-specific switching of partners relevant to cell migration, allowing the sequential and coordinated participation of GRKs in several steps of the cell motility process [10,11].

Lessons and potential bias from experimental models should also be considered. Most studies about GRKs in migration are based in in vitro systems (2D-random motility, wound healing and transwell assays), and may therefore need further analysis. Transwell assays mainly assess cell polarization and persistence, overlooking the influence of the structural architecture/biophysical properties of the extracellular matrix. 2D-substrates favour the mesenchymal migration mode, what may override other molecular repertoires that could determine an intrinsically different mode of migration (for instance amoeboid-blebby) in physiological/3D environments [49, 50]. Furthermore, transwell assays neither reproduce the in vivo complexity of superimposing soluble gradients, nor of gradients of matrix-bound factors, which intertwinement might alter the mechanisms engaged in the guiding response. We have reported that in transwell assays, GRK2 downregulation enhanced migration of endothelial cells to both VEGF and FN. However, migration of GRK2-deficient endothelial cells in vivo was impaired during the postnatal development of the retinal vasculature, as denoted by the reduced advance of endothelial tip cells and impaired filopodia formation and/or stabilization [51]. For proper migration in vivo, VEGF is anchored to fibrillar fibronectin depots assembled on the cellular projections of retinal astrocytes, which provide a guiding scaffold [52,53], in contrast to the presentation of these chemotactic cues in vitro. GRK2 might play in vivo additional roles in the integration of fibronectin and VEGF signals, positively influencing guiding persistence and migration, as previously described for the intertwinement of integrins and S1P receptors in epithelial cells [14]. Combined experiments in classical, 3D and in vivo experimental models will help to better assess the role of GRKs in cell migration and to define the underlying molecular mechanisms.

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Figure legends

Figure1.- Proposed model for the role of GRK2 in the modulation of chemotactic gradient sensing. Local fraction of receptor occupancy by chemoattractants would trigger stimulatory signals for the generation of internal signalling asymmetry, whereas global levels of occupied receptors would inhibit this parameter [16]. In the presence of lower GRK2 levels/activity, stimulated GPCR would remain longer/more functional in the membrane, thus eliciting robust directional sensing responses and the specification of stable pseudopodia as if the gradient was steeper. Conversely, excessive GRK2 activity would diminish the global level of occupied receptors, resulting in the inability to differentiate the cell front from the back, as occurs in the absence of gradients, resulting in cessation of movement.

Figure 2.- Different GRK2 substrates and interactors in the modulation of cell polarization membrane protrusion and adhesion. By dynamically interacting with diverse substrates and effectors in different cellular locations, GRK2 may modulate several facets of the cell migration machinery in a stimuli- and cellular context-dependent manner. FA, focal adhesions; FC, focal contacts.

Figure 3.- GRK2 phosphorylation by other kinases as a "signalling switch" governing substrate specificity and interaction with cellular partners during cell migration. Migratory stimuli would trigger GRK2 recruitment to activated GPCR in the lamellipodium membrane. At such specific locations, chemokine receptor stimulation would promote the transient Src-mediated tyrosine phosphorylation of GRK2, enhancing its interaction with GIT1 and facilitating localized activation of the Rac/Pak/Mek/Erk pathway. Subsequent phosphorylation of GRK2 at S670 by MAPK disrupts interactions with GIT-1 and GPCR, simultaneously switching on the ability of GRK2 to phosphorylate HDAC6 co-localized at the lamellipodium. Both phospho-S670-GRK2 and HDAC6 are specifically co-recruited to pseudopodia in response to pro-migratory stimuli, resulting into dynamic, local HDAC6-mediated de-acetylation of MTs. The concerted action of hipo-acetylated MTs and GIT1 signalosomes at the leading edge would contribute to cortical polarity and membrane protrusion and thus lead to enhanced cell motility.

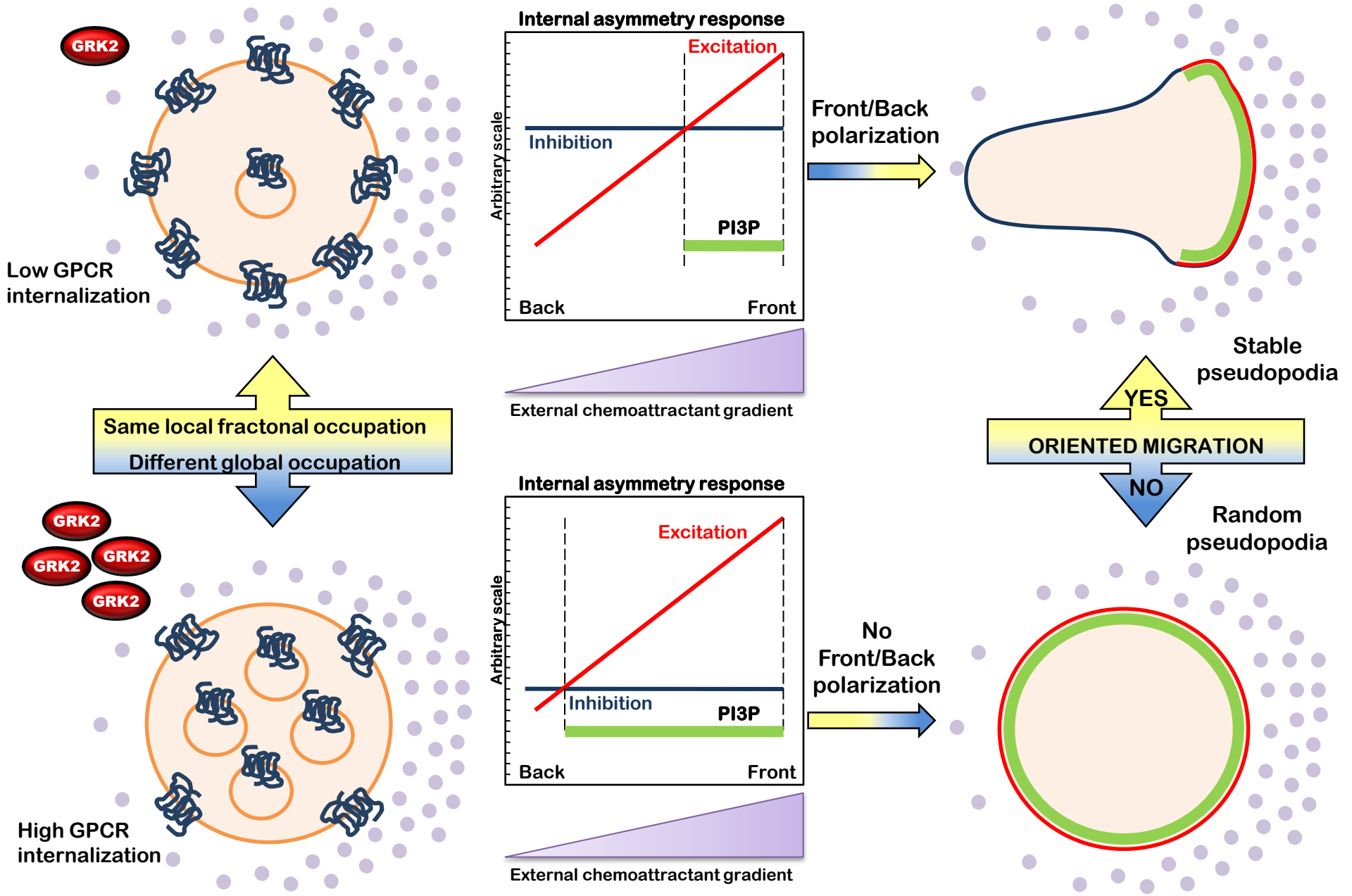

Figure 1

Fractional occupation excitation
Global occupation inhibition

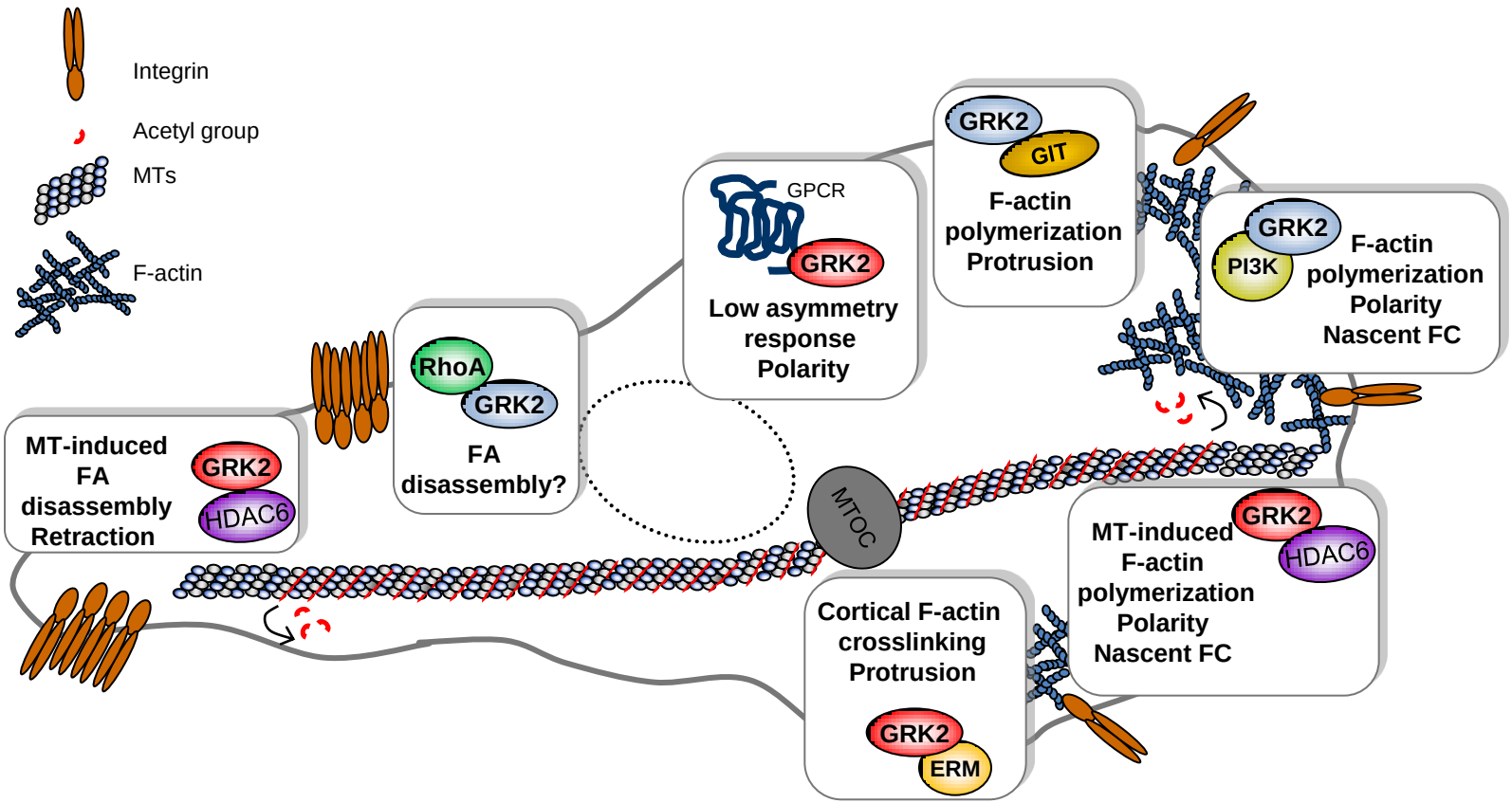
Integrin
Acetyl group
MTs
F-actin
GPCR
GRK2
Low asymmetry response Polarity
GRK2
GIT
F-actin polymerization Protrusion
GRK2
PI3K
F-actin polymerization Polarity Nascent FC
RhoA
GRK2
FA disassembly?
MT-induced FA disassembly Retraction
GRK2
HDAC6
MTOC
MT-induced F-actin polymerization Polarity Nascent FC
GRK2
HDAC6
Cortical F-actin crosslinking Protrusion
GRK2
ERM

Figure 2

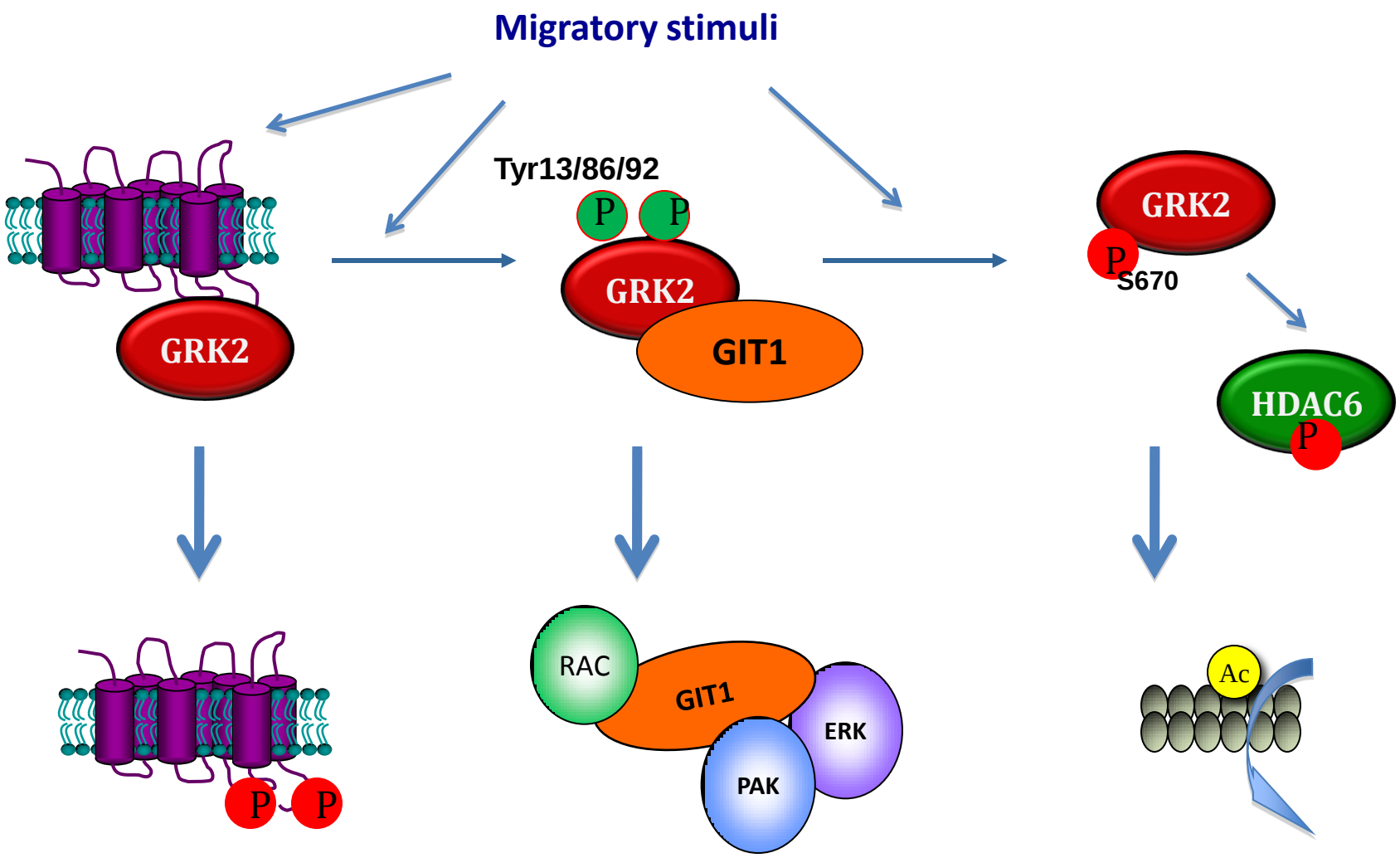
Migratory stimuli
Tyr13/86/92
P
P
GRK2
GIT1
GRK2
P
S670
HDAC6
P
GRK2
P
P
RAC
GIT1
ERK
PAK
Ac
GPCR desensitization and internalization/
Arrestin-dependent signalling
Cortical actin remodelling
and focal adhesion turnover
Dynamic Tubulin
deacetylation

Figure 3

Table1. The overall effect of altering GRK2 levels/activity on chemotactic migration depends on stimuli, cell type and migration assays

Cellular type	GRK2 expression/functionality	Migration outcome to chemoattractants	Experimental migration assay
Epithelial cells and fibroblasts	Decreased Over-expressed	Reduced to FN[14,26], serum[26,24], S1P[14] Increased to FN[14,26], serum[26], S1P[14]	Transwell[14,26] Wound-healing in vivo[14] and in vitro[26,24]
Endothelial cells	Decreased	Increased to VEGF[51], FN[51] Reduced to VEGF[51]	Transwell[51] In vivo retina[51]
Vascular smooth muscle cells	Over-expressed	Decreased to PDGF [54], and AngII [55] Unchanged to EGF[54]	Transwell[54,55]
Basophilic cells	Decreased	Reduced to fMLP*	Transwell*
Lymphocytes	Decreased	Increased to S1P[13], CCL3[56], CCL4[56] Reduced to CCL21[13]	Transwell[13, 56] In vivo lymph node entry[13]
Neutrophils	Increased Decreased	Decreased to fMLP[17], LTB4[31], IL8[31] Increased to CXCL2 [44]	Transwell[31, 44] 2-D tracking[17]
Monocytes	Decreased	Unchanged to CCL2[48] Reduced to MCP-1 plus LPS[48] Increased to CCL5[57]	Transwell[48] intraperitoneal mobilization[57]
Granulocytes	Decreased	Increased to LTB4[58] Unchanged to IL8[58], C5a[58]	Transendothelial migration[58]

*our unpublished data