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The PIX-GIT complex: a G protein signaling cassette in control of cell shape

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Abstract

Arf and Rho GTP-binding proteins coordinately regulate membrane dynamics and cytoskeletal rearrangements. The Cdc42/Rac guanine nucleotide exchange factor PIX and the Arf GTPase activating protein GIT form a stable complex in cells. The PIX-GIT complex functions to integrate signaling among Arf, Cdc42, and Rac proteins in response to cues emanating from integrins, heterotrimeric G proteins, receptor tyrosine kinases, and cell-cell interactions. A concept that emerges from the literature is that the PIX-GIT complex serves as a cassette to elicit changes in cell shape essential for polarized cell responses in a wide range of biological contexts.

Keywords

PIX; GIT; Arf; Cdc42; Rac

1. Introduction

Ras-like G proteins (small G proteins) and the molecules that regulate their activity serve as potent determinants of cell shape by impacting on the cytoskeleton, membrane dynamics, and vesicular trafficking [1-4]. In general, small G proteins cycle between an active, GTP-bound, and an inactive, GDP-bound form, and they require precise spatiotemporal activation in order to execute their proper function [5]. Accordingly, the use of live cell activation state-specific fluorescent probes in fluorescence resonance energy transfer (FRET) has demonstrated that the localization of GTP-bound forms of small G proteins is indeed highly restricted within the cell [6-8]. This is accomplished by guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), which in addition to enzymatic activity, possess protein-protein and phosphoinositide interacting domains that restrict their function to defined subcellular locations [9,10]. Moreover, GEFs and GAPs often mediate cross talk between members of the same or distinct families of small GTP-binding proteins. Such mechanisms permit coordination between, for instance, vesicular trafficking and cytoskeletal remodeling controlled by Arf and Rho proteins, respectively. Here, we discuss the role of the Cdc42/Rac exchange factor PIX, and the ArfGAP GIT in regulating cell shape and polarity in response to integrin ligation, G protein coupled receptor signaling, receptor tyrosine kinase activation, and other stimuli. While PIX and GIT proteins have recently been reviewed individually [11-13], there are compelling reasons for discussing their roles in morphogenetic events collectively, as they form a tight complex in cells and influence the stability and function of one another.

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Indeed, it is becoming apparent that the PIX-GIT complex operates as a cassette that exerts its physiological functions by coordinating the localization and activation of Arf and Rho proteins.

2. PIX: PAK-interacting exchange factor

The PIX, a.k.a. 'Cool', proteins were isolated as direct interactors with the Cdc42/Rac effector molecule PAK [14,15], and were also identified in a screen for SH3 domain-containing proteins [16]. Of the two forms, α PIX is expressed primarily in hematopoietic cells and muscle [14], whereas β PIX is ubiquitously expressed and is subject to extensive splicing, particularly in the brain [14,17]. PIX molecules are Dbl-family exchange factors for Rho proteins, defined by the presence of tandem Dbl homology (DH) and Plekstrin homology (PH) domains, and they contain several protein-protein interaction motifs that dictate PIX localization and function (Fig. 1). In addition to the DH and PH domains, all PIX proteins have an amino-terminal SH3 domain, which binds with high affinity to proline-rich sequences in PAK [14,15], c-Cbl ubiquitin ligase [18], and Rac1 [19]. Moreover, both α PIX and the predominant splice variants of β PIX harbor a GIT-binding domain [20-23], a coiled-coil motif that mediates dimerization [17,24], and a proline-rich region that is required for the interaction of PIX proteins with the POPX1 and the POPX2 phosphatases, which inactivate PAK [25] (Fig. 1). Finally, both α PIX and β PIX bind directly to Calpain 4, the regulatory subunit of the μ - and m-Calpain intracellular proteases, an interaction that intriguingly requires the SH3 domain as well as the PH and DH domains [26].

Major differences between the α PIX and β PIX include (i) an amino-terminal calponin-homology domain that is required for interaction with the focal adhesion protein β -parvin/affixin, and is found in α PIX but not in most forms of β PIX [14,15], (ii) a carboxy-terminal PDZ binding domain present only in β PIX that interacts with Scribble and Shank [27,28], and (iii) a 'T1' insert also present in β PIX that inhibits constitutive exchange factor activity [29]. Thus, while recombinant α PIX readily promotes nucleotide exchange on both Cdc42 and Rac, the enzymatic function of β PIX is barely detectable in vitro. In cells, phosphorylation of Y442 in β PIX by Src alleviates the inhibition mediated by the T1 insert to promote nucleotide exchange exclusively on Cdc42 [30]. The activity of α PIX is also subject to complex regulation. The α PIX dimer functions as a specific GEF for Rac, whereas the α PIX monomer promotes nucleotide exchange on both Cdc42 and Rac [31,32]. Upon activation of G protein coupled receptors, binding of G $\beta\gamma$ -associated PAK to the SH3 domain of the α PIX dimer stimulates dissociation of the α PIX dimer into the monomeric form, which induces its enzymatic activity towards Cdc42 [32]. Strikingly, GTP-bound Cdc42 also regulates the GEF activity of α PIX. Binding of activated Cdc42 to the DH domain of one α PIX molecule in the dimer induces a conformational change that enhances guanine nucleotide exchange on Rac [31]. This mechanism could thus permit coordinated activation of Cdc42 and Rac by α PIX at sites of membrane remodeling.

3. GIT: G protein-coupled receptor kinase interactor

GIT1 was initially identified as a direct interactor with GRK, G protein-coupled receptor kinase [33], where it regulates Arf6-dependent trafficking of β 2-adrenergic receptors [33,34]. GIT proteins exist in two forms, GIT1 and GIT2, which also have been named CATs (Cool-associated tyrosine phosphorylated protein) [20], PKL (Paxillin-kinase linker) [21], and p95-APP (ArfGAP-putative, PIX-interacting, Paxillin-interacting proteins) [23] (Fig. 2). Apparently, only one form of GIT1 exists, whereas GIT2 has numerous splice variants, although their expression pattern in tissues and cell lines remains unsettled [35]. The longest splice form of GIT2 (GIT2-long) is widely expressed [35]. Using a gene trap strategy, GIT2 expression in the mouse has recently been described [36]. The study shows that while GIT2 is almost ubiquitously expressed in mammalian tissues and cell types, GIT1 expression is more

restricted. For instance, GIT1 is confined to the vasculature and the bronchial epithelia in the lung, and is only detected in the vasculature and bile duct of the liver. Both GIT1 and GIT2 are widely expressed in the brain, except in the cerebellum, where GIT1 is restricted to Purkinje cells. Finally, in the testis, GIT1 and GIT2 expression appears to be mutually exclusive. The significance of these differences in expression patterns is presently unknown but is consistent with the existence of non-redundant functions between GIT1 and GIT2.

The amino-terminus of GIT proteins comprises an ArfGAP domain [33] (Fig. 2). While, GIT1 and GIT2 molecules exhibit catalytic activity towards all Arf family members *in vitro* [37], their localization to the cell periphery [38], effects on the actin cytoskeleton [21,22], lipid specificity [37], and modulation of endocytic trafficking [23,37,39,40], suggest that they are specific regulators of Arf6. An exception is GIT2-short, which has been localized to the Golgi apparatus and proposed to function as a GAP for Arf1 [41]. All GIT forms additionally possess ankyrin repeats that recently were implicated in intramolecular folding of GIT1 [42], and a Spa2 homology domain (SHD), which in addition to PIX, binds FAK [22], MEK [43], PLC γ [44], and Piccolo [45]. The interaction of the β PIX-GIT1 complex with PLC γ was reported to be required for activation of Cdc42 and Rac1 by the β PIX-GIT1 complex [46]. Both GIT1 and GIT2-long contain a coiled-coil region with an internal leucine zipper, which mediates homo- and hetero-dimerization [45,47,48]. The carboxyl-terminus of GIT1 comprises a domain with similarity to the focal adhesion-targeting (FAT) domain of FAK [49,50]. This FAT-homology (FAH) domain includes the previously characterized Paxillin binding sequence (PBS) [21, 22]. Since the carboxyl-termini in GIT1 and GIT2 are highly homologous, it is likely that the GIT2 carboxy-terminus similarly adopts the conformation of the FAH domain characterized for GIT1. GIT proteins contain a second, more proximal Paxillin-binding site, which is dispensable for Paxillin binding in GIT2-long [21]. In GIT2-short, however, it constitutes the sole motif for interaction with Paxillin and is required for trafficking of Paxillin from the Golgi apparatus to focal adhesions [41]. Finally, GIT1 harbors a synaptic localization domain (SLD) located between the SHD and the FAH [51]. The region comprising the SLD is most divergent between GIT1 and GIT2 proteins and likely constitutes a major determinant of distinct functions.

4. Role of the PIX-GIT complex in membrane ruffling and cell spreading

The PIX-GIT complex has been most widely studied in the context of integrin-mediated cell spreading and cell motility. Focal adhesions are macromolecular protein and phosphoinositide assemblies on the inner leaflet of the plasma membrane that connect integrins clustered by engagement with extracellular matrix constituents to the cytoskeleton [52-54]. In addition to providing a physical anchor, focal adhesions serve as scaffolds for complex bidirectional signaling networks in control of cell adhesion and motility. In spreading and migrating cells, small focal adhesion-like structures termed focal complexes are established at the leading edge of the cell where they serve as traction points to support membrane protrusion. Over time, focal complexes grow in size, translocate centripetally underneath the cell body, and undergo maturation into larger structures termed focal adhesions, which provide stable attachment. Meanwhile, a new cohort of focal complexes is formed at the leading edge by recycling of integrins and cytoplasmic constituents from focal adhesions. This process is referred to as focal adhesion turnover [55].

The adaptor protein Paxillin is among the first proteins to be incorporated into focal complexes upon integrin engagement [49]. Paxillin contains five leucine-rich regions termed LD motifs. Localization of the β PIX-GIT complex to focal complexes is mediated by direct binding of the PBS (Paxillin-binding sequence) in GIT molecules to the LD4 motif of Paxillin [21,56]. Recruitment and activation of the β PIX-GIT complex at adhesion sites is highly regulated. Phosphorylation of paxillin within the LD4 motif by PAK appears to promote binding of GIT1

to Paxillin [57]. Moreover, phosphorylation of GIT2 by a Src-FAK signaling cascade is required for its association with Paxillin, recruitment to focal complexes, and for restricting membrane protrusion [58]. β PIX has also been identified as a substrate of FAK and this phosphorylation event enhances binding of Rac to β PIX and recruitment to focal complexes [59]. Thus, perturbation of FAK activation prevents localization of the GIT-PIX complex, Rac, and PAK to focal complexes, attenuates PAK activation, and interferes with normal cell spreading and motility [58-60]. As described above, the exchange factor activity of β PIX appears to be conformationally constrained due to the presence of the T1 insert, and phosphorylation of β PIX by Src at tyrosine 442 alleviates this inhibition and activates specific exchange activity towards Cdc42 [30]. More recently, it has been discovered that β PIX can bind Rac in a nucleotide-independent manner through the direct interaction of the β PIX SH3 domain with a polybasic stretch in the C-terminus of Rac1 [19]. Taken together, these results suggest that, depending on its phosphorylation status, β PIX may function either as a scaffold, a GEF, or both to coordinate the activation of Cdc42 and Rac at the leading edge. This finding may also explain the observation that overexpression of β PIX leads to the coordinated activation of both Cdc42 and Rac1 [17]. A recent study indicates that β PIX serves exclusively as a GEF for Cdc42 and not Rac [30]. This raises the possibility that β PIX primarily serves as a scaffold for Rac, and that other GEFs, for instance smgGDS [61], may promote nucleotide exchange on β PIX-associated Rac.

The precise role of GIT proteins in regulating the activity of the β GIT-PIX complex at focal adhesions remains unsettled. Early studies implicated GIT in the activation of motile responses, as GIT overexpression was found to induce Cdc42/Rac1-dependent membrane protrusion and spreading [22,62]. In contrast, these findings are at odds with studies demonstrating that GITs are resident focal adhesion proteins, which function to limit Rac-mediated lamellipodia formation, and Cdc42-dependent focal adhesion turnover [38,63]. This conclusion is corroborated by the observation that expression of a mutant Paxillin protein where the LD4 motif has been deleted, or a mutant GIT protein without the PBS, enhances Rac1 activation, membrane protrusion, and random cell motility [56]. It is also consistent with the finding that overexpression of the GIT PBS alone, which presumably perturbs the function of GIT molecules by occupying the LD4 motif on Paxillin, elicits membrane protrusion, cell spreading, focal adhesion turnover, and random migration [21-23,62].

Exactly how GIT molecules function to restrict Cdc42/Rac activation remains unclear. Arf6 is a well-established regulator of Rac1 [4], and activation of Arf6 has been proposed to regulate the trafficking of an endosomal pool of Rac1 to the plasma membrane [64,65]. As such, in many systems activation of Arf6 has been shown to result in the subsequent activation of Rac. Accordingly, GIT proteins have been detected on endosomes and implicated in the delivery of Rac to the plasma membrane [23,40]. In this context, it is noteworthy that PIX and GIT proteins have been associated with Ca^{2+} -dependent exocytosis in neuroendocrine cells [27,66], and stimulated recycling of the TSH receptor in thyroid epithelial cells [67]. Moreover, in addition to their role in trafficking Rac, recent studies suggest that Arf6 may function to directly regulate the activity of Rac-specific GEFs [68,69]. First, ARNO, a GEF for Arf6 [70], can promote nucleotide exchange on Rac by the bipartite RacGEF Dock180/Elmo, which is activated downstream of integrins [68,71]. Second, as discussed in detail below, GIT proteins have been shown to restrict Rac1 activation, a function that requires an intact GAP domain [63]. Thirdly, GIT molecules might act downstream of activated Arf6, i.e., as effectors of Arf6 [72]. An effector function has indeed been invoked for other ArfGAPs such as ACAP1 and ASAP2 (PAG3), which bind tightly to Arf6-GTP and regulate downstream signaling events [73,74]. In this scenario, membrane-bound Arf6-GTP would regulate the localization and/or activity of the PIX-GIT complex and, for example, facilitate localization of PIX to the plasma membrane to initiate Cdc42/Rac signaling. Further work is evidently necessary to elucidate the functions of PIX and GIT discussed above. Meanwhile, the existing literature supports a

role for the PIX-GIT complex as an essential cassette that coordinates activation of Arf and Rho signaling pathways in control of cell shape and motility.

5. PIX and GIT: determinants of cell polarity and directional motility

Cell spreading is a suitable context for dissecting mechanisms of membrane ruffling and focal adhesion turnover, but it does not permit the study of the polarization events elicited by chemotactic, haptotactic, and cell-cell contact-mediated stimuli that govern directional cell migration. Several recent studies using widely different cell systems and experimental conditions support the conclusion that PIX and GIT molecules serve pivotal functions in specifying cell polarization and directionality motility. Surprisingly, these functions appear to be conserved irrespective of the mechanisms by which the PIX-GIT complex is recruited to the membrane as illustrated by the following four examples. What links these four examples together is the concept that PIX and GIT serve to locally restrict activation of the small GTP-binding proteins on which PIX and GIT act.

5.1. $\alpha 4$ integrin-directed cell migration

A succession of studies by Ginsberg and colleagues has explored the mechanism by which $\alpha 4$ integrins ($\alpha 4\beta 1$ and $\alpha 4\beta 7$) control cell polarity and directional migration. $\alpha 4$ integrins are unusual in that they bind directly to Paxillin [75], as opposed to other integrins where the interaction with Paxillin is indirect [49]. The $\alpha 4$ subunit is both an A-kinase anchoring protein (AKAP) [76], and also a substrate of PKA [77].

Phosphorylation of Ser988 in the cytoplasmic tail of $\alpha 4$ integrin negatively regulates Paxillin-binding to promote Rac activation and lamellipodia formation at the leading edge, while Paxillin is recruited by non-phosphorylated $\alpha 4$ along the rear and sides of the cell to inhibit protrusive activity [63,78]. The capacity of $\alpha 4$ integrin-associated Paxillin to restrict membrane protrusion is critically dependent on the interaction of Paxillin with GIT protein (only GIT1 was tested). Importantly, $\alpha 4$ integrin, Paxillin and GIT1 can form a ternary complex that attenuates activation of Arf6 and Rac1 in cells [63]. Thus, a model that can be derived from these studies is that in migrating $\alpha 4$ integrin-expressing cells, GTP-bound Arf6 promotes nucleotide exchange on Rac1 at the leading edge by mechanisms discussed above. In contrast, along the lateral sides and trailing edge, the $\alpha 4$ integrin-Paxillin-GIT complex represses Rac-mediated membrane protrusion by locally inactivating Arf6 [63] (Fig. 3). The contribution of the enzymatic and scaffolding activities of PIX remains to be addressed in this system.

5.2. Wound healing in cell monolayers

Another scenario in which the PIX-GIT complex plays a pivotal role as a determinant of cell shape and polarity is upon scrape wounding of monolayers where the cells extend protrusions and migrate perpendicular to the wound plane. The polarizing and motile stimulus is an aggregate of integrin and growth factor signaling as well as interactions with neighboring cells. In this process, GTP-bound Cdc42 is essential for orienting lamellipodial extensions, whereas activated Rac is required for membrane protrusion and cell motility [79]. Consistent with an important function of β PIX in establishing polarity is the observation that knockdown of β PIX expression significantly reduces the number of membrane extensions into the wound, and that overexpression of β PIX induces protrusions uniformly around the perimeter of cells [80,81]. In fibroblasts, a Cdc42-PAK- β PIX signaling cascade has been proposed to restrict Rac activation and consequently the formation of polarized lamellipodia. In this scenario targeting of β PIX to the leading edge requires Cdc42-dependent recruitment and activation of PAK followed by the direct interaction of PAK with β PIX [80]. This is consistent with previous studies of epithelial cells demonstrating that a regulated interaction of PAK with β PIX is necessary for directional cell migration [82]. In this study, expression of mutant PAK forms,

which sequestered β PIX at cell-substratum attachment sites, perturbed directional migration and significantly augmented cellular levels of Rac-GTP [82] (Zegers and Hansen, unpublished observations).

An alternate mechanism for targeting the β PIX to the wound edge is through direct interaction with Scribble, a protein that was first identified in *Drosophila melanogaster* as a tumor suppressor and basolateral polarity determinant in epithelia [83,84]. The function of Scribble in mammalian epithelial cells is not clear, but it has been shown to control directional cell motility and cadherin-mediated cell-cell adhesion [85,86]. Scribble is a LAP protein, thus containing leucine-rich repeats (LRRs) and four PDZ domains. The LRRs are necessary and sufficient for localizing Scribble to the plasma membrane [87], while the PDZ domains interact with a C-terminal PDZ binding domain that is found in the predominant splice variant of β PIX but not α PIX [27] (Fig. 1). Interestingly, upon scratch wounding of astrocyte or epithelial cell monolayers, both Scribble and β PIX are rapidly recruited to the leading edge [81,86]. Perturbation of Scribble function by RNA interference or overexpression of mutant Scribble forms results in failure to reorient the Golgi apparatus and to initiate membrane protrusion [81,86]. In astrocytes, β PIX requires Scribble for localization to membrane ruffles, but not to focal complexes. In contrast, Scribble recruitment to lamellipodia is independent of β PIX. Cell polarization, including Golgi reorientation, is perturbed by overexpression of catalytically inactive β PIX as well as by abrogation of the β PIX-Scribble interaction. Thus, in a model consistent with these findings, Scribble targets β PIX to membrane protrusions at wound edge, which elicits activation of Cdc42 and resulting polarization of the cytoskeleton and secretory apparatus permissive for directional cell migration [81] (Fig. 4). The role of GIT proteins in wound healing remains to be investigated.

5.3. Neutrophil chemotaxis

A third setting in which PIX and GIT orchestrate polarized cell migration is in neutrophil chemotaxis. Chemoattractants such as complement C5a and the formyl peptide fMLP (N-formyl-Met-Leu-Phe) bind serpentine receptors to activate a G protein signaling cascade (Fig. 5). In this scenario, $G\beta\gamma$ subunits bind directly to PAK1 [88]. Two internal $G\beta\gamma$ interaction motifs exist in mammalian PAK1 that are distinct from the carboxy-terminal $G\beta\gamma$ binding site sequence previously identified in the yeast PAK homologue Ste20 [88,89]. As described above, upon interaction with $G\beta\gamma$, PAK1 recruits and activates α PIX to promote nucleotide exchange on Cdc42, which in turn binds PAK1 to stimulate kinase activity. Consequently, a feed forward cycle is established, which polarizes the actin cytoskeleton [88]. Neutrophils isolated from either α PIX or GIT2 knock-out mice fail to mount this response and exhibit attenuated directional migration in chemotactic gradients [88,90].

The studies further documented that α PIX and GIT2 are required for the reciprocal localization of the PI3-kinase effector AKT and PTEN in neutrophils exposed to chemoattractants [88, 90]. It has been demonstrated that lipid products of the PI3-kinase play a pivotal role in establishing 'frontness' and that the PI3-phosphatase PTEN is involved in specifying 'rearsness' of chemotaxing neutrophils [91-93]. Moreover, it was independently shown that α PIX can be found in a complex with the regulatory p85 subunit of PI3-kinase, and that PI3-kinase enhances the GEF activity of α PIX, thus lending further support to the existence of cross-talk between the PIX-GIT complex and PI3-kinase signaling pathways [94]. Interestingly, in fMLP-treated neutrophils from GIT2 knock-out mice, hydrolysis of Arf1-GTP, but not Arf6-GTP is perturbed. However, it is not clear whether this results from depletion of GIT2-long or a shorter splice variant. Indeed, prior studies have implicated GIT2-short as a GAP for Arf1, but not Arf6 [41]. The significance of the PIX-GIT complex in controlling directional cell migration is highlighted by the demonstration that neutrophils from GIT2-deficient mice fail to reach peripheral inflammatory regions [90].

5.4. Neuronal polarization

The last example discussed here of the PIX-GIT complex playing an essential role in determining cell shape and polarity is in neuronal cells. Neuronal morphogenesis is a multi-step process initiated by the formation of lamellipodia from which short neurites extend, followed by elongation of first the axon and then dendrites. Next, synapses are formed through the interaction of dendritic filopodia with axonal partners and the maturation of dendritic filopodia into mushroom-shaped dendritic spines [95-97]. The existing literature implicates the PIX-GIT complex in several of these steps. In NGF-stimulated PC12 cells, PAK and β PIX act upstream to promote activation of Rac, which is essential for lamellipodia formation [98]. Neurite extension in response to β FGF is repressed when two major ERK/PAK phosphorylation sites (Ser525, Thr526) in β PIX are mutated [99]. Further consistent with a requirement for regulated interaction of PIX and GIT, is the inhibition of neurite extension in chick embryonic retinal neurons by overexpression of GIT1 fragments predicted to sequester PIX [40].

The PIX-GIT complex regulates cytoskeletal changes required for proper spine morphogenesis and synapse formation [100,101] (Fig. 6). In hippocampal neurons, GIT1 is targeted to synapses by a synaptic localization domain (SLD; Fig. 2) [51]. Overexpression of the SLD on its own increases the number of dendritic protrusions and perturbs spine formation [51]. These effects are blocked by co-expression of dominant-negative Rac. Spine formation is similarly perturbed by overexpression of β PIX or mutant GIT1, which cannot bind β PIX [51]. These findings suggest that GIT1 regulates spine morphogenesis by sequestering β PIX and restricting activation of Rac, although it remains to be determined whether the latter events are directly linked. This conclusion is supported by experiments using FRET to detect activated Rac. While activated Rac normally localizes selectively to the base of dendritic protrusions, it is detected throughout the elongated protrusions observed upon overexpression of β PIX [101]. β PIX and Rac have further been implicated in activation of PAK and in PAK-mediated phosphorylation of myosin light chain (MLC), events that are required for spine formation [101]. These findings are consistent with established mechanisms by which PAK regulates actin cytoskeletal rearrangements [102]. Collectively, the data suggest an essential role for a β PIX-Rac-PAK-MLC signaling cascade to promote extension of dendritic protrusions, whereas GIT1 in turn serves as a brake on this process to ensure that the protrusive activity is restricted to permit morphogenesis of synaptic spines. β PIX and GIT1 also appears to function in organizing pre- and postsynaptic multiprotein complexes and neurotransmitter receptor targeting through direct interaction with proteins such as Shank [28], Piccolo [45], and liprin- α [103].

Bi-directional signaling of Eph receptor tyrosine kinases and their ephrin ligands regulates numerous processes in neuronal morphogenesis through effects on the actin cytoskeleton elicited by regulating the activities of Rho family GTP-binding proteins [104-106]. Interestingly, GIT1 was recently implicated in spine morphogenesis elicited by reverse signaling downstream of ephrinB [107] (Fig. 6). This was demonstrated in experiments where ephrinB in hippocampal neurons was activated by a soluble preclustered form of the EphB2 receptor ectodomain. Activation of ephrinB elicits phosphorylation of GIT1 on Tyr392, which is located within the SLD. This event creates a docking site for the SH2-SH3 adaptor Grb4, which binds to the tail of activated ephrinB. Activated ephrinB thus forms a ternary complex with Grb4 and GIT1. Interfering with the formation of the ephrinB-Grb4-GIT1 complex blocks reverse signaling and perturbs normal spine morphogenesis and synapse formation [107]. The latter example constitutes a fourth known mechanism of recruiting PIX and/or GIT to membrane domains, where the molecules play an essential role in orchestrating membrane and cytoskeletal architecture to yield polarization required for specialized cell functions.

6. Concluding remarks

The PIX-GIT complex regulates membrane ruffling, focal adhesion turnover, and polarity in motile cells, and consequently cell spreading as well as directional migration. These functions are executed irrespectively of the mechanisms by which the PIX and GIT are recruited to the membrane, thus suggesting that the complex serves as a cassette that can be utilized in a wide range of biological contexts. The examples given above undoubtedly underestimate the physiological significance of the PIX-GIT complex in morphogenesis. For instance, in T cells, the PIX-GIT complex plays a pivotal role in PAK activation and downstream transcriptional responses upon formation of the immunological synapse [108,109]. Yet the mechanism of membrane targeting remains to be defined, with Scribble representing an exciting lead to pursue [110]. Given such diversity, it is hardly surprising that the molecular mechanisms underlying cellular functions of PIX and GIT have been difficult to tease apart in the decade after PIX and GIT were first discovered. In most cellular contexts the substrate specificity of PIX towards Cdc42/Rac is not settled. Likewise, while prevailing evidence suggests that GIT molecules mainly function as GAPs for Arf6 in cells, further work is required to determine the extent to which GIT proteins act on Arf1 and other Arf proteins. The task of defining the protein-protein interactions that mediate specific functions of the PIX-GIT complex in cells is daunting and complicated by the fact that PIX and GIT form homodimers and exist as larger oligomeric assemblies in cells. However, the engineering of mutant PIX and GIT molecules that selectively abrogate functional domains, combined with an accurate mapping of which PIX and GIT forms are expressed in specific tissues and cell types, yield promise for future studies [14,45,111]. These advances will permit the replacement of endogenous PIX/GIT proteins with defined mutants expressed at physiological levels, which should yield progress in our understanding of PIX and GIT biology. The incentive to conduct such studies is substantial, as PIX and GIT have been implicated in a wide range of disorders including cell transformation and oncogenesis [30,112,113], mental retardation [114], neurodegenerative disorders [115], vascular development and homeostasis [116,117], inflammation [90], and infectious disease [118,119].

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Fig. 1.

Domain structure of PIX proteins. The figure illustrates the domain structure of αPIX (Cool-2), the predominant form of βPIX (p85Cool-2), and a splice variant of βPIX (p50Cool-2). In addition to the DH-PH cassette common to all Dbl family GEFs, PIX proteins contain several protein-protein interaction domains with functions that are summarized in the figure and discussed in further detail in the text. It should be noted that although the figure shows the CH domain to be absent from βPIX, a longer CH domain-containing splice variant exists, which recently has been implicated in vascular stability in zebrafish. Moreover, splice variants of βPIX, which include the GBD and the CC but are missing the PDZ binding motif, exist primarily in the brain.

Fig. 2.

Domain structure of GIT proteins. The figure illustrates the domain structure of GIT1 and the predominant form of GIT2 (GIT2-long) as well as of GIT2-short. GIT2 mRNA undergoes extensive alternative splicing but to date only GIT2-long and GIT2-short have been detected in cells. Although the SLD has only been characterized in GIT1, it remains to be determined whether a similar localization motif exists in GIT2.

Fig. 3.

GIT orients cell migration by restricting membrane protrusion. This model is derived from studies of $\alpha 4$ integrins by Ginsberg and coworkers and partially supported by our own work. It establishes a function of GIT proteins to repress Rac-dependent cell spreading. At the leading edge, engagement with ECM leads to integrin activation and phosphorylation of the $\alpha 4$ tail, which disrupts the association of $\alpha 4$ integrin with Paxillin. In turn, phosphorylated $\alpha 4$ integrins elicit Arf6- and Rac1-dependent membrane protrusion by a mechanism that presently is not known but may involve β PIX. In contrast, at the trailing edge and along the lateral sides of the cell, the non-phosphorylated tail of $\alpha 4$ integrin forms a ternary complex with Paxillin and GIT protein (GIT1 and/or GIT2), which hydrolyzes GTP-bound Arf6 and consequently locally represses Rac1 activation. The net role of GIT action in this context is to restrict Arf6 and Rac1 activation to the leading edge to promote directional cell motility on ECM substrata. The role of β PIX proteins in this setting is not clear but work from our laboratory does not support a mechanism whereby GIT restricts Rac activation by repressing the GEF activity of β PIX.

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Fig. 5.

The α PIX-GIT2 cassette controls neutrophil chemotaxis. Neutrophils polarize towards a chemotactic gradient by a mechanism where the α PIX-GIT complex plays an obligatory role. The signaling cascade is initiated by binding of chemoattractant to serpentine receptors followed by targeting of PAK to the membrane through interaction with $\beta\gamma$ subunits. PAK then recruits α PIX, which promotes local Cdc42 activation that in turn binds and further activates PAK, thus establishing a self-amplifying cascade. The net result is polarization of the actin cytoskeleton and membrane protrusion towards the chemoattractant. GIT2 has moreover been shown to be essential for this response. In this context, GIT2 is implicated as GAP for Arf1, and functions to restrict Rac activation and suppress the production of reactive oxygen species.

Fig. 6.

Requirement for the PIX-GIT1 complex in spine morphogenesis. Recruitment of the PIX-GIT1 complex to the dendritic membrane elicits local Rac and PAK activation to initiate dendritic protrusion. Subsequently, upon contact with axonal membrane, Eph-ephrin reverse signaling leads to phosphorylation of GIT1 and formation of a ternary complex consisting of ephrin ligand, Grb4 and GIT1, which restricts membrane protrusion to shape dendritic spines. The role of GIT2 and of specific PIX forms in spine morphogenesis remains to be determined.