The multifunctional GIT family of proteins

Ryan J. Hoefen and Bradford C. Berk*

Cardiovascular Research Institute and Department of Medicine, University of Rochester School of Medicine and Dentistry, Rochester, NY 14642, USA

*Author for correspondence (e-mail: bradford_berk@urmc.rochester.edu)

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Summary

The G-protein-coupled receptor (GPCR)-kinase-interacting proteins 1 and 2 (GIT1 and GIT2) are ubiquitous multidomain proteins involved in diverse cellular processes. They traffic between three distinct cellular compartments (cytoplasmic complexes, focal adhesions and the cell periphery) through interactions with proteins including ARF, Rac1 and Cdc42 GTPases, p21-activated kinase (PAK), PAK-interacting exchange factor (PIX), the kinase MEK1, phospholipase Cγ (PLCγ) and paxillin. GITs and PIX cooperate to form large oligomeric complexes to which other proteins are transiently recruited. Activation of Rac1 and Cdc42 drives association of PAK with these oligomers, which unmasks the paxillin-binding site in GITs that recruits them to focal complexes. There, they regulate cytoskeletal dynamics by feedback inhibition of Rac1. GITs also participate in receptor internalization by regulating membrane trafficking between the plasma membrane and endosomes, targeting ARF GTPases through their ARF GTPase-activating protein (ARF-GAP) activity. Furthermore, GITs act as scaffolds to control spatial activation of several signaling molecules. Finally, recent results suggest pathogenic roles for GIT proteins in Huntington's disease and HIV infection.

Key words: GIT1, G-protein-coupled receptor, Endosomes, Phospholipase C

Introduction

Among the largest gene families in the mammalian genome are the G-protein-coupled receptors (GPCRs). The diverse downstream signaling events stimulated by GPCR ligands require multiple signaling complexes. Many GPCR-interacting proteins recruited during signal initiation and termination, such as G proteins themselves, arrestins and clathrin, have been identified. Among the first of these proteins to be discovered were the GPCR kinases (GRKs), which are crucial for receptor phosphorylation and internalization. A search for proteins that interact with GRKs led to the identification of the GRK-interacting proteins (GITs), a family of scaffolding proteins (Premont et al., 1998). GITs were almost simultaneously found to bind to the focal adhesion protein paxillin (Turner et al., 1999) and phospholipase Cγ (PLCγ) (Haendeler et al., 2000) (Table 1). Variously termed 'cool-associated tyrosine phosphorylated proteins 1 and 2' [Cat-1 and Cat-2 (Bagrodia et al., 1999)], 'ADP ribosylation factor (ARF)-GTPase activating protein, PIX-, paxillin-interacting proteins 1 and 2' [APP-1 and APP-2 (Di Cesare et al., 2000)] and 'p95 paxillin kinase linker' [(p95PKL) (Turner et al., 1999)], GIT1 and GIT2 are the names now most commonly used.

The GIT proteins have a complex domain structure and are now known to bind many proteins. They appear to have important functions in the control of cytoskeletal dynamics and membrane trafficking between the plasma membrane and recycling endosomes. Here, we review current understanding of their structure and functions.

The GIT family

All mammals and birds studied to date express two GIT proteins: GIT1 and GIT2. The GIT proteins are very highly conserved across mammalian and avian species. GIT1 is 98% identical between human and mouse, and 85% identical between human and chicken. GIT2 is 86% identical between human and mouse, and 89% identical between human and chicken. The sequence differences between orthologs occur mainly in the central region of the proteins, which suggests that the conserved N- and C-termini contain important functional domains.

Human GIT1 and GIT2 share 65% sequence identity and 85% similarity. A major difference between these proteins is that, whereas GIT1 exists almost entirely in its full-length form, GIT2 is extensively alternatively spliced in a tissue-specific manner. It has at least ten distinct splice variants and potentially 32 different transcripts (Premont et al., 2000). A truncated GIT2 isoform is created by inclusion of an alternative exon that contains a stop codon and has a distinct 3′ untranslated region (Ohara et al., 1997). We term this 'GIT2-short' and the longer isoform 'GIT2-long'. GIT1 and GIT2 are widely distributed. The highest levels occur in testis and there is relatively little in liver and spleen (Ohara et al., 1997; Paris et al., 2002; Premont et al., 1998; Premont et al., 2000). GIT2-long is found in all tissues analyzed, whereas GIT2-short seems to be restricted to immune cells (Premont et al., 2000).

GIT proteins form homo- and heterodimers and localize to focal complexes at the cell periphery, focal adhesions on the ventral surface of the cell, and cytoplasmic structures throughout the cell (Di Cesare et al., 2000; Loo et al., 2004; Manabe et al., 2002; Mazaki et al., 2001; Paris et al., 2002; Turner et al., 1999; West et al., 2001; Zhao et al., 2000). The presence of GIT1 and GIT2 at centrosomes has also been

Table 1. GIT-interacting proteins

GIT1 domain	Interacting proteins	Function
ANK repeat	Endosomes	GIT binding to endosomes
SHD	PIX MEK1 PLCγ	Through PIX, GIT interacts with PAK, Rac1 and Cdc42
Coiled coil	GIT1, GIT2	Homo- and heterodimerization
PBS	Paxillin	Location at focal complexes
Unknown	GRKs	Receptor internalization

reported recently (Zhao et al., 2005). Overexpressed green fluorescent protein (GFP)-tagged GIT1 partially co-localizes with endosomes but not with markers of the Golgi compartment or membranes (Di Cesare et al., 2000; Manabe et al., 2002). GIT2-short has been reported to localize to the cell periphery and perinuclear region (Mazaki et al., 2001).

GIT protein domain structure

The full-length GIT proteins have an N-terminal ARF GTPase-activating protein (ARF-GAP) domain, three ankyrin (ANK) repeats, a Spa2-homology domain (SHD), a coiled-coil domain and a paxillin-binding site (PBS) (Fig. 1). The truncated GIT2-short protein lacks the coiled-coil domain and PBS.

ARF-GAP domain

ARFs are small GTP-binding proteins that regulate membrane traffic by interacting with vesicular coat proteins and phospholipid-metabolizing enzymes. They are regulated by ARF-GAPs, all of which (including GIT1 and GIT2) contain a zinc-finger-like motif ($CX_2CX_{16}CX_2C$) essential for their GAP activity (Cukierman et al., 1995; Goldberg, 1999). GIT1 and GIT2 exhibit ARF-GAP activity towards ARF1, ARF2, ARF3 and ARF5 in vitro (Mazaki et al., 2001; Premont et al., 1998; Premont et al., 2000; Premont and Vitale, 2001; Vitale et al., 2000). Unusually among ARF-GAPs, the GIT proteins also act on ARF6 (Uchida et al., 2001). Moreover, their ARF-GAP activity is stimulated by phosphatidylinositol (3,4,5)-trisphosphate [PtdIns(3,4,5)P_3] but not by phosphatidylinositol 5-phosphate, phosphatidylinositol (4,5)-bisphosphate [PtdIns(4,5)P_2] or diacylglycerol (DAG) (Vitale et al., 2000). GIT proteins might therefore be recruited to the plasma membrane and activated in response to signaling events, regulating signal-mediated membrane trafficking through ARF6.

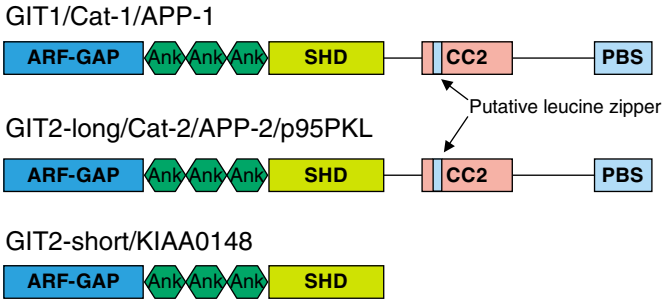

Fig. 1. GIT1 and GIT2 domain structure. The full-length GIT proteins have an N-terminal ARF GTPase-activating protein (ARF-GAP) domain, three ankyrin (ANK) repeats, a Spa2-homology domain (SHD), a coiled-coil (CC) domain and a paxillin-binding site (PBS). The truncated GIT2-short protein lacks the CC domain and PBS.

Deleting the ARF-GAP domain, or introducing an R39K mutation that abolishes ARF-GAP activity, causes GIT1 to associate with very large recycling endosomes that are not seen in normal cells (Di Cesare et al., 2000; Matafora et al., 2001; Paris et al., 2002). This finding suggests that ARF-GAP activity is necessary to maintain proper membrane trafficking and peripheral localization of GIT1 (Matafora et al., 2001). Interestingly, the large vesicles induced by overexpression of GIT2 ARF-GAP mutants accumulate the early endosomal marker EEA1 but not the recycling endosome marker Rab11 (Paris et al., 2002). GIT1 and GIT2 thus appear to have separate roles in trafficking.

Ankyrin repeats

Juxtaposed to the ARF-GAP domain of GIT1 and GIT2 are three ANK repeats. ANK repeats commonly mediate protein-protein interactions but exhibit no consensus binding sequence and have diverse binding partners. The region surrounding the first ANK repeat has sequence similarity to the paxillin-binding region of vinculin (Turner et al., 1999) and some affinity for paxillin in GIT2-short but not GIT1 and GIT2-long (see below) (Mazaki et al., 2001). The first ANK repeat appears to mediate binding of GITs to endosomes, since small N-terminal fragments including only the ARF-GAP and the first ANK repeat localize to endosomes, but deletion of the ANK repeat results in a diffuse cytoplasmic localization (Di Cesare et al., 2000; Paris et al., 2002).

Spa2-homology domain (SHD)

The SHD is a tandem repeat present only in GIT1 and GIT2 orthologs and yeast Spa2 and Sph1 proteins. The yeast proteins are essential for the maintenance of cell polarity, localize to sites of directional cell growth and are required for morphological changes during yeast mating (Arkowitz and Lowe, 1997; Gehrung and Snyder, 1990; Snyder, 1989). A complex set of protein interactions occurs at the SHD to form a large oligomeric complex (Fig. 2). The main binding partner for this domain is p21-activated kinase interacting exchange factor (PIX). Through PIX, GITs can also interact with p21-activated kinase (PAK) and the small GTPases Rac1 and Cdc42.

PIX

PIX proteins contain Dbl-homology and pleckstrin-homology (DH/PH) domains, which are characteristic of guanine nucleotide exchange factors (GEFs). They serve as GEFs for Rac2, Cdc42 and, to a lesser extent, Rho (Feng et al., 2002; Loo et al., 2004; Manser et al., 1998). The two PIX proteins are termed α-PIX and β-PIX; the latter is alternatively spliced to 85 kDa and 50 kDa forms. Only α-PIX and the longer form of β-PIX bind to GIT1. An α-helical region of the C-terminus appears to mediate binding to the SHD (Feng et al., 2002; Loo et al., 2004).

GIT and PIX proteins interact through the SHD in two-hybrid assays (Premont et al., 2000) and co-immunoprecipitation experiments (Bagrodia et al., 1999; Brown et al., 2002; Di Cesare et al., 2000; Lamorte et al., 2003;

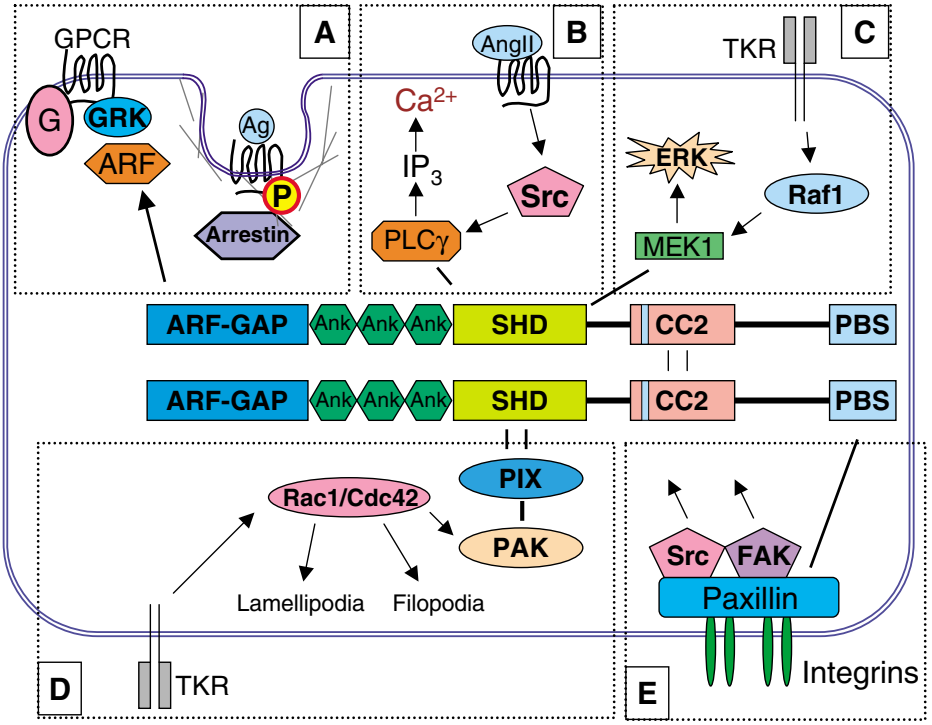

Fig. 2. Summary of GIT1 protein interactions and functions. Major signaling pathways include (A) agonist (Ag)-receptor (GPCR) endocytosis; (B) angiotensin-II (AngII)-stimulated PLCγ activation and Ca^{2+} mobilization; (C) scaffolding MEK1 activation in response to TRK activation; (D) cytoskeletal regulation through PAK and Rho-family GTPases; and (E) focal complex assembly and disassembly. G, heterotrimeric G proteins; GRK, GPCR kinase; GPCR, G-protein-coupled receptor; IP_3, inositol (1,4,5)-trisphosphate; P, phosphorylation; TKR, tyrosine kinase receptor.

Loo et al., 2004; Premont et al., 2000; Zhao et al., 2000). Since both proteins can homodimerize, they might form large aggregates in the cell (Fig. 2). Indeed, GIT and PIX are predominantly found in very high molecular weight (1-2 MDa) fractions in cell lysates separated by gel filtration and sucrose density gradients (Paris et al., 2003; Premont et al., 2004). GITs and PIX appear to be the major constituents of the aggregates and form complexes of a similar size (approximately 10-25 subunits) when co-expressed in sf9 cells (Premont et al., 2004). However, such complexes cannot be created in vitro, which suggests they are formed co-translationally (Premont et al., 2004).

PIX and GITs co-localize at focal complexes, the cell periphery and cytoplasmic complexes, which suggests that these proteins exist as oligomers at each of these sites (Loo et al., 2004; Turner et al., 1999). In fact, oligomerization appears to be essential for localization to these sites since mutations that disrupt either GIT-PIX association or PIX homodimerization result in diffuse cytoplasmic localization of both proteins (Kim et al., 2001; Loo et al., 2004).

PAK

PAKs are serine/threonine kinases that are activated by Rac1 and Cdc42 (Hofmann et al., 2004). PAK activity produces major effects on cell morphology by catalyzing disassembly of actin stress fibers at focal adhesions (Zhao et al., 2000). PAK also mediates the Rac1 and/or Cdc42 (Rac1/Cdc42)-induced activation of several mitogen-activated protein kinases (MAPKs): extracellular signal-regulated kinases 1 and 2 (ERK1/2), Jun N-terminal kinase (JNK) and p38 (Bagrodia et al., 1999). PAK interacts directly with PIX and is thus recruited to GIT-PIX oligomers (Bagrodia et al., 1999; Brown et al., 2002; Di Cesare et al., 2000; Premont et al., 2000; Zhao et al., 2000).

Small GTPases

RhoA, Rac1 and Cdc42 have major roles in regulating cell morphology and motility. RhoA activity promotes actin-myosin contraction, leading to formation of actin stress fibers and steady-state focal complexes termed focal adhesions. Rac1 activation causes actin polymerization and formation of lamellipodia. Cdc42 activation stimulates formation of filopodia. Rac1 and Cdc42, but not RhoA, bind directly to PAK when in the active (GTP-bound) state but not in the inactive (GDP-bound) state (Bagrodia et al., 1999). It was initially thought that GTP-bound Rac1/Cdc42 directly activates PAK kinase activity. However, recent evidence suggests an essential role for GIT-PIX complexes in Rac1/Cdc42-mediated activation of PAK kinase activity (Fig. 3). These new data indicate that association with GTP-bound Rac1/Cdc42 induces a conformational change in PAK that exposes its N-terminal PIX-binding region, permitting association of Rac1/Cdc42-PAK with GIT-PIX oligomers (Brown et al., 2002). High local concentrations of PAK at GIT-PIX complexes permit autophosphorylation and consequently activation of PAK (Loo et al., 2004).

The Rac1/Cdc42-induced association of PAK with GIT-PIX oligomers also recruits GIT-PIX to focal complexes. Translocation of GITs to the cell periphery inhibits Rac1 activity, thus acting as negative feedback that prevents prolonged Rac1 activity through an undefined mechanism that requires ARF-GAP activity (Nishiya et al., 2005; West et al., 2001). The role of PIX, and its potential ability to stimulate Rac1 and Cdc42 by virtue of its DH/PH domains, is not well understood.

Stimulation of endothelial cells with thrombin recruits GIT1 to focal adhesions in a RhoA-dependent manner (Shikata et al., 2003a; van Nieuw Amerongen et al., 2004), although constitutively active RhoA does not induce translocation of

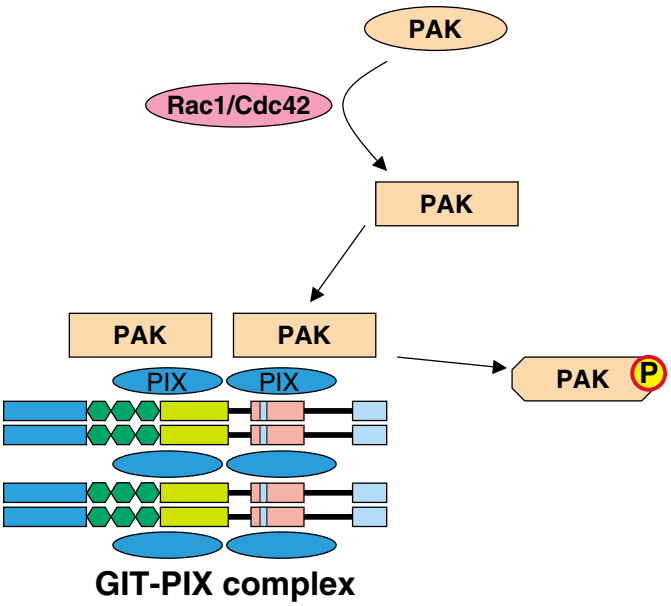

Fig. 3. Rac1/Cdc42-stimulated PAK activation requires GIT-PIX oligomers. Activated Rac1 and Cdc42 interact with inactive PAK, causing a conformational change that allows it to bind to GIT-PIX oligomers through a direct interaction with PIX. High local concentrations of PAK permit autophosphorylation (P), stimulating the kinase activity of PAK and its subsequent disassociation from the GIT-PIX oligomers.

GIT2 to focal adhesions (Brown et al., 2002). This suggests an essential difference between GIT1 and GIT2, although the reason for this disparity is currently unknown. Since RhoA does not directly interact with or activate PAK, its recruitment of GIT1 to focal adhesions must occur through a mechanism different from that by which Rac1/Cdc42 induces recruitment of GITs to focal adhesions.

MEK1

MEK1 is the upstream kinase that phosphorylates and activates ERK1/2. GIT1 interacts with MEK1 through the SHD (Yin et al., 2004). Interestingly, the SHD region of yeast Spa2 also interacts with yeast MEK1 orthologs (Mkk1p, Mkk2p and Ste7p), which suggests that this is an evolutionarily conserved function (Sheu et al., 1998). GIT1 might act as a scaffold that exerts spatial control of ERK1/2 activation since (1) GIT1 co-localizes with ERK1/2 at focal adhesions (Yin et al., 2005), (2) overexpressing GIT1 prolongs epidermal growth factor (EGF) stimulation of ERK1/2, and (3) knocking down GIT1 expression with short interfering (si)RNA inhibits EGF-stimulated ERK1/2 activity (Yin et al., 2004).

PLCγ

PLCγ catalyzes the hydrolysis of PtdIns(4,5)P_2 to DAG and inositol (1,4,5)-trisphosphate [Ins(1,4,5)P_3], in common with other PLC isoforms, but is uniquely regulated by tyrosine phosphorylation. GIT1 binds to a PLCγ SH2 domain through the SHD region but does not bind to PLCδ or PLCβ (Haendeler et al., 2003; Schmitz et al., 1997). GIT1 and PLCγ are both phosphorylated in a Src-dependent manner in response to various agonists, although their association is not affected (Haendeler et al., 2003). Furthermore, knocking down GIT1 expression inhibits agonist-mediated PLCγ phosphorylation and Ca^{2+} release (Haendeler et al., 2003). These data suggest that GIT-PIX oligomers act as a scaffold that stably binds PLCγ and recruits an upstream kinase in response to agonist stimulation.

The coiled-coil domain

GIT1 and GIT2-long each contain three coiled-coil structures. Two occur in the SHD region and the C-terminal PBS (Zhao et al., 2000). The remaining coiled-coil, which lies between the SHD and the PBS, is not known to be a part of a larger domain and is thus simply referred to as the 'coiled-coil domain'. Computational predictions that GIT1 and GIT2 homo- and heterodimerize by using a putative leucine zipper motif within this region have been confirmed by co-immunoprecipitation (Kim et al., 2003; Paris et al., 2003; Premont et al., 2004). GIT1 and GIT2 are also found in fractions whose mobility is consistent with the existence of dimers when cell lysates are separated by sucrose density gradients or gel filtration (Kim et al., 2003; Paris et al., 2003; Premont et al., 2004). These interactions, together with PIX interaction and PIX homodimerization, probably provide the basis for the formation of the large GIT-PIX oligomers discussed above.

The paxillin-binding site

Paxillin is a focal adhesion adaptor protein that associates with binding partners through five N-terminal LDXLLXXL motifs (termed LD motifs) and four C-terminal LIM domains. GIT1 and GIT2-long bind to the LD4 region of paxillin through their PBS (residues 643-679) (Brown et al., 2002; Di Cesare et al., 2000; Lamorte et al., 2003; Matafora et al., 2001; Mazaki et al., 2001; Turner et al., 1999; Zhao et al., 2000). Hic-5 and leupaxin, members of the paxillin superfamily that contain LD motifs, also bind to GIT1 and GIT2-long (Gupta et al., 2003; Nishiya et al., 2002; Turner et al., 1999). The interaction with paxillin localizes GITs to focal complexes; deletion of either the paxillin LD4 motif or the GIT PBS region prevents GITs from associating with focal complexes (Brown et al., 2002; Matafora et al., 2001; West et al., 2001).

A C-terminal fragment of GIT1 containing only the PBS localizes to focal complexes more robustly than does the full-length protein, which suggests that the PBS region is normally not available for binding to paxillin (Brown et al., 2002; Di Cesare et al., 2000; Manabe et al., 2002; Matafora et al., 2001; Paris et al., 2002). It is likely that association of PAK with GIT-PIX unmasks the PBS region, promoting paxillin binding and recruitment to focal complexes. Two results support this idea. First, activated Rac1 and Cdc42, which cause PAK to associate with GIT-PIX complexes, translocate GIT-PIX complexes to focal complexes (Brown et al., 2002; Loo et al., 2004; Manser et al., 1997; Matafora et al., 2001; Shikata et al., 2003b). Second, a PAK mutant that is unable to bind PIX blocks localization of GIT-PIX to focal complexes (Brown et al., 2002).

Other binding sites

GRKs

GRKs bind to and phosphorylate agonist-bound GPCRs, leading to their internalization through clathrin-coated pits. The GITs interact directly with the GRKs through an unknown

binding site (Premont et al., 1998; Premont et al., 2000). Since recruitment of GRKs to the plasma membrane is an early response to binding of agonists to GPCRs, this interaction might cause agonist-dependent recruitment of GITs to the plasma membrane. Indeed, GRK2-dependent recruitment of GIT to membranes has been demonstrated in vitro (Premont et al., 1998).

GIT phosphorylation

The kinases known to phosphorylate GIT are Src and FAK. Overexpressing active forms of these kinases increases GIT1 phosphorylation (Bagrodia et al., 1999; Kawachi et al., 2001; Yin et al., 2004), and we have shown Src-mediated phosphorylation of GIT1 in response to agonists including angiotensin II, EGF and thrombin (Haendeler et al., 2003; van Nieuw Amerongen et al., 2004; Yin et al., 2004). FAK-mediated phosphorylation of GITs is only partially blocked by a kinase-inactive FAK mutant, which suggests that the scaffolding function of FAK is also important for phosphorylation of GITs (Bagrodia et al., 1999). Recent data suggest that phosphorylation of GIT2 is required for its recruitment to focal complexes, although GIT1 does not appear to have such a requirement (Brown et al., 2005). The only phosphatase shown to dephosphorylate GIT1 is protein tyrosine phosphatase ζ (PTPζ) (Kawachi et al., 2001), which also appears to bind GIT1 (Kawachi et al., 2001).

GIT functions

Cytoskeletal dynamics

A major function of the GIT proteins appears to be the regulation of cytoskeletal dynamics during cell spreading and migration. GIT phosphorylation and movement of these proteins from the cytoplasm to focal complexes occur during both processes (Bagrodia et al., 1999; Lamorte et al., 2003; Manabe et al., 2002). Mutations of GITs or paxillin that prevent recruitment of GIT-PIX oligomers to focal complexes result in the formation of numerous abnormal broad lamellipodia during cell spreading owing to increased Rac1 activity (Brown et al., 2005; West et al., 2001). Rac1 is activated by cell attachment, which leads to association of PAK with GIT-PIX and the series of events discussed above that ultimately leads to inhibition of Rac1 by GIT ARF-GAP activity.

GITs have an essential role maintaining proper directionality of cell movement, probably by restricting Rac activity to the leading edge of the cell. GIT-containing complexes move from the trailing edge to the leading edge in migrating cells (Manabe et al., 2002). Blocking the GIT-paxillin interaction increases random cell motility, probably reflecting a disinhibition of Rac1 activity that allows uncontrolled cell protrusion and retraction (Turner et al., 1999; West et al., 2001). GITs also negatively regulate RhoA and therefore might participate in the disassembly of focal adhesions at the trailing edge (Mazaki et al., 2001; Shikata et al., 2003b; van Nieuw Amerongen et al., 2004; Zhao et al., 2000).

The GITs appear to have specialized cytoskeletal functions in neurons. Neurite outgrowth requires Rac and ARF6 activity, and GIT1 mutations that abrogate ARF-GAP activity inhibit this process (Albertinazzi et al., 2003). GIT1 is enriched in pre- and postsynaptic densities (Kim et al., 2003; Ko et al., 2003; Zhang et al., 2003), and inhibiting formation of GIT-PIX oligomers in cultured hippocampal neurons decreases the number of synapses formed along dendrites (Zhang et al., 2003).

Receptor internalization and membrane trafficking

The GITs play an important role in regulation of receptor internalization through clathrin-coated pits. Overexpressing GIT1 and GIT2-long disrupts the normal internalization of some agonist-bound receptors from the plasma membrane, including the β_1 and β_2-adrenergic, adenosine 2B, μ-opioid, M1 muscarinic, and EGF receptors (Claing et al., 2000; Premont et al., 1998; Premont et al., 2000). As a result, cells do not resensitize to the associated agonists. Other receptors, including the VIP_1, M2 muscarinic, endothelin B and angiotensin II receptors, which may internalize through mechanisms separate from clathrin-coated pits, are not affected by overexpression of GITs (Claing et al., 2000).

ARF6 is a central mediator in membrane trafficking between the cell surface and endosomes. ARF6 mutants constitutively bound to either GDP or GTP inhibit receptor internalization and resensitization in a similar manner to overexpression of GITs (Claing et al., 2001). Removal of ARF-GAP activity abolishes the effect of overexpressed GITs on receptor internalization (Premont et al., 1998). It is unclear how ARF-GAP activity regulates trafficking of agonist-bound receptors from the cell surface to recycling endosomes, then back to the cell surface. Recruitment of GITs to the cell membrane through their interaction with GRKs might be involved, but further work will be necessary to elucidate the mechanism.

Recent evidence suggests that GIT1 could be involved in the altered membrane trafficking that contributes to Huntington's disease. GIT1 associates with the pathogenic huntingtin protein mutant (Goehler et al., 2004). Aggregation of this mutant increases in response to expression of a GIT1 mutant that does not possess ARF-GAP activity (Goehler et al., 2004). In neurons cultured from patients with Huntington's disease, lower-molecular-weight variants of GIT1 are observed, and these might represent N-terminal deletions lacking ARF-GAP activity (Goehler et al., 2004). GITs also interact with the presynaptic neuronal proteins piccolo and scribble, which are involved in organization and release of presynaptic vesicles (Audebert et al., 2004; Kim et al., 2003). In addition, they interact with the postsynaptic protein liprin-α, which anchors AMPA glutamate receptors to post-synaptic densities (Ko et al., 2003).

Several reports suggest that GIT proteins have a role in HIV infectivity. Downregulation of cell-surface CD4 and major histocompatibility complex (MHC) class I on the host cell by the viral protein Nef occurs soon after infection. Internalization of MHC class I is mediated by ARF6 (Blagoveshchenskaya et al., 2002). The mechanism by which Nef promotes this process is unknown, although it binds to and activates PAK, and in so doing associates with GIT-PIX oligomers. This raises the possibility that it affects ARF6 activity through GITs (Brown et al., 1999).

Conclusion

The GIT proteins function in the regulation of cytoskeletal structure, membrane trafficking and the scaffolding of signaling cascades. It is clear that they have important roles in spatial localization of signaling molecules and hence in cell polarity. Increasing interest in the GIT proteins has led to the description of numerous interacting proteins, which has hinted

that they have specialized roles in neurons and other cell types. Exciting recent results also suggest possible pathogenic roles for GIT proteins in Huntington's disease and HIV infection.

Future studies will further elucidate the mechanisms by which these proteins regulate diverse cellular functions, including membrane trafficking, and focal adhesion assembly and disassembly. As more is learned, the links and commonalities between this diverse set of functions might shed further light on the manner by which cells coordinate these activities to achieve more complex tasks – for example, the coordination of cytoskeletal and membrane dynamics for cell movement. In vivo studies will be important to verify the physiological importance of the large number of findings that have already been documented in cellular systems. Finally, the further application of our knowledge of these proteins to what is known of HIV infection and Huntington's disease, as well as other disease states, might yield new diagnostic or therapeutic tools.

References

Albertinazzi, C., Za, L., Paris, S. and de Curtis, I. (2003). ADP-ribosylation factor 6 and a functional PIX/p95-APP1 complex are required for Rac1B-mediated neurite outgrowth. *Mol. Biol. Cell* **14**, 1295-1307.

Arkowitz, R. A. and Lowe, N. (1997). A small conserved domain in the yeast Spa2p is necessary and sufficient for its polarized localization. *J. Cell Biol.* **138**, 17-36.

Audebert, S., Navarro, C., Nourry, C., Chasserot-Golaz, S., Lecine, P., Bellaiche, Y., Dupont, J. L., Premont, R. T., Sempere, C., Strub, J. M. et al. (2004). Mammalian scribble forms a tight complex with the betaPIX exchange factor. *Curr. Biol.* **14**, 987-995.

Bagrodia, S., Bailey, D., Lenard, Z., Hart, M., Guan, J. L., Premont, R. T., Taylor, S. J. and Cerione, R. A. (1999). A tyrosine-phosphorylated protein that binds to an important regulatory region on the Cool family of p21-activated kinase-binding proteins. *J. Biol. Chem.* **274**, 22393-22400.

Blagoveshchenskaya, A. D., Thomas, L., Feliciangeli, S. F., Hung, C. H. and Thomas, G. (2002). HIV-1 Nef downregulates MHC-I by a PACS-1- and PI3K-regulated ARF6 endocytic pathway. *Cell* **111**, 853-866.

Brown, A., Wang, X., Sawai, E. and Cheng-Mayer, C. (1999). Activation of the PAK-related kinase by human immunodeficiency virus type 1 Nef in primary human peripheral blood lymphocytes and macrophages leads to phosphorylation of a PIX-p95 complex. *J. Virol.* **73**, 9899-9907.

Brown, M. C., West, K. A. and Turner, C. E. (2002). Paxillin-dependent paxillin kinase linker and p21-activated kinase localization to focal adhesions involves a multistep activation pathway. *Mol. Biol. Cell* **13**, 1550-1565.

Brown, M. C., Cary, L. A., Jamieson, J. S., Cooper, J. A. and Turner, C. E. (2005). Src and FAK kinases cooperate to phosphorylate paxillin kinase linker, stimulate its focal adhesion localization, and regulate cell spreading and protrusiveness. *Mol. Biol. Cell* **16**, 4316-4328.

Claing, A., Perry, S. J., Achiriloaie, M., Walker, J. K., Albanesi, J. P., Lefkowitz, R. J. and Premont, R. T. (2000). Multiple endocytic pathways of G protein-coupled receptors delineated by GIT1 sensitivity. *Proc. Natl. Acad. Sci. USA* **97**, 1119-1124.

Claing, A., Chen, W., Miller, W. E., Vitale, N., Moss, J., Premont, R. T. and Lefkowitz, R. J. (2001). beta-Arrestin-mediated ADP-ribosylation factor 6 activation and beta 2-adrenergic receptor endocytosis. *J. Biol. Chem.* **276**, 42509-42513.

Cukierman, E., Huber, I., Rotman, M. and Cassel, D. (1995). The ARF1 GTPase-activating protein: zinc finger motif and Golgi complex localization. *Science* **270**, 1999-2002.

Di Cesare, A., Paris, S., Albertinazzi, C., Dariozzi, S., Andersen, J., Mann, M., Longhi, R. and de Curtis, I. (2000). p95-APP1 links membrane transport to Rac-mediated reorganization of actin. *Nat. Cell Biol.* **2**, 521-530.

Feng, Q., Albeck, J. G., Cerione, R. A. and Yang, W. (2002). Regulation of the Cool/Pix proteins: key binding partners of the Cdc42/Rac targets, the p21-activated kinases. *J. Biol. Chem.* **277**, 5644-5650.

Gehrung, S. and Snyder, M. (1990). The SPA2 gene of Saccharomyces cerevisiae is important for pheromone-induced morphogenesis and efficient mating. *J. Cell Biol.* **111**, 1451-1464.

Goehler, H., Lalowski, M., Stelzl, U., Waelter, S., Stroedicke, M., Worm, U., Droege, A., Lindenberg, K. S., Knoblich, M., Haenig, C. et al. (2004). A protein interaction network links GIT1, an enhancer of huntingtin aggregation, to Huntington's disease. *Mol. Cell* **15**, 853-865.

Goldberg, J. (1999). Structural and functional analysis of the ARF1-ARFGAP complex reveals a role for coatomer in GTP hydrolysis. *Cell* **96**, 893-902.

Gupta, A., Lee, B. S., Khadeer, M. A., Tang, Z., Chellaiah, M., Abu-Amer, Y., Goldknopf, J. and Hruska, K. A. (2003). Leupaxin is a critical adaptor protein in the adhesion zone of the osteoclast. *J. Bone Miner. Res.* **18**, 669-685.

Haendeler, J., Hojo, Y., Aebersold, R., Yan, C. and Berk, B. C. (2000). Angiotensin II-stimulated phosphorylation of GIT1 determines the relative activation of PLCγ and ERK1/2. *Circulation* **102**, II-15.

Haendeler, J., Yin, G., Hojo, Y., Saito, Y., Melaragno, M., Yan, C., Sharma, V. K., Heller, M., Aebersold, R. and Berk, B. C. (2003). GIT1 mediates Src-dependent activation of phospholipase Cgamma by angiotensin II and epidermal growth factor. *J. Biol. Chem.* **278**, 49936-49944.

Hofmann, C., Shepelev, M. and Chernoff, J. (2004). The genetics of Pak. *J. Cell Sci.* **117**, 4343-4354.

Kawachi, H., Fujikawa, A., Maeda, N. and Noda, M. (2001). Identification of GIT1/Cat-1 as a substrate molecule of protein tyrosine phosphatase zeta /beta by the yeast substrate-trapping system. *Proc. Natl. Acad. Sci. USA* **98**, 6593-6598.

Kim, S., Lee, S. H. and Park, D. (2001). Leucine zipper-mediated homodimerization of the p21-activated Kinase-interacting factor, beta Pix. Implication for a role in cytoskeletal reorganization. *J. Biol. Chem.* **276**, 10581-10584.

Kim, S., Ko, J., Shin, H., Lee, J. R., Lim, C., Han, J. H., Altrock, W. D., Garner, C. C., Gundelfinger, E. D., Premont, R. T. et al. (2003). The GIT family of proteins forms multimers and associates with the presynaptic cytomatrix protein piccolo. *J. Biol. Chem.* **278**, 6291-6300.

Ko, J., Kim, S., Valtschanoff, J. G., Shin, H., Lee, J. R., Sheng, M., Premont, R. T., Weinberg, R. J. and Kim, E. (2003). Interaction between liprin-alpha and GIT1 is required for AMPA receptor targeting. *J. Neurosci.* **23**, 1667-1677.

Lamorte, L., Rodrigues, S., Sangwan, V., Turner, C. E. and Park, M. (2003). Crk associates with a multimolecular Paxillin/GIT2/beta-PIX complex and promotes Rac-dependent relocalization of Paxillin to focal contacts. *Mol. Biol. Cell* **14**, 2818-2831.

Loo, T. H., Ng, Y. W., Lim, L. and Manser, E. (2004). GIT1 activates p21-activated kinase through a mechanism independent of p21 binding. *Mol. Cell. Biol.* **24**, 3849-3859.

Manabe, R., Kovalenko, M., Webb, D. J. and Horwitz, A. R. (2002). GIT1 functions in a motile, multi-molecular signaling complex that regulates protrusive activity and cell migration. *J. Cell Sci.* **115**, 1497-1510.

Manser, E., Huang, H. Y., Loo, T. H., Chen, X. Q., Dong, J. M., Leung, T. and Lim, L. (1997). Expression of constitutively active alpha-PAK reveals effects of the kinase on actin and focal complexes. *Mol. Cell. Biol.* **17**, 1129-1143.

Manser, E., Loo, T. H., Koh, C. G., Zhao, Z. S., Chen, X. Q., Tan, L., Tan, I., Leung, T. and Lim, L. (1998). PAK kinases are directly coupled to the PIX family of nucleotide exchange factors. *Mol. Cell* **1**, 183-192.

Matafora, V., Paris, S., Dariozzi, S. and de Curtis, I. (2001). Molecular mechanisms regulating the subcellular localization of p95-APP1 between the endosomal recycling compartment and sites of actin organization at the cell surface. *J. Cell Sci.* **114**, 4509-4520.

Mazaki, Y., Hashimoto, S., Okawa, K., Tsubouchi, A., Nakamura, K., Yagi, R., Yano, H., Kondo, A., Iwamatsu, A., Mizoguchi, A. et al. (2001). An ADP-ribosylation factor GTPase-activating protein Git2- short/KIAA0148 is involved in subcellular localization of paxillin and actin cytoskeletal organization. *Mol. Biol. Cell* **12**, 645-662.

Nishiya, N., Shirai, T., Suzuki, W. and Nose, K. (2002). Hic-5 interacts with GIT1 with a different binding mode from paxillin. *J. Biochem.* **132**, 279-289.

Nishiya, N., Kiosses, W. B., Han, J. and Ginsberg, M. H. (2005). An alpha4 integrin-paxillin-Arf-GAP complex restricts Rac activation to the leading edge of migrating cells. *Nat. Cell Biol.* **7**, 343-352.

Ohara, O., Nagase, T., Ishikawa, K., Nakajima, D., Ohira, M., Seki, N. and Nomura, N. (1997). Construction and characterization of human brain cDNA libraries suitable for analysis of cDNA clones encoding relatively large proteins. *DNA Res.* **4**, 53-59.

Paris, S., Za, L., Sporchia, B. and de Curtis, I. (2002). Analysis of the subcellular distribution of avian p95-APP2, an ARF-GAP orthologous to mammalian paxillin kinase linker. *Int. J. Biochem. Cell Biol.* **34**, 826-837.

Paris, S., Longhi, R., Santambrogio, P. and de Curtis, I. (2003). Leucine-zipper-mediated homo- and hetero-dimerization of GIT family p95-ARF GTPase-activating protein, PIX-, paxillin-interacting proteins 1 and 2. *Biochem. J.* **372**, 391-398.

Premont, R. T. and Vitale, N. (2001). Purification and characterization of GIT family of ADP-ribosylation factor (ARF) GTPase-activating proteins. *Methods Enzymol.* **329**, 335-343.

Premont, R. T., Claing, A., Vitale, N., Freeman, J. L., Pitcher, J. A., Patton, W. A., Moss, J., Vaughan, M. and Lefkowitz, R. J. (1998). beta2-Adrenergic receptor regulation by GIT1, a G protein-coupled receptor kinase-associated ADP ribosylation factor GTPase-activating protein. *Proc. Natl. Acad. Sci. USA* **95**, 14082-14087.

Premont, R. T., Claing, A., Vitale, N., Perry, S. J. and Lefkowitz, R. J. (2000). The GIT family of ADP-ribosylation factor GTPase-activating proteins. Functional diversity of GIT2 through alternative splicing. *J. Biol. Chem.* **275**, 22373-22380.

Premont, R. T., Perry, S. J., Schmalzigaug, R., Roseman, J. T., Xing, Y. and Claing, A. (2004). The GIT/PIX complex: an oligomeric assembly of GIT family ARF GTPase-activating proteins and PIX family Rac1/Cdc42 guanine nucleotide exchange factors. *Cell. Signal.* **16**, 1001-1011.

Schmitz, U., Ishida, M., Ishida, T. and Berk, B. C. (1997). Angiotensin II stimulates tyrosine phosphorylation of PLC-γ associated proteins: characterization of a c-Src-dependent 97 kD protein in vascular smooth muscle cells. *Circ. Res.* **81**, 550-557.

Sheu, Y. J., Santos, B., Fortin, N., Costigan, C. and Snyder, M. (1998). Spa2p interacts with cell polarity proteins and signaling components involved in yeast cell morphogenesis. *Mol. Cell. Biol.* **18**, 4053-4069.

Shikata, Y., Birukov, K. G., Birukova, A. A., Verin, A. and Garcia, J. G. (2003a). Involvement of site-specific FAK phosphorylation in sphingosine-1 phosphate- and thrombin-induced focal adhesion remodeling: role of Src and GIT. *FASEB J.* **17**, 2240-2249.

Shikata, Y., Birukov, K. G. and Garcia, J. G. (2003b). S1P induces FA remodeling in

human pulmonary endothelial cells: role of Rac, GIT1, FAK, and paxillin. *J. Appl. Physiol*. **94**, 1193-1203.

Snyder, M. (1989). The SPA2 protein of yeast localizes to sites of cell growth. *J. Cell Biol*. **108**, 1419-1429.

Turner, C. E., Brown, M. C., Perrotta, J. A., Riedy, M. C., Nikolopoulos, S. N., McDonald, A. R., Bagrodia, S., Thomas, S. and Leventhal, P. S. (1999). Paxillin LD4 motif binds PAK and PIX through a novel 95-kD ankyrin repeat, ARF-GAP protein: a role in cytoskeletal remodeling. *J. Cell Biol*. **145**, 851-863.

Uchida, H., Kondo, A., Yoshimura, Y., Mazaki, Y. and Sabe, H. (2001). PAG3/Papalpha/KIAA0400, a GTPase-activating protein for ADP-ribosylation factor (ARF), regulates ARF6 in Fcgamma receptor-mediated phagocytosis of macrophages. *J. Exp. Med*. **193**, 955-966.

van Nieuw Amerongen, G. P., Natarajan, K., Yin, G., Hoefen, R. J., Osawa, M., Haendeler, J., Ridley, A. J., Fujiwara, K., van Hinsbergh, V. and Berk, B. C. (2004). GIT1 mediates thrombin signaling in endothelial cells: role in turnover of RhoA-type focal adhesions. *Circ. Res*. **94**, 1041-1049.

Vitale, N., Patton, W. A., Moss, J., Vaughan, M., Lefkowitz, R. J. and Premont, R. T. (2000). GIT proteins, A novel family of phosphatidylinositol 3,4,5-trisphosphate-stimulated GTPase-activating proteins for ARF6. *J. Biol. Chem*. **275**, 13901-13906.

West, K. A., Zhang, H., Brown, M. C., Nikolopoulos, S. N., Riedy, M. C., Horwitz, A. F. and Turner, C. E. (2001). The LD4 motif of paxillin regulates cell spreading and motility through an interaction with paxillin kinase linker (PKL). *J. Cell Biol*. **154**, 161-176.

Yin, G., Haendeler, J., Yan, C. and Berk, B. C. (2004). GIT1 functions as a scaffold for MEK1-extracellular signal-regulated kinase 1 and 2 activation by angiotensin II and epidermal growth factor. *Mol. Cell. Biol*. **24**, 875-885.

Yin, G., Zheng, Q., Yan, C. and Berk, B. C. (2005). GIT1 is a scaffold for ERK1/2 activation in focal adhesions. *J. Biol. Chem*. **280**, 27705-27712.

Zhang, H., Webb, D. J., Asmussen, H. and Horwitz, A. F. (2003). Synapse formation is regulated by the signaling adaptor GIT1. *J. Cell Biol*. **161**, 131-142.

Zhao, Z. S., Manser, E., Loo, T. H. and Lim, L. (2000). Coupling of PAK-interacting exchange factor PIX to GIT1 promotes focal complex disassembly. *Mol. Cell. Biol*. **20**, 6354-6363.

Zhao, Z. S., Lim, J. P., Ng, Y. W., Lim, L. and Manser, E. (2005). The GIT-associated kinase PAK targets to the centrosome and regulates Aurora-A. *Mol. Cell* **20**, 237-249.