GIT Proteins, A Novel Family of Phosphatidylinositol 3,4,5-
Trisphosphate-stimulated GTPase-activating Proteins for ARF6

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ADP-ribosylation factor (ARF) proteins are key players in numerous vesicular trafficking events ranging from the formation and fusion of vesicles in the Golgi apparatus to exocytosis and endocytosis. To complete their GTPase cycle, ARFs require a guanine nucleotide-exchange protein to catalyze replacement of GDP by GTP and a GTPase-activating protein (GAP) to accelerate hydrolysis of bound GTP. Recently numerous guanine nucleotide-exchange proteins and GAP proteins have been identified and partially characterized. Every ARF GAP protein identified to date contains a characteristic zinc finger motif. GIT1 and GIT2, two members of a new family of G protein-coupled receptor kinase-interacting proteins, also contain a putative zinc finger motif and display ARF GAP activity. Truncation of the amino-terminal region containing the zinc finger motif prevented GAP activity of GIT1. One zinc molecule was found associated per molecule of purified recombinant ARF-GAP1, GIT1, and GIT2 proteins, suggesting the zinc finger motifs of ARF GAPs are functional and should play an important role in their GAP activity. Unlike ARF-GAP1, GIT1 and GIT2 stimulate hydrolysis of GTP bound to ARF6. Accordingly we found that the phospholipid dependence of the GAP activity of ARF-GAP1 and GIT proteins was quite different, as the GIT proteins are stimulated by phosphatidylinositol 3,4,5-trisphosphate whereas ARF-GAP1 is stimulated by phosphatidylinositol 4,5-bisphosphate and diacylglycerol. These results suggest that although the mechanism of GTP hydrolysis is probably very similar in these two families of ARF GAPs, GIT proteins might specifically regulate the activity of ARF6 in cells in coordination with phosphatidylinositol 3-kinase signaling pathways.

ADP-ribosylation factors (ARFs),1 a family of 20-kDa gua-

nine nucleotide-binding proteins originally identified as activators of the ADP-ribosylation of Gαs by cholera toxin, play a critical role in vesicular trafficking (for review, see Ref. 1). Members of the family include the six ARF proteins, the ARF-like (ARL) proteins, and the related, much larger ARD1 protein (2).

Like other small guanine nucleotide-binding proteins, ARFs cycle between inactive GDP-bound and active GTP-bound states. Dissociation of GDP and binding of GTP to ARFs is strongly accelerated by guanine nucleotide-exchange proteins. Several ARF guanine nucleotide-exchange proteins have been isolated and characterized (reviewed in Ref. 2). Inactivation of ARFs requires hydrolysis of bound GTP. Because members of the ARF family have an extremely low intrinsic GTPase activity, an additional GAP protein is required to catalyze GTP hydrolysis. One 48-kDa GAP, ARF-GAP1, has been purified from rat liver (3, 4) and cloned (5).

ARF-GAP1 is recruited to Golgi membranes by oligomerized ERD2 (6). This membrane receptor recognizes soluble proteins from the endoplasmic reticulum that contain a KDEL carboxy-terminal sequence for retrieval from the Golgi apparatus (7). ARF-GAP1 then inactivates ARF1 and produces a phenotype identical to that observed when ARF guanine nucleotide-exchange proteins are inactivated (6). A distinct activity, termed ARF-GAP2, has also been purified from the same tissue (4). ARF-GAP1 and ARF-GAP2 have different phospholipid dependence, with PIP2, phosphatidic acid, and PS stimulating ARF-GAP2 more robustly than ARF-GAP1 (4). Recombinant ARF-GAP1 has also been reported to be stimulated by diacylglycerol (8). For these two GAPs, substrate specificity is restricted to ARF1–5 (4). They share significant similarities with the yeast Gcs1 protein that is also a GAP for ARF1 (9). An apparently distinct ARF GAP of ~50-kDa, purified from rat spleen, has a broader specificity, which also includes ARF6 and ARL1 (10).

Two distinct protein families containing putative zinc finger ARF GAP domains similar to ARF-GAP1 have been characterized recently, the GIT family and the ASAP1/DEF-1/PAP family. The GIT1 protein was identified in a two-hybrid screen with G protein-coupled receptor kinase 2 (GRK2) (11). The mRNA for the highly similar GIT2 protein, the product of the KIAA0148 gene undergoes extensive tissue-specific alternative splicing (12, 35). Both GIT proteins stimulate hydrolysis of GTP bound to ARF1 (11, 35). The GIT proteins share a common structure, with an amino-terminal zinc finger-like motif, three

1 The abbreviations used are: ARF, ADP-ribosylation factor; ARD, ARF-domain protein; CTA, cholera toxin-catalyzed ADP-ribosyltransferase activity; DTT, dithiothreitol; GAP, GTPase-activating protein; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol 3,4,5-trisphosphate; PS, phosphatidylserine; GTPγS, guanosine 5′-O-(thiotriphosphate).

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ankyrin repeats, and a carboxyl-terminal GRK interaction domain. One major splice variant of GIT2, termed GIT2-short, lacks the carboxyl-terminal GRK interaction domain (35). Overexpression of GIT1 leads to reduced β2-adrenergic receptor signaling and increased receptor phosphorylation, which appear to result from reduced receptor internalization and resensitization (11). These cellular effects of GIT1 require an intact ARF GAP activity, suggesting a critical role of the coupling of GIT1 and ARF in β2-adrenergic receptor endocytosis.

Both ASAP1/DEF-1 and PAP/KIAA0400 were identified through interactions with known proteins, the Src and Pyk2 kinases, respectively (13, 14). They share a similar organization, with a PH domain, central ARF GAP-like putative zinc finger domain, multiple ankyrin repeats, and a carboxyl-terminal SH3 domain. Both were active as GAPs for ARF1 and ARF5, but poor GAPs for ARF6, and both were activated by PI(3,4,5)P3 (13, 14). PAP was localized to the Golgi complex and shown to prevent the ARF-dependent generation of post-Golgi vesicles, in vitro (14), whereas ASAP1/DEF-1 promoted the differentiation of fibroblasts into adipocytes (15). To understand the similarities and differences among ARF GAP proteins, we compared the zinc binding, ARF GAP activity, ARF family specificity, and lipid regulation of GIT1 and GIT2 to those of ARF-GAP1.

**EXPERIMENTAL PROCEDURES**

**Materials—**TLC plates were purchased from VWR Scientific and lipids from Sigma. Sources of other materials have been published (11, 16–18).

**Preparation of Recombinant ARF Proteins—**For large-scale production of recombinant ARF proteins, 10 ml of overnight culture of the appropriate transformed bacteria were added to a flask with 500 ml of LB broth and ampicillin, 50 µg/ml, followed by incubation at 37 °C with shaking. When the culture reached an A600 of 0.6, 250 µl of 1 mM isopropyl-β-D-thiogalactopyranoside was added (0.5 mM isopropyl-β-D-thiogalactopyranoside final concentration). After incubation for an additional 3 h, bacteria were collected by centrifugation (Sorvall GSA, 6000 rpm, 4 °C, 10 min), and stored at −20 °C. Bacterial pellets were dispersed in 5 ml of cold phosphate-buffered saline, pH 7.4, with trypsin inhibitor (20 µg/ml), leupeptin and aproitin (each 5 µg/ml), and 0.5 mM phenylmethylsulfonyl fluoride. Lysozyme (10 mg in 5 ml) was added. After 30 min at 4 °C, cells were disrupted by sonication and centrifuged (Sorvall SS34, 16,000 rpm, 4 °C, 20 min). The supernatant was applied to a column (2.5 × 100 cm) of Ultrogel ACA 54 equilibrated and eluted with TENDS buffer (20 mM Tris-HCl, pH 8.0, 1.0 mM EDTA, 100 mM NaCl, 2 mM DT, 250 mM sucrose, 5 mM MgCl2, 1 mM Na3). Fractions that had both high ARF activity and high purity were pooled and further purified on a column (1.5 × 40 cm) of DEAE, eluted with a linear gradient of 50 to 500 mM NaCl (0.6-ml fractions), and by gel filtration on Ultrogel AcA 34 (1.5 × 30 cm) before storage in small portions at −20 °C. For large-scale production of recombinant myristoylated ARF GAP proteins, expression was induced in bacteria containing the N-myristoyltransferase gene, in the presence of 0.5 µM myristic acid and 0.06 µM bovine serum albumin as described by Franco et al. (19). Myristoylated ARF6 was purified by hydrophobic interaction HPLC on a TSKgel Phenyl-5PW column (Supelco, Belfote, PA) using the method described by Randazzo (4). A single protein peak eluted in the decreasing salt gradient and was shown to stimulate cholera toxin-catalyzed ADP-ribosylation.

**Construction and Expression of GAP Proteins—**Rat GIT1/6xHis, Δ45GIT1/6xHis were purified from baculovirus-infected Sf9 cells as described previously (11), using nickel affinity and ion exchange chromatography. The human GIT2-short/6xHis (35) was transferred into viruses by recombination in Sf9 cells with Baculo-Gold virus DNA (Pharmingen). The GIT2-short/6xHis protein was purified from infected Sf9 cells using ProBond metal chelate resin (Invitrogen) batchwise, followed by chromatography on a HiTrap-Q column (Amersham Pharmacia Biotech), as described for GIT1/6xHis (11). Full-length rat ARF-
GAP1 cDNA (5) was amplified from a rat brain cDNA library and subcloned into a modified pBK-CMV vector (Stratagene) using EcoRI and XhoI. The entire cDNA was then re-amplified using an antisense primer that inserted a 6XHis tag immediately before the stop codon, and subcloned as before into pBK-CMV. The pBK-Δ45ARF-GAP1 construct was prepared by amplification using a 5'-primer that adds an initiator Met codon immediately before codon 46. All constructs were sequenced on both strands from specific primers by using automated dye terminator chemistry with AmpliTaq FS reagents (Applied Biosystems) and an ABI 377 instrument. The ARF-GAP1/6XHis and Δ45ARF-GAP1/6XHis proteins were purified from the soluble extract of infected Sf9 cells using nickel affinity and ion exchange chromatography, essentially as described for GIT1/6XHis (11).

**Assay of GTPase Activity**—The indicated amounts of ARF were incubated for 30 min at 30 °C in 20 mM Tris, pH 8.0, 10 mM DTT, 2.5 mM EDTA with bovine serum albumin, 0.3 mg/ml, and phosphatidylserine (PS), 30 μg/ml (160 μM), with 0.5 μM [α-32P]GTP (3000 Ci/mmol) and 10 mM MgCl₂. 10 μl samples were incubated at 30 °C for 5 to 30 min, with the indicated amounts of GAP protein or an equal volume of buffer (total volume 50 μl), in the presence of the indicated phospholipids (Fig. 3), before proteins with bound nucleotides were collected on nitrocellulose by vacuum filtration (18). Bound nucleotides were eluted in 250 μl of 2 M formic acid, of which 3–4 μl samples were analyzed by TLC on polyethyleneimine-cellulose plates developed with 1 M formic acid, 1 M LiCl. TLC plates were subjected to autoradiography at ~80 °C for 18–36 h. The remaining solution was used to quantify the total amount of nucleotide bound in the assay (GDP plus GTP) by scintillation counting.

**Assay of CTA-catalyzed ADP-ribosylagmatine Formation**—GAP1 (0.5 μg ~ 50 pmol) with [α-32P]GTP bound was incubated for 10 min at 30 °C with 200 ng of ARF-GAP1 (4 pmol), GIT1 (2.1 pmol), or GIT2-short (3.3 pmol) and the indicated concentration of PIP (open circle), PIP₂ (closed circle), PIP₃ (open triangle), or diacylglycerol (open square). Data are mean ± SEM; the range of values from triplicate assays. Error bars smaller than symbols are not shown. Each experiment was repeated at least twice.

**Effect of phosphoinositides**—The indicated concentration of PIP (3), before proteins with bound nucleotides were collected on polyethyleneimine-cellulose plates. The ARF-GAP1/6XHis and Δ45ARF-GAP1/6XHis proteins were purified from the soluble extract of infected Sf9 cells using nickel affinity and ion exchange chromatography, essentially as described for GIT1/6XHis (11).

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**Assay of CTA-catalyzed ADP-ribosylagmatine Formation**—GAP1 (0.5 μg ~ 50 pmol) with [α-32P]GTP bound was incubated for 10 min at 30 °C with 200 ng of ARF-GAP1 (4 pmol), GIT1 (2.1 pmol), or GIT2-short (3.3 pmol) and the indicated concentration of PIP (open circle), PIP₂ (closed circle), PIP₃ (open triangle), or diacylglycerol (open square). Data are mean ± SEM; the range of values from triplicate assays. Error bars smaller than symbols are not shown. Each experiment was repeated at least twice.
GIT1, GIT2, and ARF-GAP1 were constant for at least 20 min before slightly slowing down (Fig. 2A), so for further assays we used 10-min incubations. In addition, the assays appeared linear until nearly 50% of the added GTP-bound ARF substrate was converted to GDP-bound ARF. GIT1, GIT2, and ARF-GAP1 stimulated hydrolysis of GTP bound to ARF1 in a concentration-dependent manner (Fig. 2, B and C). GIT1 was slightly more potent than ARF-GAP1 and GIT2-short was the least potent GAP (Fig. 2C). These results suggest that the intrinsic GAP activity of GIT1, GIT2, and ARF-GAP1 is very similar in the absence of additional cofactors.

Phospholipids seem to play a critical role in the control of ARF activities by ARF regulatory proteins. It was concluded that the effect of phospholipids on ARF GAP activity was to increase the GAP concentration at the membrane where GTP-bound ARF resides (8). Investigations with ARF-GAP1 have shown that it is indeed stimulated by PIP2 and dioleylglycerol (4, 8), but the phospholipid dependence of GIT proteins has not been explored. We compared the effects of PIP, PIP2, and PIP3 on the GAP activity of GIT1, GIT2-short, and ARF-GAP1. Confirming previous observations (4, 5, 20), 200 μM PIP3 dramatically stimulated the GAP activity of ARF-GAP1 from 0.64 to 2.46 nmol/min/mg, but not that of GIT1 (from 0.88 to 0.91 nmol/min/mg) or GIT2 (0.56 to 0.65 nmol/min/mg) (Fig. 3). On the other hand, 200 μM PIP3 significantly increased the hydrolysis of GTP bound to ARF1 induced by GIT1 (0.82 to 2.56 nmol/min/mg) and GIT2 (0.56 to 1.01 nmol/min/mg) but not by ARF-GAP1 (0.64 to 0.87 nmol/min/mg) (Fig. 3). PIP did not increase GTP hydrolysis by any of the ARF GAPs tested here (Fig. 3). Because dioleylglycerol, produced mainly from phosphatidylincholine hydrolysis by phospholipase D (an effector of ARF), dramatically increased the activity of a recombinant fragment of ARF-GAP1, it was suggested that phospholipase D activity could be a major regulator of ARF GAPs (8). Dioleylglycerol had similar effects on Gcs1, an analogous ARF GAP from yeast (9). We confirm that DAG (C18:1-(3)C18:1) stimulated the GTPase activity of ARF-GAP1, but no effect on the activity of GIT1 or GIT2 was found (Fig. 3). The ARF GAPs that are activated by PIP2 or other phosphoinositides are presumably subject to diverse kinds of regulation. From these results it seems plausible that GIT proteins and ARF-GAP1 are involved in distinct signaling pathways, which is also suggested by their distinct cellular localization. Centaurin-α has been described to be a potential PIP3-binding protein with similarity to ARF GAPs that could complement a yeast strain deficient in the yeast ARF GAP Gcs1 (21), suggesting that several ARF GAPs could be regulated by PIP3. In many cell types, the agonist-stimulated PI 3-kinase utilizes predominantly PIP2 as a substrate to generate PIP3 (22, 23). The ratio of PIP2 and PIP3 seems to play a critical role in many aspects of vesicular trafficking (24). It will now be of particular interest to know if signaling pathways involving PI 3-kinases or other enzymes involved in the synthesis or hydrolysis of PIP3 may contribute to intracellular regulation of GIT proteins.

It was demonstrated that the amino-terminal GATA-like zinc finger motif of ARF-GAP1 (5) and ARD1, an ARF-related protein that has an amino-terminal GAP domain (18), are critical for GTP hydrolysis. In the presence of GTP or a non-hydrolyzable analogue, all members of the ARF family serve as allosteric activators of cholera toxin (CTA) ADP-ribosyltransferase (25). As expected, addition of ARF-GAP1 and GIT1 reduced the ability of ARF1 to activate CTA in the presence of GTP, but not GTPγS (Fig. 4). This result confirms that GIT1, like ARF-GAP1, influences the biological activity of ARF1 by promoting GTP hydrolysis. Deletion of 45 amino acids from the amino terminus of GIT1 or ARF-GAP1 (containing the conserved zinc finger motif) completely prevented inhibition of ARF-induced CTA activation (Fig. 4), suggesting that the zinc finger motif is required for GAP activity of both proteins. We can exclude that this loss of activity results from complete misfolding of the Δ45GIT1 protein, because several additional GIT interacting proteins (GRK2, PIX, pauxillin) do bind normally to Δ45-GIT1 in co-immunoprecipitation assays.2

![Substrate specificity of ARF-GAP1, GIT1, and GIT2-short.](http://www.jbc.org/)

**TABLE I**

**Zinc content of ARF GAPs assessed by inductively-coupled plasma emission spectroscopy**

The indicated amounts of Hi-Trap-Q chromatography purified His-tagged ARF-GAP1 (GAP1), GIT1, and GIT2-short proteins were analyzed for metal ions by inductively-coupled plasma emission spectroscopy. The detection limit for zinc was 0.1 μg/ml. No significant amounts of the other metal ions were found in any samples, except for Na+ from the buffer and Ca2+ in single samples of ARF-GAP1 and GIT2-short.

| Protein name | Sample | Zinc content (μg/ml nmol) |
|--------------|--------|--------------------------|
| GAP1 prep 1  | 2.0    | 37                      | 2.9  | 31  |
| GAP1 prep 2  | 2.2    | 46                      | 2.9  | 45  |
| GIT1         | 0.8    | 9                       | 0.44 | 7   |
| GIT2 prep 1  | 2.1    | 40                      | 1.7  | 20  |
| GIT2 prep 2  | 2.1    | 40                      | 1.8  | 21  |

2 R. T. Premont, unpublished observations.

Fig. 5. Substrate specificity of ARF-GAP1, GIT1, and GIT2-short. A, samples (0.5 μg = 25 pmol) of ARF1, ARF2, ARF3, ARF5, ARF6, ARL1, or ARL2 or 2 μg (≈31 pmol) of ARD1 with [α-32P]GTP bound were incubated for 10 min at 30°C with 400 ng of ARF-GAP1 (GAP1), GIT1 (4.2 pmol), or GIT2-short (6.6 pmol). Intrinsic GTPase activity of individual ARF proteins (control) is in the open columns. Data are mean ± one-half the range of values from triplicate assays. Each experiment was repeated twice. B, 0.5 μg (25 pmol) of ARF5 (open symbols) or ARF6 (closed symbols) with [α-32P]GTP bound were incubated for 10 min at 30°C with increasing amounts of ARF-GAP1 (circles), GIT1 (squares), or GIT2-short (diamonds). Data are mean ± one-half the range of values from triplicate assays. Each experiment was repeated twice.
Members of the ARF GAP family identified to date share a conserved domain containing a C_C_C_C_C putative zinc finger (5, 11, 13, 14). To investigate whether ARF GAP proteins actually bind to a metal through this domain, we subjected three purified ARF GAP proteins to plasma emission spectroscopy, a sensitive technique that allows for the simultaneous detection of up to 20 metal ions (Table I). For each of the three ARF GAP proteins analyzed, the only metal ions detected over background were zinc and sodium (from the NaCl buffer) except for one instance, where significant calcium was also detected in ARF-GAP1/6xHis and GIT2/short/6xHis. The nanomole of zinc detected was similar to the nanomole of protein analyzed for each ARF GAP, consistent with a near one-to-one complex of zinc with protein. Recent publication of the crystal structure of the ARF-GAP1 protein amino-terminal domain bound to ARF1 reveals that one zinc ion is indeed bound by the four conserved cysteine residues of the ARF GAP domain (26). Interestingly, the metal ion does not contact the ARF protein, but appears to play a role in determining the overall structure of the GAP domain (26). Mutation of single cysteine residues within this zinc finger abrogated GAP activity of ARF-GAP1 (5) and ARD1 (18), presumably because such mutants could not bind zinc. As GIT1 and GIT2 also appear to bind zinc, we predict that zinc chelation by this C_C_C_C_C motif is a common feature of ARF GAP proteins. The effect of removing the zinc to form the cognate apoproteins, or of replacing it with similar metal ions, remains unexplored. It is now well established that RasGAPs and RhoGAPs share a common mechanism of the GAPase-rate enhancement involving a critical arginine residue (27, 28). A putative arginine finger motif has also been postulated within the zinc fing region in ARF- GAP1 (29) and this specific arginine residue is present also in GIT1 and GIT2. Recently, data from the crystal structure of the ARF1-ARF-GAP1 complex suggested that the postulated arginine in ARF-GAP1 did not make contact with the active site of the GAPase (26). The function of this conserved arginine residue in ARF GAP proteins remains to be established.

We tested further the GAP activities of GIT1, GIT2, GIT2, and ARF-GAP1 on different members of the ARF family. ARF-GAP1 stimulated hydrolysis of GTP bound to ARF1, ARF2, ARF3, and ARF5, but not to ARF6 (Fig. 5A), confirming previous observations that this GAP affects the class I and class II, but not class III ARFs (4). On the other hand, GIT1 and GIT2 promoted GTP hydrolysis by all five ARFs tested (Fig. 5A). Neither ARL1 nor ARL2, members of the ARF-like family, nor ARD1, the ARF-related protein with its intrinsic GAP domain, were substrates of GIT1, GIT2, or ARF-GAP1 (Fig. 5A). Over a range of GAP concentrations where the two GIT proteins were quite active, ARF-GAP1 failed to accelerate the GAPase activity of ARF6 (Fig. 5B). These results suggest that ARF-GAP1 and GIT proteins are indeed specific GAPs for ARF proteins. More importantly, they indicate that unlike ARF-GAP1, both GIT1 and GIT2 can stimulate the efficient hydrolysis of GTP bound to ARF6.

Native ARF proteins in the cell are myristoylated on their amino termini, while the bacterially expressed recombinant ARF proteins used in the preceding assays in this study are not. The effect of myristoylation of ARF on the GAP activity of GIT1, GIT2, and ARF-GAP1 was also tested. No significant difference in GAP activities was detected whether or not ARF1 or ARF6 were myristoylated (Fig. 6A). These results are in agreement with previous observations made with a different ARF GAP activity purified from rat spleen (10). It is notable that myristoylated ARF6, the form found in cells, was a particularly good substrate for GIT1 and GIT2 compared with ARF-GAP1 (Fig. 6B).

Of all ARF GAP proteins characterized to date, the GIT proteins appear to be the best GAPs for ARF6, a type III ARF with several unusual features including a predominantly plasma membrane localization. ARF6 also has been localized on endosomes (30, 31) and on secretory granules (32), where it is believed to participate in endocytic and exocytic trafficking events. Interestingly, GIT proteins were identified in a yeast two-hybrid screen through their ability to interact with GRK2. Overexpression of GIT1 leads to increased receptor phosphorylation and reduced β2-adrenergic signaling, resulting from attenuated receptor internalization and resensitization (11). These cellular effects do not reflect regulation of GRK kinase activity, but require an intact amino terminus, suggesting a function for ARF in regulating β2-adrenergic receptor endocytosis (11). The involvement of ARF6 in this specific recycling pathway will now have to be investigated.

In addition to their role as ARF GAPs, GIT proteins also appear to have other important cellular functions. A two-hybrid screen with GIT1 identified an interaction with β2-PIX, a putative rac1/cdc42 guanine nucleotide-exchange factor that binds the rac1/cdc42-activated PAK kinases (35). Both GIT1 and GIT2 interact with α2- and β2-PIX proteins, in a multiprotein complex which also contains p21-activated kinase (PAK) (35). Bagrodia et al. (33), starting with PAK kinase, recently identified the GIT-PIX-PAK complex, as did Turner et al. (34), who additionally discovered the interaction of a distinct third GIT family member (p95-PKL) with paxillin. Together, these studies document a role for GIT proteins in anchoring the PIX-PAK

**Fig. 6. Effect of myristoylation of ARFs on the GAP activity of ARF-GAP1, GIT1, and GIT2-short.** A. samples (0.5 μg ~ 25 pmol) of ARF1, myrARF1, ARF6, or myrARF6 with [α-32P]GTP bound were incubated for 10 min at 30 °C with 400 ng of ARF-GAP1 (GAP1 ~ 8 pmol), GIT1 (4.2 pmol), or GIT2-short (6.6 pmol). Intrinsic GAPase activity of each ARF protein (control) is in open columns. Data are mean ± one-half the range of values from triplicate assays. Each experiment was repeated twice. B. 0.5 μg (25 pmol) of myrARF1 (open symbols) or myrARF6 (closed symbols) with [α-32P]GTP bound were incubated for 10 min at 30 °C with increasing amounts of ARF-GAP1 (circles), GIT1 (squares), or GIT2-short (diamonds). Data are mean ± one-half the range of values from triplicate assays. Each experiment was repeated twice.
complex, via paxillin, in cellular focal adhesions. These results suggest that the multidomain proteins of the GIT family could be at the cross-roads of several signal transduction pathways involving multiple small GTPases in vesicular trafficking and cytoskeletal remodeling.

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