The GIT Family of ADP-ribosylation Factor GTPase-activating Proteins

FUNCTIONAL DIVERSITY OF GIT2 THROUGH ALTERNATIVE SPLICING*

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Richard T. Premont‡, Audrey Claing, Nicolas Vitale§, Stephen J. Perry, and Robert J. Lefkowitz¶

From the Departments of Medicine and Biochemistry, Howard Hughes Medical Institute, Duke University Medical Center, Durham, North Carolina 27710, and §Centre de Neurochimie, INSERM U-338, 67084 Strasbourg Cedex, France

We recently characterized a novel protein, GIT1, that interacts with G protein-coupled receptor kinases and possesses ADP-ribosylation factor (ARF) GTPase-activating protein activity. A second ubiquitously expressed member of the GIT protein family, GIT2, has been identified in data base searches. GIT2 undergoes extensive alternative splicing and exists in at least 10 and potentially as many as 33 distinct forms. The longest form of GIT2 is colinear with GIT1 and shares the same domain structure, whereas one major splice variant prominent in immune tissues completely lacks the carboxyl-terminal domain. The other 32 potential variants arise from the independent alternative splicing of five internal regions in the center of the molecule but share both the amino-terminal ARF GTPase-activating protein domain and carboxyl-terminal domain. Both the long and short carboxy-terminal variants of GIT2 are active as GTPase-activating proteins for ARF1, and both also interact with G protein-coupled receptor kinase 2 and with p21-activated kinase-interacting exchange factors complexed with p21-activated kinase but not with paxillin. Cellular overexpression of the longest variant of GIT2 leads to inhibition of β2-adrenergic receptor sequestration, whereas the shortest splice variant appears inactive. Although GIT2 shares many properties with GIT1, it also exhibits both structural and functional diversity due to tissue-specific alternative splicing.

The ADP-ribosylation factor (ARF)1 small GTP-binding proteins comprise a family of highly conserved and functionally important regulatory proteins. Like other GTP-binding proteins, ARF proteins are activated by binding of GTP and deactivated by hydrolysis of the bound GTP to GDP (1, 2). Activated ARF proteins are associated with cellular membranes, primarily in the Golgi; however, the ARF6 subtype localizes predominantly to the plasma membrane and endosomes (1, 2). Activated ARF proteins target specific ARF effector proteins to the membrane, such as the β-coatomer protein in the Golgi. ARF proteins are known to play important roles in vesicular budding and targeting (1, 2) and can directly activate phospholipase D (3, 4). ARF protein activation is facilitated by interaction with specific guanine nucleotide exchange factors, several of which have been identified recently (5). However, ARF proteins have little intrinsic GTPase activity, and for their deactivation, they require accessory GTPase-activating proteins (GAPs) (5–7).

The characterization of the GTPase-activating protein for ARF1 in the Golgi apparatus, ARF-GAP1 (8), has allowed the identification of additional ARF GAP family members. These include the GIT1 (9), ASAP1/DEF-1 (10, 11), and βPAP (12) proteins as well as several uncharacterized sequences in the data bases. Whereas mutagenesis and structural studies have shown that about half of the ARF-GAP1 protein is required for GAP activity (8, 13), many of the more recently identified ARF GAP proteins are significantly larger and contain diverse identifiable functional domains. Furthermore, these more recently characterized ARF GAPs were generally isolated not because they are ARF GAPs but as proteins that interact with important signal transduction proteins. Indeed, GIT1 was isolated by interaction with G protein-coupled receptor kinase 2 (GRK2), ASAP1/DEF-1 with the Src tyrosine kinase, and βPAP with the Pyk2 tyrosine kinase (9–12). The rationale for ARF GAP proteins interacting with these distinct cellular signaling networks is unknown but appears to be a common feature among diverse ARF GAP family members.

We recently identified one such ARF GAP protein, GIT1, due to its interaction with members of the GRK family (9). GIT1 functions as a GAP for ARF1 (9) and other ARF family members (14). GIT1 overexpression profoundly affects the signaling and regulation of the G protein-coupled β2-adrenergic receptor, not through altering the activity of the GRK2 but by inhibiting the agonist-promoted sequestration of the β2-adrenergic receptor from the cell surface (9). Furthermore, this regulation appears to require the intact ARF GAP domain of GIT1, hinting at a role for ARF protein function in G protein-coupled receptor trafficking to and from the plasma membrane (9). Thus, one reason for the existence of these large, modular ARF GAPs may be to coordinate ARF activity at the plasma membrane (and elsewhere) with other cellular signaling events. We now have identified and characterized a second member of the GIT family of GTPase-activating proteins for ARF small GTP-binding proteins, GIT2. Although sharing many features with GIT1, GIT2 differs markedly from GIT1 in exhibiting molecular and functional diversity through extensive alternative splicing.
EXPERIMENTAL PROCEDURES

Materials—General laboratory reagents were from Sigma. Thermostable polymerases, DNA ligase, and restriction enzymes were from Promega. Radioisotopes were from NEN Life Science Products, and cycle sequencing reagents were from Applied Biosystems/Perkin-Elmer. The GIT1 antisemur (9) and GRK2/3 antisemur (15) have been described previously. The p50-cool/β-PIX antisemur (16) and p5H-PK

-α/β-PIX (p5H-PK) expression plasmid (17) were obtained from Dr. R. Cerione. Polyclonal OctA(FLAG)-probe, hemagglutinin probe, and 6×His-probe antibodies were obtained from S. cerevisiae. Anti-FLAG M2 monoclonal antibody and pAK3 (22374 from Transduction Laboratories. 6×His-probe antibodies were obtained from Dr. R. Cerione. Polyclonal OctA(FLAG)-probe, hemagglutinin probe, and 6×His-probe antibodies were obtained from Santa Cruz Biotechnology. Anti-paxilin monoclonal antibody was obtained from Transduction Laboratories. Anti-FLAG M2 monoclonal antibody and M2 covalently coupled to Sepharose were obtained from Sigma. The pRK5-GRK2 and pRK5-GRK5 (18) and pBK(α)-GIT1 and pBK(α)-GIT1/ FLAG (9) expression plasmids have been described.

GIT2 Gene Mapping—The human GIT2 gene was localized to human chromosome band 12q24.1 by amplification of the 3′-untranslated region (based on human EST sequence W94986) of the GIT2-long variant. The template DNA was genomic DNA isolated from individual cell clones of the Stanford G3 radiation hybrid cell panel (Research Genetics), each of which contains defined fragments of human chromosomes (19). The primers used were 5′-AGAAACACAACTGACAAGGGCAGG and 5′-TTGCTTGAAAGAGATGCTAAT-TAG and 5′-TTCTTCTTGAGGGCTTTCATC was used directly as isotype. Amplified cDNA fragments were sequenced to verify fidelity. The GIT2 amino-terminal “common” probe was the GIT2 short variant with a carboxyl-terminal His tag. The carboxyl-terminal fragment was subcloned, sequenced, and then ligated into the amino-terminal fragment in pBKcα vector at the internal HindIII site to reconstruct the complete open reading frame. Additional GIT2-long variants containing carboxyl-terminal FLAG or 6×His tags were created by the same technique using primers that inserted the appropriate epitope tags immediately before the stop codon and the addition of anti-FLAG antibody.

For yeast two-hybrid library screening, the yeast strain Y190 was transformed with pBR322 containing the GAL1 promoter and β-galactosidase reporter gene. Yeast cells were tested for reporter gene expression by measuring β-galactosidase activity. The β-galactosidase activity was assayed by quantifying the amount of β-galactosidase produced in the presence of the reporter plasmid. The β-galactosidase activity was normalized to the amount of β-galactosidase produced in the absence of the reporter plasmid. The results were expressed as a percentage of the control value.
protein (9). From the EST data base, we noted the existence of mouse and human GIT1 homologs as well as an additional GIT1-related sequence that we tentatively termed GIT2. EST clones W94986 and AA153040 appear to encode human and mouse sequences related to the extreme carboxyl-terminal of GIT1. However, the scarcity of related EST clones did not allow identification of more amino-terminal sequences from this presumed GIT2 cDNA. The chromosomal localization of the human GIT2 gene was determined by amplification of a portion of the human GIT2 3′-untranslated region, based on the human EST W94986 sequence, from genomic DNA isolated from a panel of radiation hybrid cell lines containing defined fragments of human chromosomes. The human GIT2 gene is present at band 12q24.1, an estimated 700 kb from marker D12S1583. The human GIT1 gene has been previously mapped to band 17p11.2 (9).

Functional Diversity of GIT2

Variants of the GIT2 Protein—Sequencing of amplified fragments allowed determination of the full-length human GIT1 and GIT2 cDNA sequences (Fig. 2). The longest splice variant of human GIT2 (GIT2-long) is 759 amino acids long, with a predicted size of 84.5 kDa. This form is co-linear with and 65% identical to rat GIT1 (9) and 64% identical to human GIT1 (Fig. 2). Two recent reports have described additional GIT family members; the CAT2 protein is a splice variant of GIT2 apparently lacking exons B and C (see below), whereas the paxillin kinase linker (PKL) protein encodes a distinct third GIT family member with 89% identity with GIT2 and 65% identity with human GIT1 and human GIT2.

Using a forward primer from the KIAA0148 sequence and a reverse primer from the W94986 EST sequence, amplification reactions were performed with 1 μl of cDNA library from the indicated human tissues. Product fragments were separated and visualized in a 1% agarose gel. By analogy to GIT1, these primers were predicted to yield a single product band of 1500 bp, which corresponds to the longest product band observed in all tissues (indicated by the bracket), which were isolated separately for direct DNA sequencing. St., skeletal.

FIG. 1. Amplification of human GIT2 extended carboxyl-terminal. Using a forward primer from the KIAA0148 sequence and a reverse primer from the W94986 EST sequence, amplification reactions were performed with 1 μl of cDNA library from the indicated human tissues. Product fragments were separated and visualized in a 1% agarose gel. By analogy to GIT1, these primers were predicted to yield a single product band of 1500 bp, which corresponds to the longest product band observed in all tissues (arrowhead). However, many distinct smaller bands were also evident in all tissues (indicated by the bracket), which were isolated separately for direct DNA sequencing. St., skeletal.

FIG. 2. Alignment of the deduced amino acid sequences of human GIT1 and human GIT2. The five internal alternative sequences (putative exons A through E) within the long carboxyl-terminal variant of human (hum) GIT2 are indicated by alternating bold and thin underlining. The GIT2-short alternative carboxyl-terminal sequence (exon D) is also thin underlined.
The shortest variant of GIT2 (GIT2-short), which possesses a foreshortened carboxyl-terminal sequence and a distinct 3′-untranslated region relative to the nine sequenced GIT2-long forms, corresponds precisely to the previously described KIAA0148 cDNA sequence (Fig. 3). This variant differs from all the GIT2-long variants in lacking the extended carboxyl-terminal region beyond exon E. In GIT1, the carboxyl-terminal half of the molecule is responsible for interaction with GRKs (9), and much of the equivalent domain is absent in this GIT2-short variant. The truncated carboxyl-terminal sequence arises from the use of a distinct alternative exon D′ encoding seven amino acids before a termination codon, which precludes the presence of sequences from the putative alternative exon encoding region E or the common extended carboxyl-terminal region found in the GIT2-long sequences.

**Distribution of GIT2 Variants**—The KIAA0148 mRNA has been reported to be widely expressed, with highest expression in kidney and heart (20). GIT2 cDNA fragments were amplified readily from all human tissue and cell cDNA libraries tested (see Fig. 1) but, as noted previously, with clearly evident tissue-specific alternative splicing. To assess the tissue distribution of the human GIT2 mRNA as well as to independently assess the extent of alternative splicing, human tissue Northern blots were probed with DNA probes specific to the two major carboxyl-terminal splice variants as well as with a common amino-terminal probe (Fig. 4). A probe that recognizes the common amino-terminal half of GIT2 identifies multiple distinct mRNA bands that are widely distributed in human tissues. There is a major band at 6.5 kb and several closely spaced bands at 2.5–3.0 kb in all 16 tissues tested. Bands within the 2.5–3.0-kb range vary greatly in intensity among tissues, and there are additional distinct bands seen in only a few tissues (4.0 kb and 9.0 kb in brain, 7.5 kb in leukocytes, 2.8 kb in testis). By contrast, a full-length human GIT1 probe hybridizes to only a single 4.0-kb band, with a wide tissue distribution similar to that seen previously for rat GIT1 (data not shown). The tissue distribution of the PKL mRNA remains unknown. Although the PKL and GIT2 proteins exhibit quite high identity, their nucleotide sequences are much more divergent; however, there remains a possibility that some weaker bands on the Northern blots may result from cross-reactivity in the probes used.

The Northern blots were then hybridized separately with probes specific to the two alternative carboxyl-terminal splice variants, one from the GIT2-short (KIAA0148) variant, 3′-untranslated region and the other from a domain common to all the GIT2-long variants (encoding amino acids 578–759 of GIT2-long). The GIT2-long carboxyl-terminal probe hybridized to the widely expressed 6.5-kb band and to the 2.8-kb band in testis, with some additional minor bands. The 2.3-kb band in spleen and leukocytes that was prominent with the common amino-terminal probe was notably absent with the GIT2-long carboxyl-terminal probe. The GIT2-short carboxyl-terminal probe hybridized predominantly to a 2.3-kb band in spleen and leukocytes and to a lesser extent in thymus. An additional 4.4-kb band was observed in brain. Thus the GIT2-short mRNA is expressed primarily in immune cells with little expression in other tissues, whereas the GIT2-long variants are widely expressed.

**GIT2 Is a Functional GTPase-activating Protein for ARF1**—GIT1 has been previously demonstrated to be active as a GAP for the ARF1 small GTP-binding protein (9). By analogy to the ARF-GAP1 protein (8), the amino-terminal CX3CX4C zinc finger motif of GIT1 was thought to be a critical component of the GAP domain. Deletion of this zinc finger region in the Δ45-GIT1 protein renders it inactive in GAP assays (9). Since the equivalent GAP-like domain of GIT2 is very similar to that of GIT1, we were interested in knowing if GIT2 also could serve as a GAP for the ARF1 protein. The human GIT2-long and GIT2-short (KIAA0148) variants were modified by the addition of 6×His residues at their carboxyl termini, and the GIT2-long/6×His and GIT2-short/6×His proteins were purified to near homogeneity from Sf9 cells infected with recombinant baculovirus. Expression and yield of the GIT2-long protein was significantly poorer than that of the GIT2-short protein. The addition of purified GIT2-long or GIT2-short proteins to [α-32P]GTP-bound ARF1 protein led to the conversion of bound GTP to GDP, as expected for a GAP protein (Fig. 5). GIT proteins did not alter the total amount of guanine nucleotide bound to ARF1 nor convert GTP to GDP in the bulk solution, either alone or in the presence of ARF1 (data not shown),
FIG. 5. GIT2 is an active ARF GAP. A, purified GIT2-long/6×His protein (1 μg) was added to 0.5 μg of ARF1 with bound [α-32P]GTP, and conversion of bound GTP to GDP was assessed after 20 min. Data are shown as the mean ± S.E. from two experiments done in duplicate. B, the indicated amounts of purified GIT2-short/6×His protein (KIAA0148 variant) were added to 0.5 μg of ARF1 with bound [α-32P]GTP, and conversion of bound GTP to GDP was assessed after 20 min. Data are shown as the mean ± S.E. from three experiments done with triplicate samples. GIT2 significantly increased the conversion of GTP to GDP without altering total guanine nucleotide binding to ARF1. The assay was linear through 30 min.

indicating that activity is not due to nonspecific GTP hydrolysis in solution. Thus, like GIT1, these two GIT2 variants are active as GTPase-activating proteins for ARF1.

GIT2 Interacts with GRK2—GIT1, through its carboxyl-terminal half, has been shown to interact with several GRK subtypes, in yeast 2-hybrid assays and in co-immunoprecipitation assays from transfected COS cells (9). This GRK binding region of GIT1 contains stretches that are highly conserved with the equivalent region of GIT2, mainly in the extended carboxyl-terminal (long) variants. To determine whether the GIT2 protein shares with GIT1 the ability to bind to GRKs, GRK2 was expressed in COS-7 cells along with FLAG-tagged GIT proteins. The GIT1/FLAG, GIT2-long/FLAG, and GIT2-short/FLAG proteins were immunoprecipitated using M2 FLAG antibody, and the co-immunoprecipitation of GRK2 was measured by immunoblotting (Fig. 6). The longest carboxyl-terminal variant of GIT2, like the GIT1 protein, appears to interact with GRK2. Unexpectedly, the short carboxyl-terminal splice variant of GIT2 (KIAA0148), which specifically lacks much of this carboxyl-terminal putative GRK interaction region, also co-immunoprecipitated GRK2. Thus, GIT1 and the long and short variants of GIT2 all share the ability to interact with GRK2, suggesting that the GRK interaction site may be located between residues 370 and 470 of GIT2, rather than being located exclusively in the extreme carboxyl-terminal regions beyond residue 470.

GIT2 Overexpression Alters β2-Adrenergic Receptor Sequestration—Overexpression of GIT1 in HEK293 cells leads to a marked decrease in agonist-promoted sequestration of the β2-adrenergic receptor from the cell surface (9). This inhibition of receptor sequestration led to both increased receptor phosphorylation and increased receptor desensitization. These effects of GIT1 overexpression were shown to require the intact ARF GAP domain of GIT1. To assess whether GIT2 shares the ability to alter β2-adrenergic receptor function in living cells, HEK293 cells were cotransfected with the longest splice variant of GIT2 and the FLAG-tagged β2-adrenergic receptor, and agonist-stimulated β2-adrenergic receptor sequestration was measured after 30 min of treatment with 10 μM isoproterenol (Fig. 7). As with GIT1, overexpression of GIT2-long attenuated the agonist-stimulated sequestration of the β2-adrenergic receptor from the cell surface. However, experiments using the GIT2-short carboxyl-terminal splice variant failed to show any effect on β2-adrenergic receptor sequestration, despite expression at levels equivalent to that of the GIT2-long variant, hinting at a specific role for the carboxyl-terminal domain of the GIT proteins in this activity.

FIG. 6. GIT2 interacts with GRKs. GRK2 was transiently expressed in COS-7 cells along with pBK-CMV empty vector or pBK(Δ) containing FLAG-tagged GIT1 (1), GIT2-long (2), or GIT2-short (2 s) as indicated. Cells were harvested and lysed, and the soluble fraction was separated by SDS-PAGE and immunoblotted using a GRK2-cross-reactive anti-GRK3 polyclonal antiserum to test for co-immunoprecipitation of GRK2. Blots were subsequently probed using an anti-FLAG-probe polyclonal antibody to assess the amount of GIT/FLAG protein immunoprecipitated. A representative experiment (of four) is shown.
The ability of the full-length GIT and PIX proteins to interact in a mammalian cell was tested using co-immunoprecipitation. COS-7 cells were transfected with GIT1/FLAG, GIT2-long/FLAG, GIT2-short/FLAG, or control empty vector, and the expressed GIT proteins were immunoprecipitated with M2 anti-FLAG monoclonal antibody immobilized on agarose beads. Since immunoblotting of COS-7 cell extracts with a β-PIX antiseraum (16) indicated significant endogenous expression of the β-PIX protein, we examined GIT/FLAG immunoprecipitates for the presence of co-immunoprecipitated β-PIX protein (Fig. 8). β-PIX protein was readily detected in immunoprecipitates from cells transfected with all three GIT/FLAG constructs, whereas none was seen from cells transfected with only empty vector. Thus, GIT1 and both the long and short variants of GIT2 can interact with endogenous PIX proteins in a mammalian cell. The extreme carboxyl terminus of the GIT2-long protein (a region absent in the GIT2-short protein) does not appear to be required for this interaction.

**GIT Proteins Interact with PAKs through PIX Exchange Factors**—Since the SH3 domain of the PIX proteins has been reported to bind to a polyproline domain of the PAK kinases, we tested whether the GIT-PIX complex immunoprecipitated from COS-7 cells contained PAK kinase. COS-7 cells were transfected with hemagglutinin-tagged PAK3 and either GIT1/FLAG, GIT2-long/FLAG, GIT2-short/FLAG, or control empty vector. M2 FLAG antibody immunoprecipitates were blotted with anti-hemagglutinin epitope antibody to reveal the presence of co-immunoprecipitated PAK kinase along with the endogenous PIX protein (Fig. 9). In the absence of transfected GIT/FLAG, no PAK kinase or PIX protein was detected. However, immunoprecipitation of the GIT/FLAG proteins indicates that GIT1 and GIT2 can also associate with PAK kinase, presumably through PIX. We conclude that the ability of GIT protein variants to bind the PIX-PAK complex does not appear to correlate with their ability to alter β2-adrenergic receptor sequestration.

**GIT1, but Not GIT2, Interacts with Paxillin**—The association of the focal adhesion adaptor protein paxillin with the paxillin kinase linker protein p95-PKL, a GIT protein family member
highly similar to GIT2, was recently reported (23). We therefore examined GIT/FLAG protein immunoprecipitates for the presence of co-immunoprecipitated paxillin. Endogenous COS cell paxillin was highly enriched in FLAG immunoprecipitates from cells expressing the GIT1/FLAG protein compared with cells expressing no epitope-tagged GIT1 protein (Fig. 10). However, neither the GIT2-long nor GIT2-short proteins appeared to bind strongly to paxillin, which is unexpected as the human GIT2 and human PKL proteins share 89% amino acid identity and complete identity within the site proposed to bind paxillin (residues 645-681 of GIT2-long) (23). GIT2 undergoes extensive alternate splicing of its carboxy-terminal domain, and it is possible that some of these additional variants might regulate the binding of paxillin or paxillin-like proteins. In any case, the ability of GIT proteins to bind paxillin does not appear to correlate with their ability to alter β2-adrenergic receptor sequestration. We conclude that some additional unrecognized function of the GIT protein carboxy-terminal domain, important for regulation of the sequestration of the β2-adrenergic receptor, appears to be lost in the GIT2-short splice variant.

**DISCUSSION**

We have identified and characterized a member of the GIT protein family, GIT2. The GIT family is now known to be composed of three members: GIT1, GIT2/KIAA0148/CAT2, and PKL (9, 22, 23). All GIT family members share a structure composed of an amino-terminal zinc finger ARF GAP domain, three ankyrin repeats, and a conserved carboxy-terminal region. GIT1 and GIT2 are active as GAPs for ARF1, bind GRK2, and can reduce agonist-dependent sequestration of β2-adrenergic receptors from the cell surface. A splice variant of GIT2 (GIT2-short), which lacks the carboxy-terminal 300 residues conserved among the other family members, loses the ability to alter receptor sequestration while retaining the ability to interact with GRK2. All three GIT proteins appear to bind to a complex of PIX exchange factors and PAKs. However, GIT2 appears unable to interact with paxillin, whereas both GIT1 and PKL interact with paxillin robustly.

The recently determined x-ray crystal structures of the GAP domains of ARF-GAP1 and PAP show that the zinc finger-like sequence does in fact bind one atom of zinc (13, 25). Using spectroscopic methods, we have shown that the GIT2, GIT1, and ARF-GAP1 proteins all bind stoichiometric quantities of zinc (14). GIT1 and GIT2 share the ability to GAP all ARF family members, including ARF6, and the GAP activity of GIT1 and GIT2 is stimulated by phosphatidylinositol 3,4,5-trisphosphate but not phosphatidylinositol 4,5-bisphosphate (14). GIT2 is expected to share other properties with GIT1, such as the ability to alter β2-adrenergic receptor signaling and phosphorylation (9) and to alter the sequestration of other G protein-coupled receptors utilizing the clathrin-coated pit pathway for their internalization (26).

As the GIT2 and GIT1 proteins are expressed ubiquitously in human tissues, the GIT protein family is likely to regulate many G protein-coupled receptors. In addition, the GIT proteins may have functions in other key cellular signaling events involving ARF and GRK proteins. The association of GIT proteins with PIX exchange factors, PAKs, and paxillin and the finding that these proteins can co-localize at focal complexes suggest that GIT proteins may function in regulation of the cytoskeleton (23).

Unlike GIT1, GIT2 undergoes extensive alternative splicing to generate at least 10 distinct mRNA forms. There appears to be a high degree of tissue specificity to this alternative splicing such that different tissues express very distinct complements of GIT2 variants. Two general types of splicing occur. One type deletes one to five exons within the central region of the GIT2 sequence, producing proteins with common amino and carboxy termini. A second type of alternative splicing substitutes a novel carboxy-terminal exon and leads to a prematurely terminated protein. There do not appear to be similar splice variants of the human or rat GIT1 proteins (data not shown), although alternative splicing of PKL has been suggested (23).
Functional Diversity of GIT2

Why do there exist GIT2 variants with and without the carboxyl-terminal domain? Experiments using GIT2-short indicate that it is deficient in the ability to antagonize agonist-promoted sequestration of the β2-adrenergic receptor compared with GIT1 or GIT2-long. Thus, ARF GAP activity and GRK binding are insufficient to confer on the GIT2-short protein the ability to interfere with G protein-coupled receptor function. This activity difference between GIT2-long and GIT2-short is not due to altered interactions with PIX-PAK complexes or paxillin. This suggests that some unknown function of the conserved carboxyl-terminal domain, either regulatory or structural, is important for G protein-coupled receptor regulation activity. Experiments focusing on identifying protein interactions with the GIT protein carboxyl-terminal region may be informative.

What is the functional role of the tissue-specific internal splicing of GIT2? This central portion of the GIT proteins exhibits high conservation and may be an important regulatory protein interaction domain. The interaction of GRK2 with the GIT1 carboxyl terminus and with the GIT2-short variant suggests that GRK binding to GIT proteins may require the presence of alternative regions A, B, or C. Furthermore, the PIX proteins (16, 21, 24) bind to this region of the GIT proteins (22, 23). A further interaction of PKL, a GIT family member highly similar to GIT2, with paxillin has also been described (23). The paxillin binding site was mapped to the carboxyl-terminal region of PKL. Binding to PIX or paxillin proteins may be affected by deletion of some of these five alternative exons, altering the ability of GIT2 to serve an adaptor function.

In summary, we have characterized GIT2, a member of the GIT family of GRK-interacting ARF GAP proteins. GIT2 shares many properties with GIT1 but also exhibits a distinct character due to extensive tissue-specific alternative splicing, particularly the loss of the ability to interfere with β2-adrenergic receptor sequestration. Further examination of the GIT proteins and their functions may uncover the molecular logic of this variability within the GIT2 protein.

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