Purification and Cloning of a Brefeldin A-inhibited Guanine Nucleotide-exchange Protein for ADP-ribosylation Factors*

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Activation of ADP-ribosylation factors (ARFs), ~20-kDa guanine nucleotide-binding proteins that play an important role in intracellular vesicular trafficking, depends on guanine nucleotide-exchange proteins (GEPs), which accelerate replacement of bound GDP with GTP. Two major families of ARF GEPs are known: ~200-kDa molecules that are inhibited by brefeldin A (BFA), a fungal metabolite that blocks protein secretion and causes apparent disintegration of Golgi structure, and ~50-kDa GEPs that are insensitive to BFA. We describe here two human brain cDNAs that encode BFA-inhibited GEPs. One is a ~209-kDa protein 99.5% identical in deduced amino acid sequence (1,849 residues) to a BFA-inhibited ARF GEP (p200) from bovine brain. The other smaller protein, which is ~74% identical (1,785 amino acids), represents a previously unknown gene. We propose that the former, p200, be named BIG1 (for brefeldin A-inhibited GEP) and the second, which encodes a ~202-kDa protein, BIG2. A protein containing sequences found in BIG2 had been purified earlier from bovine brain. Human tissues contained a 7.5-kilobase BIG1 mRNA and a 9.4-kilobase BIG2 transcript. The BIG1 and BIG2 genes were localized, respectively, to chromosomes 8 and 20. BIG2, synthesized as a His6 fusion protein in SF9 cells, accelerated guanosine 5'-3-O-(thio)triphosphate binding by recombinant ARF1, ARF5, and ARF6. It activated native ARF (mixture of ARF1 and ARF3) more effectively than did any of the nonmyristoylated recombinant ARFs. BIG2 activity was inhibited by BFA in a concentration-dependent manner but not by B17, a structural analog without effects on Golgi function. Although several clones for ~50-kDa BFA-insensitive ARF GEPs are known, these new clones for the ~200-kDa BIG1 and BIG2 should facilitate characterization of this rather different family of proteins as well as the elucidation of mechanisms of regulation of BFA-sensitive ARF function in Golgi transport.

ADP-ribosylation factors (ARFs) are ~20-kDa guanine nucleotide-binding proteins, initially identified by their ability to enhance cholera toxin-catalyzed ADP-ribosylation of the GTP-binding protein GαS (1). ARFs are ubiquitous in eukaryotic cells from *Giardia* to human and are known to play an important role in intracellular vesicular trafficking (for review, see Ref. 2), as well as to activate phospholipase D (3, 4). Six mammalian ARFs, identified by cDNA cloning, are grouped into three classes based on size, amino acid sequence, phylogenetic analysis, and gene structure: ARFs 1–3 in class I, ARFs 4 and 5 in class II, and ARF6 in class III (5, 6).

Like other GTPases that regulate many kinds of intracellular processes, ARFs are active and associate with membranes when GTP is bound, whereas inactive ARF-GDP is cytosolic. Replacement of GDP by GTP is accelerated by ARF GEPs or guanine nucleotide-exchange proteins, several of which have been identified (7). These include Gea1 and Gea2 from yeast (8), mammalian B2-1 or cytohesin-1 (9) and cytohesin-2 or ARNO (10), which are ~83% identical in amino acid sequence, and GRP1 (general receptor for phosphoinositides), a third member of the cytohesin group (11).

All ARF GEPs of known structure contain Sec7 domains (8–13). Sec7 was identified in a group of conditionally lethal yeast mutants as a gene involved in Golgi vesicular trafficking and secretion (14). The Sec7 gene product is a ~230-kDa phosphoprotein that moves between membrane and cytosolic fractions (15). Its Sec7 domain was demonstrated relatively recently to function as a brefeldin A (BFA)-inhibited ARF GEP (16). BFA is a fungal fatty acid metabolite with a monocyclic lactone ring that blocks protein secretion reversibly and causes apparent collapse of Golgi membranes into the endoplasmic reticulum (17, 18). These effects result from BFA inhibition of GEP-catalyzed ARF activation (GTP binding) (19, 20).

The two major types of ARF GEPs differ in size and susceptibility to inhibition by BFA. Their Sec7 domains contain the determinants of GEP activity as well as its BFA sensitivity. The larger ~200-kDa GEPs (e.g. Sec7, Gea1, and Gea2 from yeast) are inhibited by BFA, whereas those of the cytohesin family are BFA-insensitive, ~50-kDa proteins. Morinaga et al. (21, 22) reported the purification and cloning of a BFA-inhibited ARF GEP from bovine brain, which was referred to as p200. We describe here the cloning from a human brain library of two GEP cDNAs. One, with a deduced amino acid sequence (1,849 residues) 99.5% identical to that of p200, probably represents the human form of p200. The other, encoding a 202-kDa protein 74% identical in sequence, is a new BFA-inhibited human GEP. We propose that because of their BFA sensitivity and size relative to the cytohesins, these mammalian ARF GEPs be named BIG1 (for BFA-inhibited GEP) and BIG2, respectively.

**EXPERIMENTAL PROCEDURES**

**Materials—**[α-32P]dTTP, [γ-32P]dATP, and [γ-35S]GTP[S] were purchased from NEN Life Science Products. All restriction enzymes and Taq polymerase used to prepare BIG1-In and BIG2-In were purchased from Roche Molecular Biochemicals (formerly Boehringer Mannheim). Sources of other materials are noted below or have been reported (22).

**DNA Sequencing—**All plasmids were purified using Qiagen Plasmid Purification and Cloning of a Brefeldin A-inhibited Guanine Nucleotide-exchange Protein for ADP-ribosylation Factors* (Received for publication, October 1, 1998, and in revised form, December 28, 1998)

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ADP-ribosylation factors (ARFs)* are ~20-kDa guanine nucleotide-binding proteins, initially identified by their ability to...
Midi kits (Qiagen, Chatsworth, CA). An Applied Biosystems Inc. 373 DNA sequencer was used for sequencing. 500 ng of plasmid DNA, 3.2 pmol of primer, and 8 μl of Terminator Ready Reaction Mix (Perkin-Elmer) were used for cycle sequencing (25 cycles of 96 °C for 30 s, 50 °C for 15 s, and 60 °C for 2 min), followed by incubation at 90 °C for 4 min in total volume of 20 μl.

Characterization of the cDNAs from Human Brain—Probes for screening a human frontal cortex ZAP cDNA library (1.2 × 10⁹ plaque-forming units) (Strategene) were prepared by PCR with bovine BIG1 cDNA (22) as template. Sequences were: nucleotides 306–762 (probe 1), 2086–2728 (probe 2), and 5207–5541 (probe 3). Prehybridization overnight at 42 °C in 10× dextran sulfate, 1% SDS, 50 μl Tris, pH 7.4, 0.5 μl NaCl, and 50 μl formamide was followed by hybridization with [α-32P]dCTP-labeled probe (2 × 10⁶ cpm/ml) in 100 ml of the same buffer overnight at 42 °C. Filters were then washed with 2 × SSC and 0.1% SDS at room temperature for 10 min twice and 1 × SSC and 0.1% SDS at 50 °C for 10 min. Two positive plaques were obtained with probe 1. The first contained bases 1–2467 of the BIG2 coding region named BIG2-N. (Base numbers are relative to A of ATG, equal to 1.) The other contained bases 1–420 of BIG1 (named BIG1-N). One plaque obtained with probe 2 contained bases 1738–4657 of BIG1 (named BIG1-C), and one with probe 3 (BIG2-C) contained bases 3514–5860 of BIG2.

The sequence between BIG2-N and BIG2-C was obtained by a nested PCR. Primers for the first PCR (30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 2 min) were BIG2-N bases 2359–3385 (forward) and BIG2-C (reverse). Gene-specific primers in BIG1-C were AP-1 and AP-2. Gene-specific primers in BIG2-N (bases 2359–2385 (forward) and 3406–3432 (reverse) were used in a 100 ml PCR (30 cycles of 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min) with adapters (AP-1 and AP-2). Gene-specific primers in BIG1-C were PC0-CCC (forward primer (bases 1826–1848), and reverse primer In-R (total volume 100 nl) for the first PCR (30 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 4 min). The 1.8-kb PCR product was purified using a QIAEX II Gel Extraction kit and subcloned into pCR-Blunt vector (named BIG2-Not). BIG2-C (500 ng) and 1 μl of 1.2-kb PCR were used as templates for PCR (30 cycles of 94 °C for 1 min, 60 °C for 1 min, 72 °C for 4 min) with primers In-F and bases 4249–4222 (reverse) in a total volume of 100 μl to obtain bands 2374–4249 (1.9 kb). The 1.9-kb PCR product was subcloned into pCR-Blunt vector (named BIG2-Nsi-Clal) based on subsequent digestion with Nsi I and Clal. A 1.7-kbp DNA excised from BIG2-Not with Clal and Nsi I was ligated to BIG2-Nsi-Clal, which had been digested with Clal (base 3791) and Nsi I (named BIG2-Nsi-Not).

To construct the full-length BIG2 DNA, the 2.4-kb DNA, excised from BIG2-Nsi-Not with Nsi I (base 2986 of BIG2) and Nsi I, was ligated to BIG2-Nde-Xba which had been digested with Nde I and Xba I. The subcloned plasmid DNA was named BIG2-Full. PCR product (bases 2374–4249) excised with Nde I and Nsi I was ligated to the baculovirus transfer vector pAcHLT-C with the Hisa sequence encoded at its NH₂ terminus. A sample (2 μg) and 0.5 μg of BaculoGold DNA (PharMingen) were mixed with 2 × 10⁶ Sf9 cells in 3 ml of TNM-FH Insect medium (PharMingen). After incubation at 27 °C for 5 days, a sample (10 μl) of the cell supernatant (Sup 1) was added to 1 × 10⁶ Sf9 cells in 5 ml of medium followed by incubation for 5 days at 27 °C before the supernatant was collected (Sup 2). Sf9 cells (2 × 10⁵) were added to 1 ml of Sup 2 and 30 ml of TNM-FH.

For insertions generated by PCR and a composite BIG2, the sequence between bases 421 and 1737 in BIG1 was obtained by an analogous procedure. Primers for the first PCR (30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min) were bases 275–299 in BIG1-N (forward) and 1886–1860 in BIG1-C (reverse). A sample (2 μl) of this PCR product was used as template in a nested PCR (30 cycles of 94 °C for 1 min, 52 °C for 1 min, and 72 °C for 2 min) with bases 300–329 (forward) and 1836–1811 (reverse) as primers. The 1.5-kb product was subcloned in pCR2.1 (Invitrogen) to produce BIG2-In. Sequencing of the individual fragments generated a composite BIG2.

The sequence between bases 241 and 1737 in BIG1 was obtained by a rapid amplification of cDNA ends procedure using 2.5 units of Pfu DNA polymerase (Strategene) and a Marathon Ready cDNA kit (CLONTECH), which included the DNA template and two reverse primers with adapters (AP-1 and AP-2). Gene-specific primers in BIG2-N (bases 4486–4491 (G1, forward) and bases 4587–4611 (G1, forward) were used in the first PCR (total volume 50 μl) 1 μl of template with G1 and AP-1 was used for 30 cycles of 94 °C for 1 min, 68 °C for 1 min, and 72 °C for 3 min. 1 μl of this PCR mixture was used as template in the second PCR (total volume 50 μl) with G2 and AP-2 for 30 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 3 min. The PCR product, which was purified by II gel extraction kit (Qiagen), was subcloned in pCR-Blunt (Invitrogen) and sequenced. Sequences of these cDNAs yielded a composite BIG1 sequence.

Construction of Full-length BIG2 cDNA in pAcHLT-C—For insertion of BIG2 into the baculovirus transfer vector pAcHLT-C (PharMingen), restriction sites for Nde I and Nsi I were introduced, respectively, before the initiation and after the termination codons of BIG2. To insert the Nde I site, the forward primer was 5′-CATATGTTGAGGACGACGAGACGAGCCCAG-3′ including the Nde I site (italic) and BIG2 initiation codon (underlined) with reverse primer (bases 298–274) and 500 ng of BIG2-N as template in a PCR (100 μl) with 5 units of Taq polymerase for 30 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s. The 305-bp PCR product was subcloned into pCR2.1 vector. The DNA excised from the purified plasmid with HindIII (site in pCR 2.1 vector) and Nde I (base 219 of BIG2) was ligated to BIG2-N, which had been digested with the same enzymes, and named BIG2-Bde-Nhe.

To ligate BIG2-Nde-Nhe and BIG2-In, BIG2-In (bases 2374–3593) was the DNA template with primers In-F and In-R and 2.5 units of Pfu DNA polymerase (total volume 100 μl) for the first PCR (30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 3 min). The 1.7-kb product (1.2-kb PCR) was purified using a QIAEXII gel extraction kit and eluted in 40 μl of distilled water. 1 μl of 1.2-kb PCR product and 500 ng of BIG2-N were used as templates in the second PCR (30 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 4 min) with Pfu DNA polymerase, forward primer (bases 1826–1848), and reverse primer In-R (total volume 100 μl). The 1.7-kb PCR (bases 1836–3593), which was purified using a QIAEXII gel extraction kit, was subcloned into pCR-Blunt vector. The plasmid DNA was digested with AatII (base 1849 of BIG2) and SpeI (site in pCR-Blunt vector). The excised DNA and the BIG2-Nde-Nhe, which had been digested with AatII and Xba I, were ligated and subcloned (named BIG2-Nde-Xba).

To construct BIG2-C, the plasmid DNA (total volume 50 μl) was digested with Nde I and Not I in 10 ml of the same solution of 300 mM NaCl, 10% glycerol, 50 mM Tris–HCl (pH 8.0), 1 mM EDTA, 1 mM NaCl, 1 mM dithiothreitol, 0.25 mM sucrose, 5 mM MgCl₂, 0.5 mM AEBSF, and 30 mM NaCl overnight at 4°C.
downstream of the termination codon) for 30 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s (total volume 20 μl). An intron from BIG2 was amplified from 200 ng of human genomic DNA (CLON-TECH), with forward primer CL2-A and reverse primer (bases 2591–2568), in a volume of 100 μl for 30 cycles of 94 °C for 1 min, 65 °C for 1 min, and 72 °C for 2 min. The single PCR product was sequenced to verify the presence of the 622-bp intron at position 2533 in genomic DNA (2). Sequencing of a 7.5-kb band hybridized with BIG1 cDNA; a 9.4-kb transcript hybridized with the BIG2 probe. When quantified by densitometry and expressed as a fraction of the density of the corresponding glyceraldehyde-3-phosphate dehydrogenase mRNA, BIG1 mRNA in placenta and lung was considerably more abundant than it was in heart, brain, kidney, or pancreas. The amounts of BIG2 mRNA were relatively similar in all of those tissues.

Chromosomal Localization of BIG1 and BIG2—The BIG1 cDNA hybridized clearly with a single band (400 bp) from chromosome 8 (data not shown) and with a band of the same size in the lane containing NAIMR91 DNA (positive control). The BIG2 probe hybridized with a 700-hp band from chromosome 20, which was also present in the total human DNA (data not shown). Hybridization with DNA from other chromosomes was detected.

Function of Recombinant BIG2 Synthesized in Sf9 Cells—To demonstrate the GEP activity of BIG2, it was synthesized as a His6 fusion protein in Sf9 cells. After purification on nickel-nitrilotriacetic acid agarose, a single protein band corresponding to a molecule of about 200 kDa was detected on SDS-polyacrylamide gel electrophoresis (Fig. 5, inset). At 25 °C, the purified BIG2 accelerated GTPγS binding by rARF1 in a concentration-dependent manner (Fig. 5). The basal rate of binding was essentially constant for at least 30 min, although the initial rate of BIG2-stimulated binding was apparently beginning to decline by 5 min (Fig. 6). BIG2 itself did not bind GTPγS under these conditions (data not shown).

BFA at a concentration of 60 μg/ml (214 μM) inhibited BIG2 activity ~70%, and 20 μg/ml caused ~25% inhibition (Fig. 7). B17, a structural analog of BFA which does not interfere with Golgi function, did not inhibit BIG2 activity (Table III). As shown in Table IV, BIG2 was similarly effective in enhancing GTPγS binding by rARF1, rARF5, and rARF6. It activated the native ARF (chiefly ARF1 and ARF3) more effectively than it did any of the recombinant nonmyristoylated ARFs.

RESULTS

Human BIG1 and BIG2 cDNAs—BIG2 was initially purified as part of a macromolecular complex of ~670 kDa from bovine brain. After separation of complex components by SDS-polyacrylamide gel electrophoresis, proteins of ~200 (p200) and 190 kDa exhibited BFA-inhibited ARF GEP activity (22). Sequences of peptides from p200 were used as the basis for cloning the bovine cDNA for BIG1. Cloning of human BIG1 and BIG2 cDNAs is described under “Experimental Procedures.” The cDNA for human BIG2 encodes a protein of 1,785 amino acids with a calculated molecular weight of 202,000. Both human and bovine BIG1 are somewhat larger with 1,849 amino acids and molecular weights of 209,000.

Sequences of nine peptides produced by trypsin digestion of the p190 that had been purified from bovine brain (22) are almost identical (106 of a total of 190 amino acids) to that deduced from the human BIG2 clone. Descriptions of nine tryptic peptides from the p190 and 190 kDa bands. The primary structures of BIG1 and BIG2 exhibit a very high degree of identity, consistent with the notion that they may be representatives of a distinct branch of the BFA-inhibited, 200-kDa GEP family. In all family members the Sec7 domain constitutes only a small fraction of the entire molecule, although there are clearly some other elements of the structure which influence its GEP activity (16, 24). There are several regions outside of the Sec7 domains with sequences that are 70–89% identical in BIG1 and BIG2 and very likely have similar functions, which remain to be deter-

### TABLE I

| Peptide | Sequence | Position |
|---------|----------|----------|
| 1       | LIAHGHTNNPDSSGAPK   | 94–112    |
| 2       | QFLQECQGMSTVEDIAQF  | 663–681   |
| 3       | FEEYLSYYE       | 811–820   |
| 4       | KYLSGGGR      | 1012–1019 |
| 5       | ELANFRFQ       | 1173–1180 |
| 6       | DFLRPFHEIMK    | 1182–1192 |
| 7       | HLDVDLRQSSLSDK  | 1502–1517 |
| 8       | NFSEGQGQ       | 1518–1526 |
| 9       | NYEQT0VL      | 1631–1638 |
mined. The corresponding regions of other proteins of the GEP family (e.g. Sec7) presumably enable them to function in somewhat different pathways, involving specific interactions with a different assortment of proteins and other molecules. In addition to anchoring, scaffolding, and/or adapter roles, these proteins may have other enzymatic activities. The number of recognized ARF GEPs has increased dramatically in the last 2 years and far exceeds the five or six known ARFs (7). Clearly, FIG. 1. Deduced amino acid sequences of BIG1 and BIG2. The sequence of human BIG2 is aligned with those of human (h) and bovine (b) BIG1 (22). Dots denote identical amino acids. Conservative differences from BIG2 are in capital letters and nonconservative in lowercase. Hyphens indicate gaps; Sec7 domains are in boldface. Alignment was generated using GeneWorks 2.5.1.

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we are just beginning to glimpse the complexity of organization and operation of the pathways in which they function. It appears that the multiple GEPs may contribute in a major way to accomplishing a specificity of ARF action in cells which, at present, lacks a mechanistic explanation.

The regions of highest identity (90%) in BIG1 and BIG2 are the Sec7 domains, which in the yeast Sec7 protein are responsible for GEP activity and its BFA inhibition (16). Similarly, the BFA-insensitive GEP activities of ARNO (10) and cytohesin-1 (23) are properties of their Sec7 domains. With fewer than 200 amino acids in the Sec7 domains, 50% of which are identical in the BFA-sensitive and -insensitive GEPs, the residues responsible for BFA inhibition should soon be identified.

By comparing systematically the activities of cytohesin-1 and its Sec7 domain with a variety of ARF-related proteins, it was established that some determinants of the substrate specificity of cytohesin-1, which is much more restricted than that of its Sec7 domain, must reside elsewhere in the molecule (24). This seems likely to be true also for the other 50-kDa BFA-insensitive GEPs, ARNO (10) and GRP1 (11). Whether or not it applies to the 200-kDa BFA-inhibited GEPs remains to be determined.

FIG. 2. Alignment of deduced amino acid sequences of Sec7 domains of ARF GEPs. The amino acid sequences are presented as in Fig. 1 for hBIG2, hBIG1, ySec7 (14), EMB30 (12), yGea1 (8), hB2-1 (9), hARNO (10), and mGRP1 (11). Alignments were produced by GeneWorks 2.5.1 and adjusted by inspection.

### Table II

Percentage identity and similarity of amino acid sequences of Sec7 domains of nine proteins with ARF GEP activity

| Protein | hBIG2 | hBIG1 | ySec7 | EMB30 | Gea1 | hB2-1 | hARNO | mGRP1 |
|---------|-------|-------|-------|-------|------|-------|-------|-------|
| hBIG2   | 90    | 89    | 46    | 42    | 34   | 51    | 51    | 47    |
| hBIG1   | 95    | 95    | 47    | 42    | 35   | 51    | 50    | 47    |
| ySec7   | 76    | 69    | 46    | 42    | 34   | 46    | 45    | 45    |
| EMB30   | 60    | 61    | 61    | 61    | 61   | 61    | 61    | 61    |
| Gea1    | 48    | 48    | 48    | 48    | 48   | 48    | 48    | 48    |
| hB2-1   | 67    | 68    | 68    | 68    | 68   | 68    | 68    | 68    |
| hARNO   | 67    | 67    | 67    | 67    | 67   | 67    | 67    | 67    |
| mGRP1   | 67    | 67    | 67    | 67    | 67   | 67    | 67    | 67    |

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The two families of GEPs also differ structurally in the presence of pleckstrin homology (PH) domains in the cytohesin-1 family. PH domains, which participate in specific protein-phospholipid and protein-protein interactions, are critical regions in nucleotide-exchange proteins for many GTPases and other regulatory molecules (25). Whereas cytohesin-1 and ARNO (and their PH domains) preferentially bind phosphatidylinositol 4,5-bisphosphate (26), GRP1 has a higher affinity for phosphatidylinositol 3,4,5-trisphosphate (27). Each phosphoinositide increased the activity of the respective GEP dramatically, presumably because it concentrates the protein (along with the ARF substrate) at a membrane surface (26). PH domains have not been recognized in the larger BFA-sensitive GEPs, although their activity is also influenced by specific phospholipids.

With the recent report (28) that activated ARF induces binding of spectrin and ankyrin isoforms to Golgi membranes, it is tempting to speculate that an ARF GEP could be part of a soluble complex containing those and perhaps other anchoring or adapter molecules involved in a spectrin-ankyrin (or analogous) assembly that is important for structure and function in vesicular trafficking pathways. It was suggested that phosphatidylinositol 4,5-bisphosphate, which is bound preferentially by the PH domain of the Golgi-specific β2 spectrin, is one site of spectrin attachment to the membrane. A second is in a repeat sequence near the spectrin NH₂ terminus. Because...
BIG1 and BIG2 were purified as components of a >600-kDa protein complex. Through six steps of purification from bovine brain cytosol, including (NH₄)₂SO₄ fractionation, chromatography on DEAE-Sephadex, hydroxylapatite, and Mono Q, followed by precipitation at pH 5.8, the BFA-inhibited GEP activity remained associated with a supramolecular complex (21). It was finally eluted in a relatively symmetrical peak from a column of Superose 6B at the position of thyroglobulin (669 kDa). The active fractions contained several proteins that were separated by SDS-polyacrylamide gel electrophoresis, among them p200 and p190, the amounts of which were judged to correlate best with activity. After elution from the gel and renaturation, p200 exhibited BFA-inhibited GEP activity (21), as did p190 (data not shown).

Although the other components of the isolated complex remain to be identified, they seem likely to be of importance in the process and/or regulation of vesicular transport. It has, of course, not been established that the two GEPs, in fact, reside together in the same supramolecular structure because it appears probable that the purified preparation was a heterogeneous population of complexes, derived, obviously, from a heterogeneous population of cells. In the absence of other information, it seems plausible to consider that BIG1 and BIG2 may be components of different but similar complexes, with analogous compositions and parallel functions, probably in different intracellular pathways and/or in different cells. Separation from the preparation of a single, homogeneous macromolecular complex so that its component proteins can be characterized is clearly critical to understanding the relationship between the two very similar, albeit different, BIG gene products, their functions, and their interactions with other molecules, both within and outside of the complex.

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