cDNA Cloning and Expression of Rat and Human Protein Geranylgeranyltransferase Type-I*

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank®/EMBL Data Bank with accession number(s) L24116 (rat β subunit of GGTase-I) and L25441 (human β subunit of GGTase-I).

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The abbreviations used are: GGTase-I, protein geranylgeranyltransferase type I; GGTase-II, protein geranylgeranyltransferase type II; β, β subunit of protein farnesyltransferase; βGGTP, subunit of protein geranylgeranyltransferase; bp, base pair(s); CAAX, cysteine (C), aliphatic amino acid (A), any amino acid (X); FPP, farnesyl diphosphate; FTase, protein farnesyltransferase; G proteins, GTP-binding regulatory proteins; GGPP, geranylgeranyl diphosphate; kb, kilobase(s); MFP, mouse fibrosarcoma polyoma virus; NADH, reduced nicotinamide adenine dinucleotide; PAGE, polyacrylamide gel electrophoresis; PNGase, polypeptide N-glycosidase F; PC-R, polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; β, subunit of yeast FTase; β, subunit of mammalian FTase; RM, Rab escort protein; COOH, carboxyl-terminal; CAAX, cysteine (C), any aliphatic amino acid (A), any amino acid (X); Zn²⁺, zinc ion; Mg²⁺, magnesium ion; ABC, Rab escort protein; MBP, maltose binding protein; CAAX, cysteine (C), any aliphatic amino acid (A), any amino acid (X); NVT, sodium cyanate; NaCUC, sodium citrate.

Protein geranylgeranyltransferase type-I (GGTase-I) transfers a geranylgeranyl group to the cysteine residue of candidate proteins containing a carboxyl-terminal CAAX motif in which the "X" residue is leucine (1, 2). Known targets of GGTase-I include the γ subunits of brain heterotrimeric G proteins and Ras-related small GTP-binding proteins such as Rac1, Rac2, Rap1A, and Rap1B (3-5). Additionally, short peptides encompassing the CAAX motif of these substrates can also be recognized by the enzyme (4-6). Immobilization of one such peptide for use as an affinity matrix has led to the isolation of GGTase-I from bovine brain (7). The purified enzyme contains two subunits with molecular masses of 48 kDa and 43 kDa, which have been designated, respectively, as α and β (henceforth designated βGGT). GGTase-I is dependent on both Mg²⁺ and Zn²⁺ for optimal activity. Demonstration of the Zn²⁺ dependence required prolonged incubation against, or purification in the presence of, a chelating agent. This property led to the designation of GGTase-I as a zinc metalloenzyme (7).

The properties of GGTase-I are similar to those of a related enzyme, protein farnesyltransferase (FTase). FTase transfers the prenyl moiety from farnesyl diphosphate to the cysteine residue of substrate proteins. FTase protein substrates, like those for GGTase-I, possess a carboxyl-terminal CAAX motif. The "X" residue of mammalian FTase substrates, however, is generally methionine, serine, or glutamine as opposed to leucine for GGTase-I substrates (5, 7). Substrates for FTase include p21ras protein, lamina B, and several proteins involved in visual signal transduction (1). Like GGTase-I, FTase is dependent upon Mg²⁺ and Zn²⁺ ions for optimal activity (8). Purified mammalian FTase is composed of two nonidentical subunits, α and β (henceforth designated βFT), with apparent molecular masses of approximately 48 kDa and 46 kDa, respectively, on SDS-PAGE. (9). cDNA clones encoding the FTase α and β subunits have been isolated, and their deduced amino acid sequences are homologous to the Saccharomyces cerevisiae proteins Ram2 and Dpr1/Ram1, respectively, which encode the subunits of yeast FTase (5, 10-12).

The 48-kDa α subunits of mammalian GGTase-I and FTase have been shown to be immunologically cross-reactive, suggesting that these two enzymes share a common α subunit (7, 11, 13). Similarly, a mutation in the S. cerevisiae gene RAM2, which encodes the α-like subunit of yeast FTase, results in a strain with a small, but significant, defect in GGTase-I activity in addition to a drastic reduction in FTase activity (5, 11). Further confirmation of a common subunit for yeast FTase and GGTase-I came from the bacterial co-expression of Ram2 with Ram1 that resulted in FTase activity (12), while co-expression of Ram2 with Cdc43Cal1 resulted in GGTase-I activity (14). Since the S. cerevisiae Cdc43Cal1 subunit of GGTase-I shows amino acid similarity to yeast Ram1/Dpr1 and mammalian βFT, it may be the yeast homolog of mammalian βGGT (15).

GGTase-I differs from the related enzyme Rab geranylger-
anyttransferase, also called protein geranylgeranylationerase type-II (designated GGTase-II), which attaches a geranylgeranyl group to the COOH-terminal cysteines in small GTP-binding proteins that terminate in Cys-Cys or Cys-X-Cys motifs (5, 16, 17). Target proteins of GGTase-II include Rab1A, which resides in the endoplasmic reticulum and Golgi complex, and Rab 3A, a component of synaptic vesicles (18). GGTase-II does not recognize COOH-terminal peptides corresponding to its target proteins, rather, substrate recognition appears to involve additional determinants on the Rab proteins (5, 19). GGTase-II consists of three protein components. One component, the 95-kDa Rab escort protein, appears to bind substrate peptides and presents them to the catalytic subunits of the enzyme (20). The catalytic component of GGTase-II contains two tightly associated polypeptides with apparent molecular masses of 80 kDa and 28 kDa that, respectively, show similarity to the α and β subunits of FtsA (21).

In the current study, we obtained peptide sequence from both the α and βGII subunits of purified bovine GGTase-I. The three peptide sequences from the α subunit of GGTase-I showed 100% identity to the amino acid sequence deduced from a cDNA encoding the α subunit of bovine FtsA (11), providing direct evidence to support the hypothesis that mammalian FtsA and GGTase-I share a common α subunit. Peptide sequences from the βGII subunit were used to design probes to screen rat and human cDNA libraries. Overlapping cDNA clones from both human and rat cDNA libraries were isolated that encode nearly identical proteins with 377 amino acids. The deduced protein sequences show similarity to yeast Cdc43 as well as to the subunit of human FtsA. Peptide sequences from the α subunit of human FtsA were used to design probes to screen rat and human cDNA libraries. Overlapping cDNA clones from both human and rat cDNA libraries were isolated that encode nearly identical proteins with 377 amino acids. The deduced protein sequences show similarity to yeast Cdc43 as well as to the subunit of human FtsA. Peptide sequences from the α subunit of human FtsA were used to design probes to screen rat and human cDNA libraries. Overlapping cDNA clones from both human and rat cDNA libraries were isolated that encode nearly identical proteins with 377 amino acids. The deduced protein sequences show similarity to yeast Cdc43 as well as to the subunit of human FtsA.

EXPERIMENTAL PROCEDURES

General Methods—Standard molecular biology techniques were used (22, 23). Enzymes were obtained from New England Biolabs or Boehringer Mannheim. cDNA clones were subcloned into pGEM-4Z or pUC18 and sequenced by the dideoxy chain termination method (24). cDNA clones were subcloned into pGEM-4Z or pUC18 and sequenced by the dideoxy chain termination method (24) using universal primers or specific internal primers. All cDNAs and pUC18 were sequenced by the dideoxy chain termination method (24)

To isolate rat cDNAs encoding the βGII subunit, the PCR probe from pRD548 was labeled and used to screen a rat brain 5′-stretch cDNA library (Clontech). Duplicate filters were hybridized in 4× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate (SDS) at 66 °C. Filters were washed in 2× SSC, 0.1% SDS for 15 min at 66 °C and then in 0.2× SSC, 0.1% SDS for 30 min at 68 °C. Of the 1× 10⁶ plaques that were screened, five positives were identified by plaque-purification. The clone with the longest 5′-end (designated as clone 22) was subcloned into pGEM-4Z and sequenced on both strands. DNA sequencing of the clone indicated that it contains all but the first two codons of the rat βGII subunit.

To isolate the amino-terminal coding sequence for the rat βGII, the 5′-rapid amplification of cDNA ends (RACE) protocol was used to extend the 5′-end of clone 22 (29). Three primers were prepared based on the 5′-end sequences of clone 22. First strand cDNA was synthesized from rat brain mRNA with one of the primers, and the cDNA was then tail with dATP using terminal transferase. Two rounds of PCR reactions were performed using the two nested primers and an oligo(dT) primer. The RACE products obtained were cloned into pGEM-4Z. Twelve clones from different PCR reactions were sequenced on both strands, and all yielded identical sequences. The DNA sequence of these clones contained an in-frame ATG codon 3 base pairs upstream of the 5′-end of the cDNA in clone 22. This ATG codon is in the same position as the initiation codon found in the cDNA from human βGII (Fig. 1).

Expression and Purification of Recombinant Human GGTase-I and FtsA—To express human GGTase-I in E. coli, the cloned human βGII subunit cDNA and the previously cloned human FtsA-α subunit cDNA (28) were co-expressed from a plasmid in which their expression was directed by a carboxyl-terminal ubiquitin promoter (UbiP) and a 5′-carboxyl-terminal of α and the amino terminus of the βGII subunit coding sequences was made by recombinant PCR using pTST-hFPTase-α and pRD666 as templates (30). Fragment 2, a 1.52-kb Xhol-EcoRI fragment from pRD550 that contained the coding sequence downstream of the XhoI site. Fragments 1 and 2 were cloned into BamHI-XhoI-digested pUC18 creating pRD566 which contains the complete coding sequence for human βGII and 3′-untranslated sequences.

To isolate rat cDNAs encoding the βGII subunit, the PCR probe from pRD548 was labeled and used to screen a rat brain 5′-stretch cDNA library (Clontech). Duplicate filters were hybridized in 4× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate) and 0.1% sodium dodecyl sulfate (SDS) at 66 °C. Filters were washed in 2× SSC, 0.1% SDS for 15 min at 66 °C and then in 0.2× SSC, 0.1% SDS for 30 min at 68 °C. Of the 1× 10⁶ plaques that were screened, five positives were identified by plaque-purification. The clone with the longest 5′-end (designated as clone 22) was subcloned into pGEM-4Z and sequenced on both strands. DNA sequencing of the clone indicated that it contains all but the first two codons of the rat βGII subunit.

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Expression and Purification of Recombinant Human GGTase-I and FtsA—To express human GGTase-I in E. coli, the cloned human βGII subunit cDNA and the previously cloned human FtsA-α subunit cDNA (28) were co-expressed from a plasmid in which their expression was directed by a carboxyl-terminal ubiquitin promoter (UbiP) and a 5′-carboxyl-terminal of α and the amino terminus of the βGII subunit coding sequences was made by recombinant PCR using pTST-hFPTase-α and pRD666 as templates (30). Fragment 2, a 1.52-kb Xhol-EcoRI fragment from pRD666 that contained the part of the βGII coding sequence not in fragment 1. Fragment 3 was a 6.2-kb SpeI (partial 5′-to-3′) fragment from pTST-hFPTase-α that contained the portion of the α coding sequence not in fragment 1 and the vector and promoter sequences from pTST-hFPTase-α. Fragments 1, 2, and 3 were ligated together to create pRD577 which has the following structure: RBS

| RBS | pTST-α coding | -GAG-GAG-ATT-TTA-CTG-AGA-GTA-βGII | coding | Glu-Glu-Phe stop | Met-Val |

SEQUENCE I

The coding sequence for the human βGII subunit was translationally coupled to the α subunit coding sequence in which the ribosomal binding site for expression of βGII was contained within the Glu-Glu-Phe epitope tag.
The GGTase-I subunit is encoded by a cDNA containing the coding region and a 420-bp 3' untranslated region. Both cDNAs contain a 9-bp 5' untranslated region followed by an 1131-bp coding region and a 3' untranslated region of 526 bp. The cDNA of the pGor subunit of rat GGTase-I (not shown) was cloned as an EcoRI-HindIII fragment for about two-thirds of the pGGI subunit (data not shown). Overlapping cDNAs encoding the complete rat and human pGGI subunits of GGTase-I were obtained by a combination of screening with the 730-bp PCR probe and RACE techniques as described under "Experimental Procedures." The sequence of the overlap of the two cDNAs was identical. Nucleotide residues are numbered on the left; amino acid residues are numbered on the right. Amino acid residue 1 is the putative initiator methionine. The molecular weight of the predicted polypeptide is 42.4 kDa. Peptides that are identical with those isolated from the pGGI subunit of bovine brain GGTase-I are underlined.

RESULTS

Protein Sequencing of GGTase-I Subunit Polypeptides—Affinity chromatography of GGTase-I yielded sufficient quantities of purified protein from bovine brain to obtain the internal sequences of both the 48-kDa α subunit and the 43-kDa βGGI subunits of bovine brain GGTase-I. The purified 48-kDa α subunit and 43-kDa βGGI subunits of bovine brain GGTase-I were digested with trypsin and the resulting peptides were purified by HPLC and sequenced (see "Experimental Procedures"). The GGTase-I subunit peptides are shown compared to peptides deduced from a cDNA encoding the bovine FTase-α subunit (11). α, amino acid.

| α Peptide | Amino acid sequence | Location of peptide (aa) in rat and human | Corresponding peptide from bovine FTase α (n) |
|-----------|---------------------|------------------------------------------|---------------------------------------------|
| 1         | TIAFALSGLDMLD       | 49-62                                    | FQDVYDFPR (aa 46-54)                        |
| 2         | GSSYLGHPFPNSK       | 97-109                                   | QQV1QEFK (aa 157-164)                      |
| 3         | IFQYTNFEK           | 285-293                                  | VLWLRDSPQEL- EFEIADLTKDAQ (aa 124-148)    |

TABLE I

Amino acid sequence of GGTase-I α and βGGI peptides

The purified 48-kDa α subunit and 43-kDa βGGI subunits of bovine brain GGTase-I were digested with trypsin and the resulting peptides were purified by HPLC and sequenced (see "Experimental Procedures"). The GGTase-I subunit peptides are shown compared to peptides deduced from a cDNA encoding the bovine FTase-α subunit (11). α, amino acid.

| α Peptide | Amino acid sequence | Corresponding peptide from bovine FTase α (n) |
|-----------|---------------------|---------------------------------------------|
| 1         | TIAFALSGLDMLD       | FQDVYDFPR (aa 46-54)                        |
| 2         | GSSYLGHPFPNSK       | QQV1QEFK (aa 157-164)                      |
| 3         | IFQYTNFEK           | VLWLRDSPQEL- EFEIADLTKDAQ (aa 124-148)    |

To express human GGTase-I, pRD577 was transformed into E. coli BL21(DE3), grown, and induced with isopropyl-β-D-thiogalactoside as described (28). Recombinant, human GGTase-I was purified from the cells essentially as described for human FTase using a YL1/2 antibody column, which binds the Glu-Glu-Pho epitope tag on the α subunit and a subsequent Mono Q HR 515 column (28). The GGTase-I eluted from the Mono Q column at approximately 0.25 M NaCl.

Recombinant human FTase was expressed in E. coli and purified as described (28). Antibodies and Immunoblotting—A fragment from pRD548 encoding amino acids 49-146 of βGGI was cloned as an EcoRI-HindIII fragment into pMAL-cRI (New England Biolabs) in order to produce a maltose binding protein-fusion protein. The maltose binding protein-βGGI fusion protein was isolated as previously described (31) and sent to Hazleton Research Products (Hazelton, PA) to immunize rabbits. Immunoblotting using the immune antisera was performed as previously described (11).

GGTase-I and FTase Assays—GGTase-I and FTase activities were assayed as previously described (5). Rat protein substrates used were expressed from the plasmids [Leu"RAS1 termin.] SLKCVLS and [LeuωRAS1 termin.] KSSCALL the proteins from which were designated, respectively, as Ras-CVLS and Ras-CAIL (32, 33). 3H-Labeled farnesyl diphosphate (40 Ci/mmol) and 3H-labeled geranylgeranyl diphosphate (15 Ci/mmol) were purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

RESULTS

Protein Sequencing of GGTase-I Subunit Polypeptides—Affinity chromatography of GGTase-I yielded sufficient quantities of purified protein from bovine brain to obtain the internal sequences of both the 48-kDa α subunit and the 43-kDa βGGI polypeptide components. A number of clearly resolved peptides were obtained from HPLC purification of tryptic digest of each polypeptide (results not shown), and their sequences were determined (Table I). A sequence comparison between the peptides obtained from the 48-kDa α subunit of bovine GGTase-I and corresponding regions of bovine FTase-α as deduced from a cDNA clone (11) are shown (Table I). Three different peptides from the α subunit of GGTase-I were sequenced and found to be 100% identical with regions of FTase-α. This sequence identity provides direct evidence that both GGTase-I and FTase share a common subunit. Verification of this hypothesis comes from the finding that co-expression of cDNA encoding FTase-α and GGTase-I βGGI produce a fully active GGTase-I enzyme (see below).

Isolation of cDNA Clones Encoding GGTase-I βGGI Subunits—Degenerate oligonucleotide primers encoding parts of GGTase-I βGGI peptides 1 and 3 were prepared and used in PCRs with DNA from a bovine brain cDNA library as template as described under "Experimental Procedures." A 730-bp PCR product was identified that hybridized to a degenerate oligonucleotide probe for βGGI peptide 2. DNA sequencing of the PCR fragment indicated that it contained an open reading frame for peptides 1, 2, and 3, suggesting that it coded for about two-thirds of the βGGI subunit (data not shown). Overlapping cDNAs encoding the complete rat and human βGGI subunits of GGTase-I were obtained by a combination of screening with the 730-bp PCR probe and RACE techniques as described under "Experimental Procedures." The nucleotide sequence of the overlapping cDNAs encoding the βGGI subunit of human GGTase-I is shown in Fig. 1. The sequence contains a 312-bp 5'-untranslated region followed by a coding region of 1131 bp and a 3' untranslated region of 526 bp. The cDNA sequence for the βGGI subunit of rat GGTase-I (not shown) contains a 8-bp 5'-untranslated region compared to a 1131-bp coding region and a 420-bp 3'-untranslated region. Both cDNAs encode a protein of 377 amino acids that contains all five peptides whose sequences were obtained from tryptic digestion of the purified βGGI subunit (Table I). The predicted molecular mass of the cloned rat and human βGGI polypeptides, 42.4 and 42.5 kDa, respectively, is very similar to the 43 kDa observed on SDS-PAGE for the βGGI subunit of the enzyme purified from bovine brain (7). In addition, the human cDNA clone contains an in-frame termination codon 24 bp upstream of the initial.
methionine, further indicating that the open reading frame is as indicated.

An alignment of the amino acid sequences of the rat and human GGTase-I β subunits together with other protein prenyltransferase β subunits is shown in Fig. 2. These additional β subunits include those for rat FTase (34), rat GGTase-I (21), and the CDC43 gene product (CDC43), the β subunit of rat farnesyltransferase (rFTase), and the β subunit of rat GGTase-II (rGG-II). Sequence comparisons were performed with the CLUSTAL program of the DNA-STAR software package. By this analysis, rGG-I shows 30.2% identity and 55% similarity with hGG-I, and 28% identity and 51% similarity with rFTase.

Expression of Recombinant Human GGTase-I—The βGGI subunit was co-expressed in E. coli together with the α subunit from human FTase. Lysates from such strains showed significant GGTase-I activity, whereas lysates from strains expressing either the βGGI or FTase α subunit alone showed no GGTase-I activity (data not shown). Purification of the GGTase-I activity using the epitope tag on the FTase α subunit and a subsequent ion exchange step resulted in isolation of a heterodimeric protein with subunits of approximately 48 kDa and 43 kDa as expected for GGTase-I (Fig. 3, lane 1). Confirmation that the 43-kDa protein was in fact βGGI was done using an antibody directed to a fusion protein of part of the cloned βGGI sequence. This antibody reacted with the 43-kDa protein was in fact βGGI (Fig. 3, lane 4). Preimmune sera from the same rabbit did not react with any of these proteins (data not shown).

Enzymatic Properties of Recombinant GGTase-I—Purified recombinant human GGTase-I was assayed to see if its enzymatic properties were similar to GGTase-I isolated from a natural source. The results in Table II show that recombinant rGG-I purified from bovine brain (Fig. 3, lanes 3 and 4). This antibody did not react with the ββ subunit of human FTase (Fig. 3, lane 5). Preimmune sera from the same rabbit did not react with any of these proteins (data not shown).
human GGTase-I. Recombinant, human GGTase-I and FTase were purified as described under "Experimental Procedures." Bovine brain GGTase-I, recombinant human GGTase-I, PAGE (8% polyacrylamide). With Coomassie Brilliant Blue. For these two lanes, the dye front of the immunoblotted using antisera as described under "Experimental Procedures." The molecular masses of the protein standards are indicated in kilodaltons (kDa). The incorporation (nmol·h⁻¹·mg⁻¹) were determined. The mean value of the rates of incorporation (nmol·h⁻¹·mg⁻¹) were determined. Each assay was performed in duplicate, and the rates of Enzymatic activity using the substrates [Ras-CAIL], [Ras-CVLS] + FPP, 66 840 <1 1060

| Substrates       | FTase | GGTase-I |
|------------------|-------|----------|
| Ras-CVLS + FPP   | 840   |
| Ras-CAIL + FPP   | 66    |
| Ras-CVLS + GGPP  | 19    |
| Ras-CAIL + GGPP  | <1    |

**TABLE II**

Enzymatic activity of recombinant, human GGTase-I

We have cloned cDNAs encoding the complete βGGI subunits of rat and human GGTase-I that contain all five peptides isolated from the purified protein (Figs. 1 and 2). The proteins encoded by each of these cDNAs are 377 amino acids in length (Figs. 1 and 2). Human βGGI, when expressed in E. coli, co-migrates with the βGGI subunit of GGTase-I isolated from bovine brain, and both are recognized by antisera raised to the recombinant protein (Fig. 3).

The similarity among the amino acid sequences of the rat and human GGTase-I βGGI subunit and the S. cerevisiae CDC43 gene product indicates that the Cdc43 protein is the yeast equivalent of the βGGI subunit of mammalian GGTase-I (Fig. 2). This finding is consistent with previous studies showing that point mutations in the yeast CDC43 gene decrease GGTase-I activity and that co-expression of Cdc43 and Ram2 in bacteria produces GGTase-I activity (5, 14, 35). The deduced polypeptide sequences of the rat and human βGGI subunit of GGTase-I also show homology to the sequences of other known protein prenyltransferase β subunits (Fig. 2).

The sequences of the three peptides isolated from the α subunit of bovine GGTase-I are identical with corresponding regions of the deduced amino acid sequence of the α subunit of bovine FTase (Table I). We have cloned numerous cDNAs for the α subunit of bovine and human FTase (11, 28). DNA sequence and restriction endonuclease analysis of these clones have shown no evidence for the existence of multiple genes. Additionally, the 48-kDa α subunits of GGTase-I and FTase purified from bovine brain or fractionated from rat brain co-migrate on SDS-PAGE (7, 13) This information, combined with the demonstration that co-expression of FTase-α with GGTase-I βGGI resulted in GGTase-I enzyme activity (Fig. 3, Table II), strongly indicates that mammalian FTase and GGTase-I share an identical α subunit. A similar situation has been demonstrated in yeast with Ram2 being a common subunit for FTase and GGTase-I (12, 14).

Since both FTase and GGTase-I have an identical α subunit, the β subunits of these enzymes must determine which prenyl diphosphate and protein substrate they use. Consistent with this idea, both Ras and FPP substrates cross-link to the β subunit of FTase (8, 28). cDNA cloning of the βGGI subunit of GGTase-I will allow construction of chimeras between the β subunits of FTase and GGTase-I. Such experiments might define regions that are responsible for the substrate specificities of the two enzymes. Furthermore, overexpression systems for GGTase-I similar to those recently described for FTase will provide a route for detailed structural and functional studies of GGTase-I (28, 36).

**REFERENCES**

1. Clarke, S. (1992) Annu. Rev. Biochem. 61, 355–386
2. Casey, P. J. (1992) J. Lipid Res. 330, 1713–1740
3. Menard, L., Tomhaye, E., Casey, P. J., Uhing, E. J., Snyderman, R., and Didbury, J. R. (1992) Eur. J. Biochem. 206, 537–546
4. Casey, P. J., Thiessen, J. A., and Moomaw, J. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 8651–8654
5. Moore, S. R., Schaber, M. D., Mosser, S. D., Rands, E., O’Hara, M. B., Garson, V. M., Marshall, M. S., Pompeiano, D. L., and Gibbs, J. B. (1991) J. Biol. Chem. 266, 14603–14610
6. Yokoyama, K., Goodwin, G. W., Ghuemashki, F., Glomsset, J. A., and Gelb, M. H. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 5302–5306
7. Moomaw, J. F., and Casey, P. J. (1992) J. Biol. Chem. 267, 17436–17443
8. Reiss, Y., Brown, M. S., and Goldstein, J. L. (1992) J. Biol. Chem. 267, 6403–6408
9. Reiss, Y., Goldstein, J. L., Seabrah, M. C., Casey, P. J., and Brown, M. S. (1990) Cell 62, 81–88
10. Chen, W.-J., Andress, D. A., Goldstein, J. L., and Brown, M. S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11368–11372
11. Kahl, N. E., Diehl, R. E., Schaber, M. D., Rands, E., Soderman, D. H., Beo, Moore, S. L., Pompeiano, D. L., Ferro-Novick, S., Powers, S., Thomas, K. A., and Gibbs, J. B. (1991) J. Biol. Chem. 266, 18884–18888
12. He, B., Chen, P., Chen, S.-Y., Vancura, K. L., Michaelis, S., and Powers, S. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 1373–1377
13. Seabra, M. C., Reiss, Y., Casey, P. J., Brown, M. S., and Goldstein, J. L. (1991) Cell 65, 425–434
14. Mayer, M. L., Caplin, B. E., and Marshall, M. S. (1992) J. Biol. Chem. 267, 20589–20593
15. Ohya, Y., Goebi, M., Goodman, L. E., Petersen-Bjorn, S., Friessen, J. D., Tamaoki, F., and Amsrak, Y. (1991) J. Biol. Chem. 266, 13536–13536
16. Seabra, M. C., Goldstein, J. L., Sudhof, T. C., and Brown, M. S. (1992) J. Biol. Chem. 267, 14977–14953
17. Horiuchi, H., Kawata, M., Katayama, M., Yoshida, Y., Musha, T., Audo, S., and Takai, Y. (1991) J. Biol. Chem. 266, 16981–16984

2 R. Diehl, unpublished data.
Molecular Cloning of Mammalian GGTase-I

18. Pfeffer, S. (1992) Trends Cell. Biol. 2, 41-46
19. Khosravi-Far, R., Clark, G. J., Abe, K., Cox, A. D., McLain, T., Lutz, R. J., Sinensky, M., and Der, C. J. (1992) J. Biol. Chem. 267, 24363-24368
20. Andres, D. A., Seabra, M. C., Brown, M. S., Armstrong, S. A., Smeland, T. E., Cremers, F. P. M., and Goldstein, J. L. (1995) Cell 73, 1091-1099
21. Armstrong, S. A., Seabra, M. C., Sudhof, T. C., Goldstein, J. L., and Brown, M. S. (1992) J. Biol. Chem. 268, 24363-24368
22. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1990) Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
23. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B., and Erlich, H. A. (1988) Science 239, 487-491
24. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463-5467
25. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294-5299
26. Aebersold, R. H., Leavitt, J., Sanvedra, R. A., Hood, L. E., and Kent, S. B. H. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 6970-6974
27. Vogel, U. S., Dixon, R. A. F., Schaber, M. D., Diehl, R. E., Marshall, M. S., Scrofick, E. M., Sigal, I. S., and Gibbs, J. B. (1988) Nature 335, 90-93
28. Omer, C. A., Kral, A. M., Dohl, R. E., Prendergast, G. C., Powers, S., Allen, C. M., Gibbs, J. B., and Kohl, N. E. (1985) Biochemistry 24, 1767-1776
29. Frohman, M. A. (1990) in PCR Protocols: A Guide to Methods and Applications (Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J., eds) pp. 28-38, Academic Press, San Diego
30. Higuchi, R. (1990) in PCR Protocols: A Guide to Methods and Applications (Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J., eds) pp. 177-183, Academic Press, San Diego
31. Kellerman, O. K., and Ferenci, T. (1992) Methods Enzymol. 90, 459-463
32. Teides, G. G., Gibbs, J. B., D'Alonzo, J. S., Sigal, I. S., and Scrofick, E. M. (1985) Nature 313, 90-93
33. Gibbs, J. B., Schaber, M. D., Schofield, T. L., Scrofick, E. M., and Sigal, I. S. (1984) Proc. Natl. Acad. Sci. U. S. A. 86, 6629-6634
34. Chen, W.-J., Andres, D. A., Goldstein, J. L., Russell, D. W., and Brown, M. S. (1991) Cell 66, 327-334
35. Finegold, A. A., Johnson, D. I., Farnsworth, C. C., Gelb, M. H., Judd, S. R., Glomset, J. A., and Tamanoi, F. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 4448-4453
36. Chen, W.-J., Mooshow, J. F., Overton, L., Kost, T. A., and Casey, P. J. (1995) J. Biol. Chem. 268, 9675-9680