Human geranylgeranyl diphosphate synthase: isolation of the cDNA, chromosomal mapping and tissue expression

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Abstract **We report the nucleotide sequence of human geranylgeranyl diphosphate (GGPP) synthase cDNA isolated from a fetal heart library. The 2.5 kb cDNA encodes a protein of 34 kDa. The protein contains six domains that have been identified previously in many other prenyltransferases. Recombinant, purified histidine-tagged protein exhibited the enzymatic properties associated with GGPP synthase, namely the synthesis of GGPP from farnesyl diphosphate and isopentenyl diphosphate. Transient transfection of mammalian cells with a plasmid encoding the putative GGPP synthase resulted in a 55-fold increase in GGPP synthase activity. Taken together, these results establish that the cDNA encodes the mammalian GGPP synthase protein. The mRNA for GGPP synthase was expressed ubiquitiously. Of the 16 human tissues examined, the highest expression of the mRNA was in testis. The mRNA levels in cultured HeLa cells were unaffected by alterations in cellular sterol levels and contrasted with the significant regulation of isopentenyl diphosphate synthase mRNA under these same conditions. Fluorescent in situ hybridization was used to map the single gene encoding human GGPP synthase to chromosome 1q43.—**Ericsson, J., J. M. Greene, K. C. Carter, B. K. Shell, D. R. Duan, C. Florence, and P. A. Edwards. **Human geranylgeranyl diphosphate synthase: isolation of the cDNA, chromosomal mapping, and tissue expression. *J. Lipid Res.* 1998.** 39: **1731–1739.**

Supplementary key word prenyltransferase

A large family of enzymes termed prenyltransferases are involved in the biosynthesis of isoprenoids. The latter, naturally occurring family of compounds are ubiquitous and are composed of over 23,000 known members (1). Important isoprenoid derivatives include sterols, dolichols, ubiquinone, prenylated (farnesylated and geranylgeranylated) proteins, cholesterol-modified proteins, signaling molecules including oxygenated sterols, farnesol and geranylgeraniol, the isoprenoid side chain of heme a and plant mono-, sesqui-, and diterpenes (2–8).

The prenyltransferases catalyze three different types of reactions (2). One type, typified by farnesyl diphosphate (FPP) synthase and geranylgeranyl diphosphate (GGPP) synthase, catalyze the sequential condensation of isopentenyl diphosphate with an allylic diphosphate to synthesize linear prenyl diphosphates with various chain lengths and/or different *cis*/*trans* double bonds. The second type, typified by squalene synthase, catalyses the condensation of two allylic diphosphate substrates, followed by a 1′-1 rearrangement of the cyclopropylcarbinyl intermediate. The third type transfers allylic units to specific non-isoprenoid acceptors. Farnesyl:protein transferase is one example of this latter group.

More than 30 prenyltransferase cDNAs have been identified and characterized in the last few years (1, 9). Two aspartate-rich motifs, DDXX(XX)D, were originally identified as a result of comparison of the sequences of FPP synthase, derived from rat, human, and yeast with the yeast hexaprenyl synthase (10). The original proposal that these aspartate-rich domains represented the binding sites for both IPP and the allylic substrate (10, 11) was supported by studies in which mutagenesis of either the conserved aspartic acids, or other amino acids in the vicinity of this domain, affected the kinetic parameters of the enzyme (12–14). The subsequent crystallization of the avian FPP synthase protein provided definitive evidence that these domains were at the catalytic active site and located on opposite sides of the cavity that accommodates the two substrates (15). Interestingly, mutagenesis of two conserved amino acids that lie just outside the aspartate-rich motif affects the chain length of the product synthesized by FPP synthase or GGPP synthase (16–19). The availability of an increased number of sequences derived from eukaryotic and eubacterial prenyltransferases recently led to

Abbreviations: FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; IPTG, isopropyl-1-thio-β-d-galactoside; DAPI, 4′,6-diamidino-2-phenylindole; CMV, cytomegalovirus; TGFβ, transforming growth factor β; bp, base pairs.

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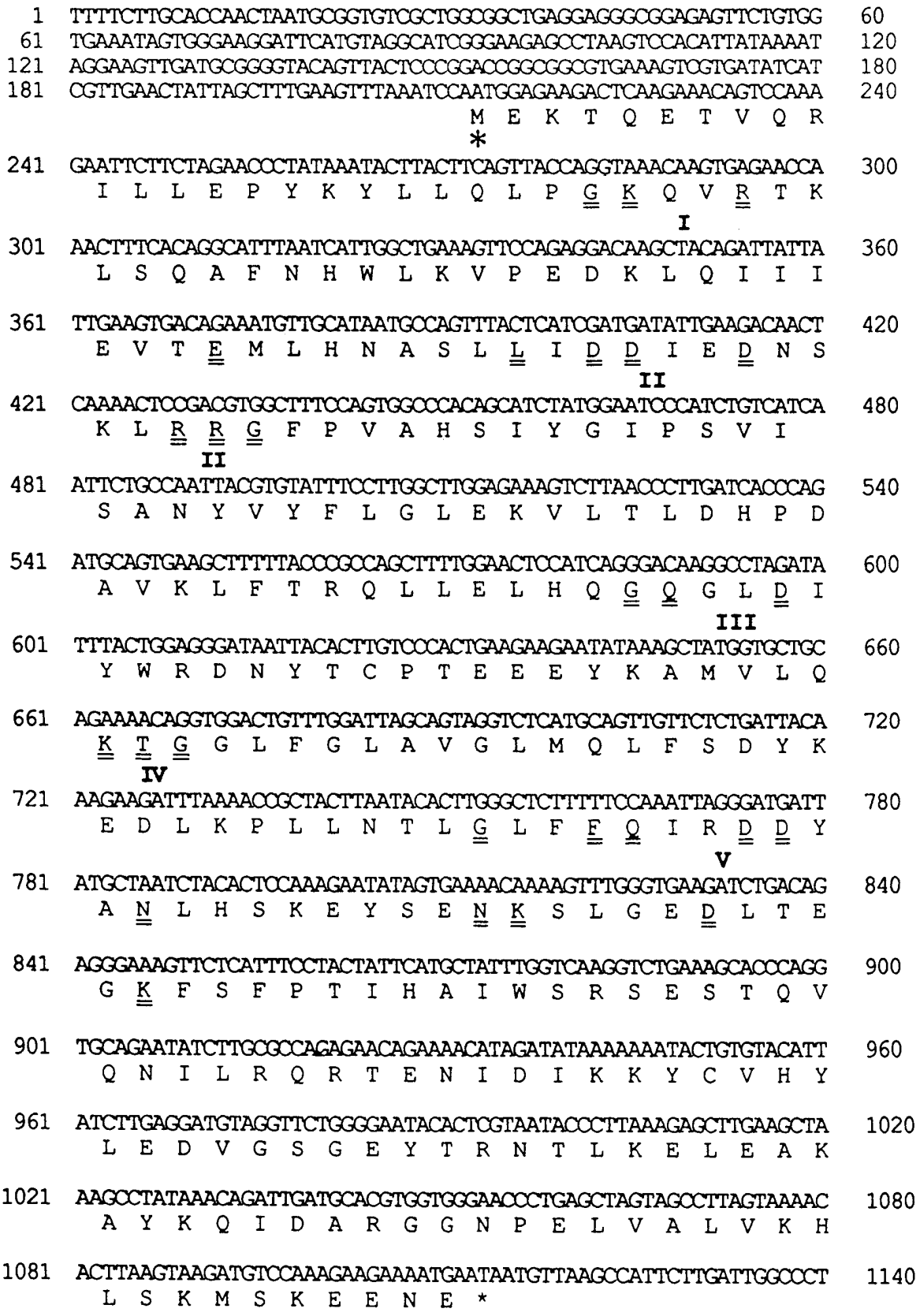

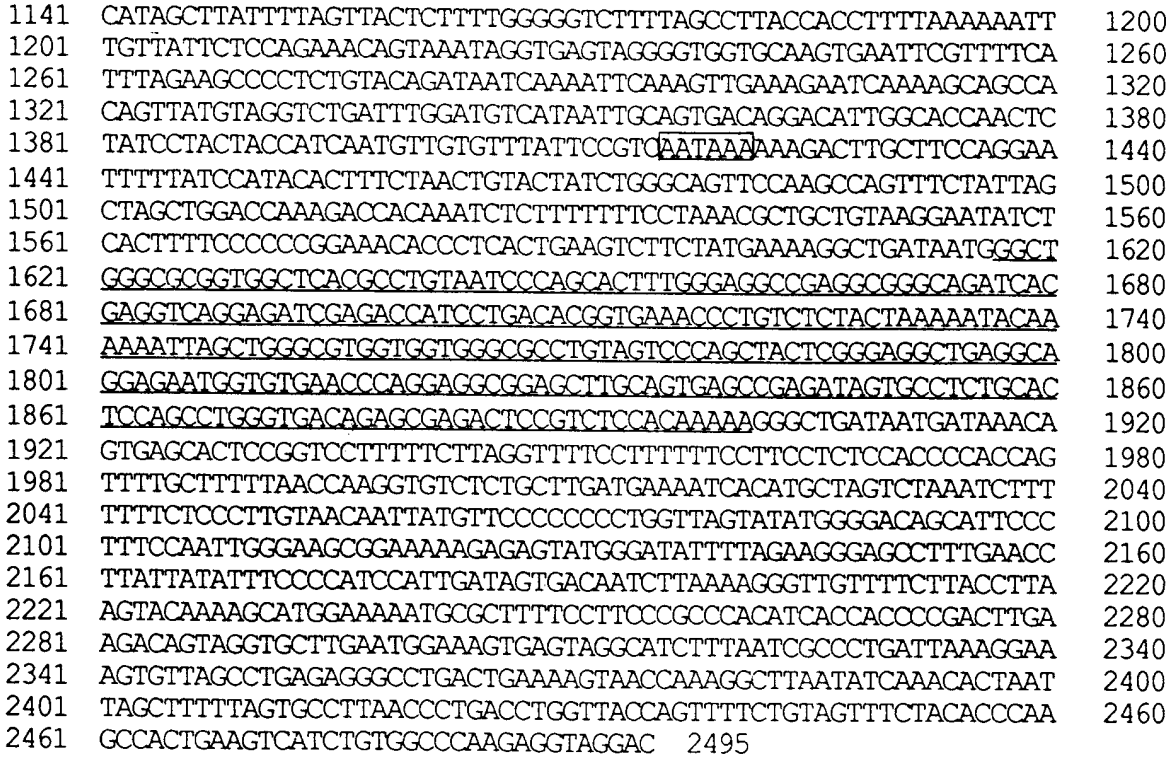

Fig. 1. The complete nucleotide sequence and predicted amino acid sequence of the human GGPP synthase cDNA. The nucleotide of the initiator methionine is designated (*); the 5′ untranslated region extends from nucleotide +1 to +212. The amino acid sequence is shown below the nucleotide sequence. Amino acids that correspond to those found in the five conserved domains, previously identified in other prenyltransferases (1, 9, 10, 20), are double underlined. The domains (**I–V**) are shown in bold type. An Alu repetitive element in the 3′ untranslated region (+1617 to +1901) is underlined. A putative polyadenylation signal in the 3′ untranslated region is boxed.

the identification of an additional five or six conserved domains that are present in many prenyltransferase members (1, 9, 10, 20).

The cDNA encoding GGPP synthase has been isolated from plants, bacteria, and yeast (21–25), but not from mammals. A bifunctional FPP synthase/GGPP synthase enzyme has been cloned from archaebacterium (26). The mammalian GGPP synthase protein was purified from bovine brain (27, 28) and, based on cell fractionation studies, appeared to be localized to the cytosol (27–29).

In the present studies we report the isolation of the full-length cDNA encoding human GGPP synthase. In addition, we report on the expression of GGPP synthase mRNA in different human tissues and on the chromosomal localization of the gene.

EXPERIMENTAL PROCEDURES

Materials

DNA restriction and modification enzymes were obtained from Gibco BRL. ^{32}P-labeled nucleotide triphosphates were obtained from Amersham Corp. Lipoprotein-deficient fetal calf serum was purchased from PerImmune. The sources of all other reagents and plasmids have been given (30–34).

Western blot analyses

HeLa cell protein (50 μg) or recombinant purified protein (5–50 ng) were resolved on 10% polyacrylamide-sodium dodecyl sulfate gels. After transfer of the proteins to nitrocellulose membranes, the Amersham ECL assay was used as described by the supplier to detect GGPP synthase. The affinity-purified GGPP synthase antibodies were used at 1:50 dilution.

Northern blot assay

Human adult tissue poly(A) blots (Clontech) were probed with a random primed ^{32}P-labeled DNA fragment containing the GGPP synthase coding region by hybridization overnight in Hybrisol (Oncor) at 65°C. The blots were washed in 2 × SSC/0.1%SDS and then again in 0.2 × SSC/0.1%SDS at 65°C for 20 min each, and exposed to film with an intensifying screen at −70°C.

HeLa cells were grown in media supplemented with 10% LPDS in the absence or presence of sterols (10 μg cholesterol and 1.0 μg 25-hydroxycholesterol per ml) or with 10% fetal bovine serum and mevinolin (5 μm). RNA was isolated and Northern blot analyses were performed as described (34). The radiolabeled probes contained either the coding sequence of GGPP synthase or human IPP isomerase. The latter cDNA was obtained by RT-PCR using RNA obtained from HepG2 cells.

Isolation of the human GGPP synthase cDNA

The sequence of the human GGPP synthase gene was detected by homology to the GGPP synthase gene of *Neurospora crassa* upon BLAST analysis (35) of human ESTs in the HGS/TIGR database. Clone HG25437 was obtained from a human fetal heart cDNA library produced in the UniZap XR vector (Stratagene), originally sequenced by the Institute for Genome Research (TIGR;36, 37). This clone was not full-length, but full-length cDNA clones (approximately 250 base pairs longer) were ob-

tained by screening the same human fetal heart library with a random primed ^{32}P-labeled fragment of HG25437 and determining the size of the inserts in positive clones by PCR, followed by DNA sequencing of the largest inserts. DNA sequencing was performed by directed primer walks using an ABI 373a automated sequencer.

Plasmids

GGPP synthase cDNA (nucleotides 213–1115, according to the numbering in Fig. 1) was cloned into pRSETB (Invitrogen), a plasmid that contains both T7 and polyhistidine tags, to produce pRSET-GGPS. GGPP synthase (nucleotides 213–1115) was also cloned into pCI-neo (Promega) to produce pCI-GGPS.

GGPP synthase expression in mammalian cells

Chinese hamster ovary cells, HEK-293, or HeLa cells were cultured as previously described (31–33). Cells were transiently transfected with pCI-GGPS using the MBS Transfection Kit (Stratagene) (33). The following day, the cells were washed twice with PBS and subsequently lysed in buffer A (25 mm HEPES, pH 7.0, 2 mm $MgCl_2$, 5 mm KF, 1 mm dithiothreitol, 1% n-octyl-β-glycopyranoside) supplemented with protease inhibitors (250 μg/ml leupeptin, 100 μm phenylmethylsulfonyl fluoride, 2 μg/ml aprotinin). Cell lysates were centrifuged at 20,000 *g* for 10 min and the activity of GGPP synthase was assayed in the soluble fraction, as described below. No activity was detected in the insoluble pellet (data not shown).

Expression and purification of recombinant GGPP synthase

The plasmid pRSET-GGPPS, containing full-length GGPP synthase with an N-terminal polyhistidine tag, was expressed in the *E. coli* strain BL21 (DE3; Novagen) and induced with IPTG (1 mm). After 4 h, cells were harvested by centrifugation and resuspended in half the original volume, using buffer A supplemented with protease inhibitors, sonicated, and the insoluble material was removed by centrifugation. Supernatants were used for nickel affinity chromatography as recommended by the supplier (Invitrogen). Recombinant GGPP synthase was eluted in buffer A containing 200 mm immidazole and dialyzed against 20 mm Tris-HCl, pH 8.0, 100 mm NaCl, and 10% glycerol. The purity of the eluted fractions was estimated after separation by SDS-PAGE and staining with Coomassie Blue. The activity of the eluted GGPP synthase fusion protein was estimated as described below.

GGPP synthase assay

GGPP synthase activity was assayed in a 20-μl reaction containing 25 mm HEPES, pH 7.0, 2 mm $MgCl_2$, 5 mm KF, 1 mm dithiothreitol, 0.5% n-octyl-β-glycopyranoside, 10 μm [^{3}H]IPP (0.125 Ci/mmol) and 10 μm of the indicated allylic isoprenoid diphosphate. The reactions were started by the addition of protein (5–200 μg for cell extracts and 2–200 ng for recombinant protein) and were allowed to proceed for 15 min at 37°C. The reactions were stopped by the addition of 2.0 ml n-butanol saturated with water, followed by extensive vortexing. After the addition of 1.0 ml 2 m KCl, the samples were vortexed once more and centrifuged at low speed in order to separate the organic and aqueous phases. The butanol phase was transferred to a new tube and an aliquot was removed for scintillation counting. To analyze the distribution of products, the remaining butanol phase was evaporated under N_2 and the residue was redissolved in 200 μl 5% n-octyl-β-glycopyranoside and dephosphorylated enzymatically as described (29). After dephosphorylation, the products were extracted with 3 ml ethyl ether–petroleum ether 1:1 and the extract was evaporated with N_2. For thin-layer chromatography (TLC), the dephosphorylated and extracted reaction products were applied to a silica gel-60 plastic sheet (Merck), developed with benzene–ethyl acetate 4:1 (solvent A). The dephosphorylated products were also analyzed by reverse-phase TLC using LCK-18 plates (Whatman) developed in acetone–water 7:1 (solvent B). The plates were subsequently sprayed with En^{3}Hance spray (NEN) and exposed to film at −80°C. The radioactive products were identified by comparison with unlabeled standards, visualized with iodine vapor.

Antibody production

Recombinant GGPP synthase, containing N-terminal T7 and polyhistidine (His_6) tags, was purified to homogeneity from *E. coli* extracts by nickel affinity chromatography as described (33). The purified protein migrated as a single band on denaturing SDS PAGE (see Fig. 2). Antibody to the purified protein was produced by Cocalico Biologicals, Inc. (PA). Purified protein (approximately 100 μg), together with Freund's complete adjuvant, was injected intradermally into two rabbits. Subsequently, 100 μg GGPP synthase protein in incomplete adjuvant was injected at 2-week intervals. Antisera that contained antibodies to GGPP synthase, as judged from Western blot analysis utilizing recombinant enzyme, were pooled.

An antigen affinity column was prepared by coupling recombinant purified GGPP synthase covalently to cyanogen bromide-activated Sepharose 4B, as per the instructions of the supplier (Pharmacia). Aliquots of the pooled antisera were diluted 1:10 in 10 mm Tris (pH 7.5), 0.5 m NaCl and applied to the affinity column. The column was washed extensively with 10 mm Tris (pH 7.5), and then 10 mm Tris (pH 7.5) containing 0.5 m NaCl (38). Bound antibodies were eluted after application of 10 bed volumes of 100 mm glycine (pH 2.5) (38). The eluted antibodies were collected in tubes containing 1 bed volume of 1 m Tris (pH 8.0) and stored at −70°C.

Fluorescent in situ hybridization

The GGPS cDNA was nick-translated using Digoxigenin-11-dUTP (Boehringer Mannheim) or Biotin-14-dCTP and fluorescence in situ hybridization was performed as detailed by Johnson, Singer, and Lawrence (39). Individual chromosomes were counterstained with DAPI and color digital images, containing both DAPI and gene signal, were recorded using a triple-band pass filter set (Chroma Technology, Inc, Brattleboro, VT) in combination with a cooled charge coupled-device camera (Photometrics, Inc, Tucson, AZ) and variable excitation wave length filters (40). Images were analyzed using the ISEE software package (Inovision Corp., Durham, NC).

RESULTS

Nucleotide sequence of human GGPP synthase cDNA

A full-length 2.495 kb cDNA clone encoding GGPP synthase was isolated from a human fetal heart library. **Figure 1** shows the complete nucleotide sequence containing 900 bp of coding sequence starting at nucleotide +213. The cDNA contains 212 bp of 5′ untranslated sequence and 1380 bp of 3′ untranslated sequence. In Fig. 1 the amino acids (double underlined) corresponding to five conserved domains (**I–V**), previously identified in many prenyltransferases (1, 9, 10, 20), are shown. In domain V, both the human (Fig. 1) and *Neurospora crassa* (25) GGPP synthase cDNAs encode asparagines at the sixth and seventh conserved amino acids.

Nearly all other prenyltransferases have a conserved aspartate and glycine, respectively, at these positions (1). An Alu repetitive element in the 3′ untranslated portion of the cDNA is shown (Fig. 1, underline).

Original homology analyses of the coding sequence by the use of the BLAST program indicated highest identity with GGPP synthase from *Neurospora crassa* (25); the identity being 55% over 266 amino acids (nucleotides 242–1039).

The cDNA clone has one potential polyadenylation signal (Fig. 1; boxed nucleotides +1416 to +1421) that would account for the smaller mRNA identified by Northern blot analyses (see below). However, it seems likely that we have not isolated the true 3′ end of the mRNA as the 2,495 bp cDNA does not contain a second polyadenylation site and also hybridizes to a second mRNA of 3.5 kb (see below).

Enzymatic activity of recombinant GGPP synthase

In order to confirm that the isolated cDNA encoded the human GGPP synthase enzyme, nucleotides 213 to 1115 were subcloned into pSETB. Expression of this plasmid was expected to produce a full length GGPP synthase protein containing an amino terminal extension of 6 histidines and an additional 20 amino acids. *Escherichia coli*, transformed with this latter plasmid and induced with IPTG (1 mm), synthesized a protein of the predicted molecular weight (**Fig. 2**, left). This protein was absent from uninduced cells (Fig. 2, left). The IPTG-induced protein bound to a nickel affinity column and was subsequently eluted in an apparently homogeneous form (Fig. 2, right, fractions 2–4).

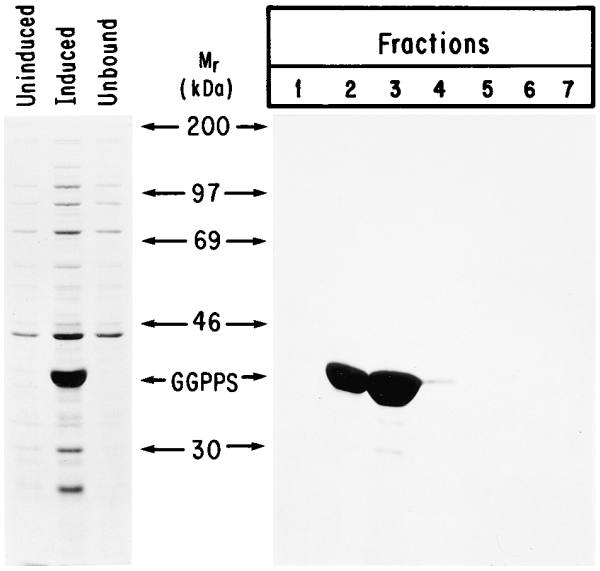

Fig. 2. Expression and purification of recombinant histidine-tagged GGPP synthase. *E. coli* were transformed with the pRSETB plasmid containing nucleotides 213 to 1115, encoding the complete open reading frame of the putative GGPP synthase. The cells were grown for 16 h and then an additional 4 h in the absence (uninduced) or presence (induced) of IPTG. Cell lysates were prepared as described in Experimental Procedures. The lysate, obtained from the IPTG-induced cells, was applied to a nickel affinity column and the unbound material was saved. The bound material was eluted in the imidazole-containing buffer and seven 1-ml fractions were collected, as described by the manufacturer. Fifty μg of the uninduced, induced, and unbound samples and equal volumes (5 μl) of each eluted fraction were analyzed on SDS-PAGE and the gels were stained with Coomassie Blue. The migration of GGPP synthase (GGPPS) is indicated.

The capacity of the histidine-tagged affinity-purified protein to catalyze condensation of radiolabeled IPP with various allylic diphosphates is shown in **Fig. 3**. The products of the reaction were dephosphorylated in order to allow separation and identification on TLC. The recombinant protein synthesized all-*trans*-GGPP in the presence of FPP + IPP > GPP + IPP >> DMAPP + IPP (Fig. 3A) in a reaction that was linear between 1 and 400 ng recombinant protein per assay (data not shown). The recombinant protein did not synthesize detectable levels of *trans, trans, cis*-GGPP (Fig. 3A), GPP or FPP (Fig. 3B). These results demonstrate that the histidine-tagged recombinant purified protein has the enzymatic properties associated with GGPP synthase, namely the synthesis of *all-trans* GGPP, resulting from the condensation of IPP with the preferred allylic substrate FPP.

Expression of GGPP synthase in mammalian cells

Chinese hamster ovary cells were transfected with plasmids encoding GGPP synthase, either in the sense or antisense orientation, under the control of a CMV-derived promoter. Twenty-four hours after transfection, the cells were lysed in buffer containing low levels of n-octyl-β-glucopyranoside and the activity of GGPP synthase was determined. Cells transfected with a plasmid containing GGPP synthase in the sense orientation exhibited a 55-fold increase in GGPP synthase enzymatic activity in the

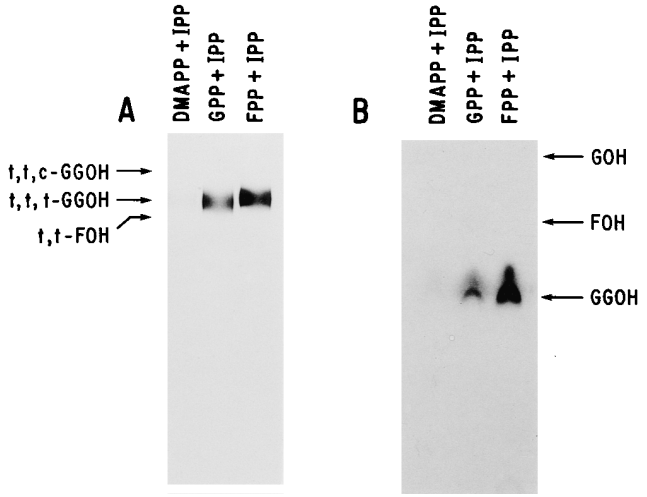

Fig. 3. Enzymatic activity of recombinant GGPP synthase. Recombinant GGPP synthase protein was purified as described in the legend to Fig. 2. Purified protein (5 ng) was assayed for prenyltransferase activity using [^{3}H]IPP and the indicated allylic diphosphate, as described in Materials and Methods. The butanol-extractable products were dephosphorylated and analyzed by thin-layer chromatography using two different solvent systems as described in Experimental Procedures. In A the solvent was benzene–ethyl acetate 4:1 (v/v) (29). In B the solvent was acetone–water 7:1 (v/v). The relative migration of the unlabeled internal standards, visualized by staining with iodine, is indicated.

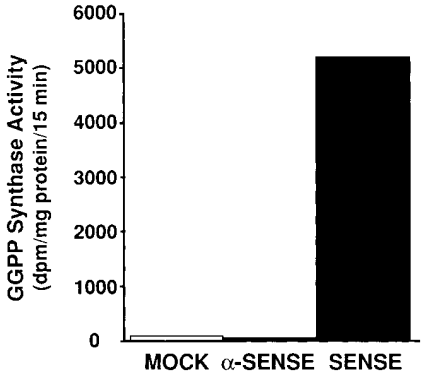

Fig. 4. Transient transfection of CHO cells with a plasmid encoding the putative human GGPP synthase cDNA results in increased GGPP synthase activity. CHO cells were mock transfected or transiently transfected with a plasmid containing the putative GGPP synthase cDNA (nucleotides 213 to 1115) in either the sense or antisense (α-sense) orientation, as indicated. After 24 h, the cells were lysed and the GGPP synthase activity (dpm product formed/mg protein/15 min) was determined, as described in Experimental Procedures. The results are representative of two experiments performed in triplicate.

soluble fraction as compared to mock transfected or antisense (α-sense) transfected cells (**Fig. 4**). The transfection efficiency was similar for both sense and antisense plasmids (data not shown). The data shown in Fig. 4 provide further support for the proposal that the isolated cDNA encodes GGPP synthase.

Western blot assay of GGPP synthase

Taken together, the results shown in Figs. 1–4 indicate that the isolated cDNA encodes human GGPP synthase. Western blot analyses indicated that the anti-GGPP synthase antibodies recognized both recombinant GGPP synthase, 36.6 kDa (data not shown), and a single 34 kDa protein present in crude HeLa cell homogenates (**Fig. 5**). Under these electrophoretic conditions, the endogenous cellular protein that reacted with the antisera migrated slightly faster than the recombinant GGPP synthase protein (data not shown), presumably as a result of the 26 additional amino acids at the amino terminus of the recombinant protein.

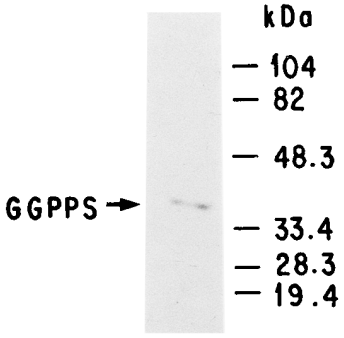

Fig. 5. Specificity of the affinity-purified antibodies to GGPP synthase. HeLa cell extract (100 μg) was analyzed by SDS-PAGE, the proteins were transferred to a nitrocellulose membrane, and the membrane was incubated with affinity-purified anti GGPP synthase antibodies (1:50 dilution) as described in Experimental Procedures. The migration of the GGPP synthase (GGPPS) and the molecular weight standards are shown.

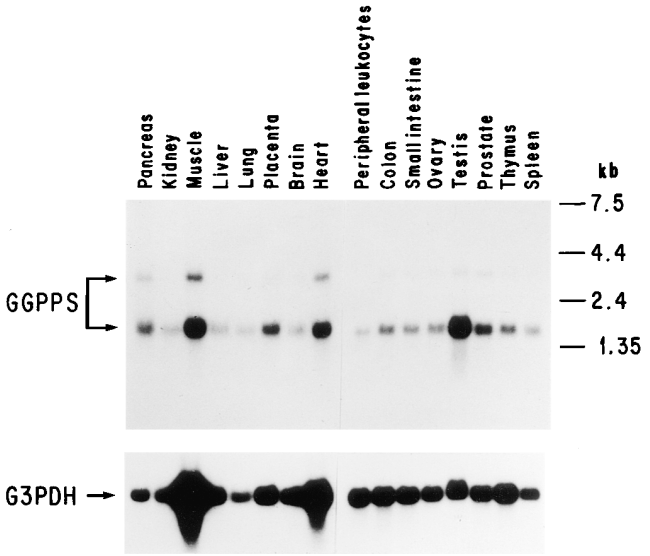

Fig. 6. Tissue expression of GGPP synthase mRNA. A human mRNA organ blot was hybridized to a radioactive GGPP synthase probe as described in Experimental Procedures. The probe did not contain the Alu sequence noted in Fig. 1. The blot was re-hybridized with glycerol-3-phosphate dehydrogenase (G3PDH) in order to normalize the results for variations in mRNA loading in the different lanes. The positions of GGPP synthase mRNA (GGPPS) and control DNA fragments (kb) are indicated.

Tissue expression and regulation

All sixteen human tissues examined contain two GGPP synthase mRNAs of approximately 3.5 and 1.7 kb (**Fig. 6**). Of these tissues, GGPP synthase mRNA is most abundant in the testis; all other tissues express similar levels of GGPP synthase mRNA after the blot was normalized for differential loading of RNA (Fig. 6).

Figure 7 shows that Northern blot analysis of 10 μg total RNA, isolated from HeLa cells, identifies one GGPP synthase mRNA of 1.7 kb. The GGPP synthase mRNA level in HeLa cells was unaffected when the cells were incubated in media supplemented with either 10% lipoprotein-deficient sera in the absence or presence of sterols or with 10% fetal bovine sera and mevinolin (5 μm). In contrast, the mRNA levels for IPP isomerase (Fig. 7) and FPP synthase (data not shown) were induced significantly when cells were incubated in the presence of media supplemented with either 10% LPDS or 10% FBS and mevinolin as compared to media supplemented with either 10% LPDS plus sterols or 10% fetal bovine sera in the absence of mevinolin, respectively. These latter results are consistent with sterol-regulated expression of FPP synthase (31, 32) and IPP isomerase transcripts. Essentially identical results were observed when the same experiments were repeated with HEK293 cells (data not shown). Recently, Paton, Shakelford, and Krisans (41) demonstrated that the expression of rat hepatic IPP

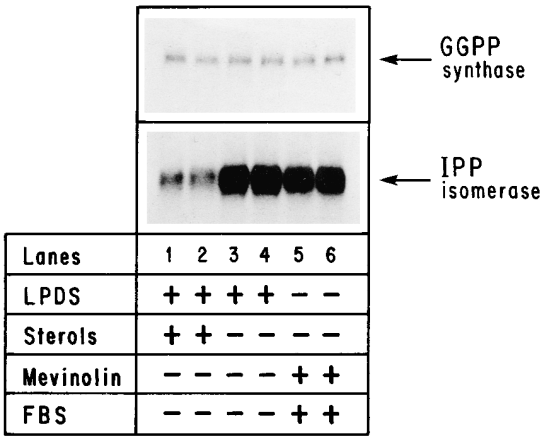

Fig. 7. Human GGPP synthase mRNA levels are unaffected by changes in cellular sterols. HeLa cells were incubated for 36 h in media supplemented with 10% lipoprotein-deficient sera (LPDS) in the absence or presence of sterols (10 μg cholesterol and 1.0 μg 25-hydroxycholesterol per ml media) or with 10% fetal bovine sera (FBS) and 5 μm mevinolin, as indicated. RNA was isolated from duplicate dishes and 10 μg total RNA was analyzed as described under Experimental Procedures. The filter was hybridized with a probe to GGPP synthase (nucleotides 213 to 1115), exposed to film, stripped, and rehybridized with a probe to human IPP isomerase. The IPP isomerase probe was generated by RT-PCR using HepG2 mRNA and primers that produced full-length human IPP isomerase cDNA (47). Equal loading of RNA in all lanes was observed when the gel was stained with ethidium bromide (data not shown).

isomerase mRNA was regulated in response to diets; the mRNA levels decreased after addition of cholesterol to the diet and increased when the animals were fed diets supplemented with hypolipidemic drugs (cholestyramine and lovastatin).

Chromosomal localization of the GGPP synthase gene

To determine the precise chromosomal location of the human GGPP synthase gene we performed single-copy gene fluorescence in situ hybridization to normal human metaphase chromosome spreads (42). Approximately 9 spreads were analyzed by eye, most of which had a doublet signal characteristic of genuine hybridization on at least one chromosome 1. Doublet signal was not detected on any other chromosome. Detailed analysis of 12 individual chromosomes, using fluorescence banding combined with high-resolution image analysis, indicated that the GGPP synthase gene is positioned within band 43 (1q43) (**Fig. 8**). Double FISH labeling indicated that GGPP synthase mapped distal to TGFβ (1q32.1–1q32.3) (data not shown).

DISCUSSION

In the current studies we identify a cDNA encoding human GGPP synthase. The identity of the cDNA is based on the GGPP synthase enzymatic activity of the recombinant protein, on the increased GGPP synthase activity in mammalian cells transiently transfected with this putative GGPP synthase cDNA, and on the presence of a number of conserved motifs, including two aspartate-rich domains, in the open reading frame encoded by the cDNA.

GGPP synthase activity is necessary for the synthesis of the 20 carbon isoprenoid GGPP. In mammals, GGPP serves as a substrate for two enzymes, GGTase-I and GGTase-II, which in turn transfer geranylgeranyl to a cysteine at or near the carboxy terminus of acceptor proteins (43). These geranylgeranylated proteins include many small G

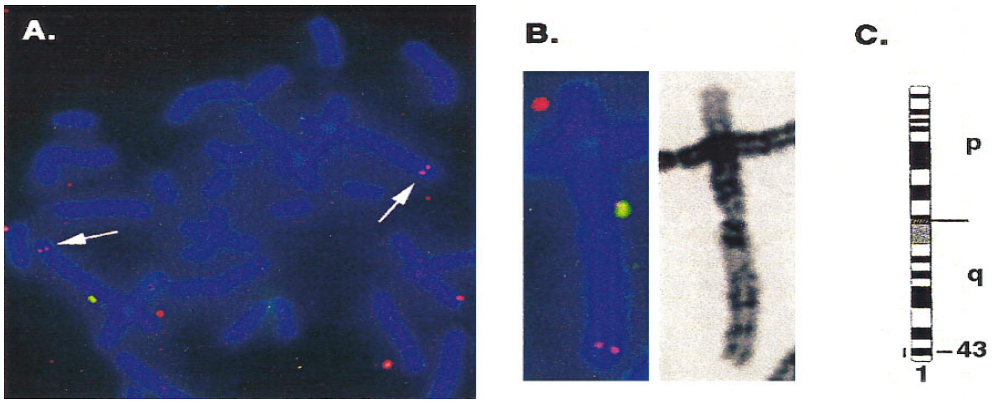

Fig. 8. Fluorescence in situ hybridization mapping of the GGPP synthase gene. The GGPP synthase gene cDNA was hybridized to normal human male chromosomes. (A) A chromosome spread from a single cell showing hybridization to the q arm of each chromosome 1 (arrows). (B) Image of a single chromosome 1 with hybridization signal. The left panel shows a DAPI-stained chromosome (blue) with gene signal (red). The right panel shows the same DAPI-stained chromosome displayed as an inverse black and white image. The g-band-like DAPI pattern was enhanced using an edge-sharpening algorithm (C) idiogram of chromosome 1 showing the positions of the GGPP synthase gene.

proteins (members of the rac, rho, and rab families) as well as other important signaling molecules (43, 44). Recently, it was shown that N-ras and K-ras are also modified by geranylgeranylation when cells are treated with an inhibitor of farnesyl:protein transferase (45). Such a finding implies that inhibitors of farnesyl:protein transferase may be incapable of preventing the oncogenic effects of activated N- or K-ras, but may be effective against activated H-ras.

Until recently, the function of GGPP in mammalian cells was thought to be limited to the modification (prenylation) of certain proteins. However, Ohizumi et al. (46) noted that addition of geranylgeraniol, the dephosphorylated form of GGPP, to cells promoted apoptosis. More recently, Polverino and Patterson (3) demonstrated that geranylgeraniol stimulates apoptosis by a process that is both rapid and involves activation of the protease caspase-3. This latter observation could account for our inability to isolate cells that stably express GGPP synthase enzymatic activity (data not shown). We hypothesize that overexpression of the GGPP synthase enzyme results in cell death as a result of geranylgeraniol-induced apoptosis.

Other studies have demonstrated that farnesol, but not geranylgeraniol or other isoprenoid alcohols, functions as a signaling molecule in two other intracellular pathways; farnesol stimulated the degradation of HMG-CoA reductase (5) and activated the farnesoid receptor:retinoid X receptor transcription factor complex (6). Thus, dephosphorylated forms of both FPP and GGPP may represent a new class of lipid signaling molecules that are biologically active in mammalian cells. The availability of the mammalian GGPP synthase cDNA should permit more detailed studies on the role of this enzyme in regulating both prenylation and apoptosis.

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