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Cloning, Analysis, and Bacterial Expression of Human Farnesyl Pyrophosphate Synthetase and Its Regulation in Hep G2 Cells

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ABSTRACT: A partial length cDNA encoding farnesyl pyrophosphate synthetase (hpt807) has been isolated from a human fetal liver cDNA library in λgt11. DNA sequence analysis reveals hpt807 is 1115 bp in length and contains an open reading frame coding for 346 amino acids before reaching a stop codon, a polyadenylation addition sequence, and the first 14 residues of a poly(A+) tail. Considerable nucleotide and deduced amino acid sequence homology is observed between hpt807 and previously isolated rat liver cDNAs for farnesyl pyrophosphate synthetase. Comparison with rat cDNAs suggests that hpt807 is about 20 bp short of encoding the initiator methionine of farnesyl pyrophosphate synthetase. The human cDNA was cloned into a prokaryotic expression vector and *Escherichia coli* strain DH5αF'IQ was transformed. Clones were isolated that express an active fusion protein which can be readily observed on protein gels and specifically stained on immunoblots with an antibody raised against purified chicken farnesyl pyrophosphate synthetase. These data confirm the identity of hpt807 as encoding farnesyl pyrophosphate synthetase. Slot blot analyses of RNA isolated from Hep G2 cells show that the expression of farnesyl pyrophosphate synthetase mRNA is regulated. Lovastatin increases mRNA levels for farnesyl pyrophosphate synthetase 2.5-fold while mevalonic acid, low-density lipoprotein, and 25-hydroxycholesterol decrease mRNA levels to 40–50% of control values.

Farnesyl pyrophosphate synthetase catalyzes the sequential condensation of isopentenyl pyrophosphate with the allylic pyrophosphates, dimethylallyl pyrophosphate, and then with the resultant geranyl pyrophosphate as shown in Scheme I.

The ultimate product of these two reactions, farnesyl pyrophosphate, is utilized in the synthesis of squalene, cholesterol, and other sterols. Farnesyl pyrophosphate synthetase has been purified from many eukaryotic sources including yeast (Eberhardt & Rilling, 1975) and liver tissue of chickens (Reed & Rilling, 1975), rats (V. Ding, unpublished results), and humans (Barnard & Popjak, 1981). The enzyme has a cytosolic localization and is found as a dimer consisting of two indistinguishable subunits of molecular weight between 38 000 and 42 000.

Farnesyl pyrophosphate synthetase is but one of a yet unknown number of enzymes generically referred to as prenyltransferases that catalyze the addition of isoprene units to a pyrophosphate primer. The microsomal enzyme dolichol phosphate synthetase or *cis*-prenyltransferase adds isopentenyl units in a cis configuration to farnesyl pyrophosphate to synthesize dolichyl phosphate, the long-chain polyisoprenoid involved in asparagine-linked glycoprotein synthesis (Adair et al., 1984). Farnesyl pyrophosphate also may be the primer utilized by a mitochondrial *trans*-prenyltransferase in the synthesis of the polyisoprenoid side chain of ubiquinone, the electron carrier in the electron transport chain of mitochondria (Nambudiri et al., 1980). To date, the prenyltransferases involved in dolichol and ubiquinone synthesis have not been purified or characterized sufficiently to know what similarities, if any, exist between them and farnesyl pyrophosphate synthetase. Likewise, relatively few details are known about the synthesis of the farnesyl moiety of the heme *a* prosthetic group of cytochromes *a* and a_3 (Weinstein et al., 1986) or the prenylation of proteins (Sinensky & Logel, 1985; Breunger

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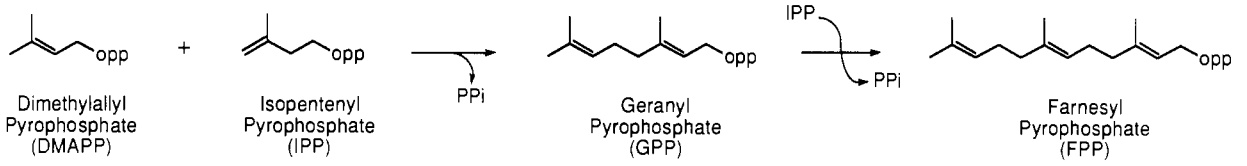
Scheme I



& Rilling, 1986) and tRNAs (Faust et al., 1980).

In animal cells, the rate-limiting enzyme in cholesterol biosynthesis is 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA)[1] reductase. The activity of this enzyme is regulated by a complex array of transcriptional and posttranscriptional controls. Sterol products are known to repress transcription (Osborne et al., 1985) and accelerate degradation of HMG-CoA reductase (Chin et al., 1985), while nonsterol components of this regulatory mechanism suppress HMG-CoA reductase activity by enhancing degradation of the enzyme and inhibiting translation of reductase mRNA (Nakanishi et al., 1988). Similar to the gene for HMG-CoA reductase (Osborne et al., 1985), genes for HMG-CoA synthase (Mehrabian et al., 1986; Gil et al., 1985) and LDL receptor (Sudhof et al., 1987a,b) are transcribed actively when cells are denied cholesterol and are repressed when sterol products accumulate. Evidence exists which suggests that other enzymes involved in cholesterol synthesis might be coordinately regulated in response to cholesterol availability (Chang & Limanek, 1980).

The potential role of farnesyl pyrophosphate as a branch-point intermediate in the synthesis of sterols, dolichol, ubiquinone, and other isoprenylated cellular metabolites has led us to study the gene expression of human liver farnesyl pyrophosphate synthetase. As such, we report the isolation of a nearly full length human fetal liver cDNA and the bacterial expression of a fusion protein that exhibits substantial farnesyl pyrophosphate synthetase activity. The human cDNA bears striking resemblance to rat liver cDNAs for farnesyl pyrophosphate synthetase, one reported earlier (Clarke et al., 1987) and recently identified (Ashby & Edwards, 1989) and others isolated in our laboratory by polysome immunoabsorption (V. Ding, unpublished results). Additionally, we show that the expression of farnesyl pyrophosphate synthetase mRNA is regulated by lovastatin, low-density lipoprotein, 25-hydroxycholesterol, and mevalonate, which are all agents known to regulate cholesterol synthesis in animal cells.

Experimental Procedures

Screening of a Bacteriophage λgt11 Library. A human fetal liver cDNA library constructed in λgt11 was obtained from Clontech Laboratories (Palo Alto, CA). The library contained 2.3×10^5 recombinants and was screened according to standard DNA cloning protocols (Maniatis et al., 1982). Two partial length rat liver cDNA clones encoding farnesyl pyrophosphate synthetase (pPRT118 and pPRT241, V. Ding, unpublished results) were isolated by polysome immunoabsorption and used as DNA probes. These rat cDNAs are 464 bp (5′-end) and 563 bp (3′-end) in length, respectively, are not overlapping clones, and code for all but 80 bp of coding sequence for rat liver farnesyl pyrophosphate synthetase. Positive clones from the human library were purified, and their DNA inserts were isolated from λ DNA after *Eco*RI digestion and subcloned into the *Eco*RI site of the phagemid vector pTZ18 (Pharmacia, Piscataway, NJ) for DNA sequence analysis.

DNA Sequence Analysis. All DNA sequences were determined by primer extension on pTZ18 single-stranded template DNA using the dideoxy chain termination method (Sanger et al., 1977). Synthetic oligodeoxynucleotides were made by phosphoramidite chemistry on a Biosearch Model 8700 DNA synthesizer (Milligen/Biosearch, Burlington, MA) and used as primers for nucleotide sequence analysis. DNA sequences were analyzed with Intelligenetics software.

Preparation of Probes for Blot Analyses. Human hpt807 cDNA was isolated from pTZ18 after digestion with *Eco*RI, agarose gel electrophoresis, and electroelution. The 1115-bp cDNA was used to probe Northern and Southern transfers as described below. Additionally, Southern blots were also probed with the 5′-end 325-bp and the 3′-end 331-bp portions of the cDNA. These fragments were isolated after digestion of the cDNA with *Bgl*II (5′-probe) or *Pvu*II (3′-probe). All DNA fragments used as probes were random primer labeled according to the manufacturer's specifications (Pharmacia, Piscataway, NJ) with [α-^{32}P]dCTP (3000 Ci/mmol, New England Nuclear, Boston, MA) to a specific activity of up to 1×10^9 dpm/μg of DNA.

Northern Blot Analysis. Human liver poly(A+) RNA was purchased from Clontech Laboratories (Palo Alto, CA). Total RNA was extracted from Hep G2 cells by the method of Chirgwin et al. (1979). Poly(A+) RNA was isolated by one cycle of chromatography on oligo(dT)–cellulose (Collaborative Research, Waltham, MA). RNA samples (2.5 μg) were resolved in 2% formaldehyde/1% agarose gels and transferred to Zeta-Probe nylon membranes (Bio-Rad Laboratories, Rockville Centre, NY) by capillary action according to instructions provided by the supplier. Hybridizations were carried out in 5× SSPE, 5× Denhardt's reagent, 0.1% (w/v) SDS, 200 μg/mL salmon sperm DNA, and 50% (v/v) formamide at 42 °C. Blots were washed twice for 15 min in 2× SSC/0.1% SDS at room temperature followed by 15-min washes in 1× SSC/0.1% SDS at room temperature and at 50 °C.

Slot Blot Analyses of Hep G2 mRNA. Hep G2 cells were seeded in 60-mm culture dishes and grown to approximately 85% confluency in Eagle's MEM containing 10% (v/v) fetal bovine serum. The medium was removed, and cells were washed three times with cold phosphate-buffered saline and fresh medium containing 10% delipidated serum (Goldstein et al., 1983) plus the test compound dissolved in the appropriate vehicle. After 24 h the medium was removed, the cells were washed three times with cold phosphate-buffered saline, and total cellular RNA was isolated by extraction with guanidine isothiocyanate. RNA samples were applied directly to nitrocellulose filters with a slot blot apparatus according to the manufacturer's specifications (Schleicher and Schuell). Nitrocellulose filters were hybridized and washed as described under Northern Blot Analysis above. For each treatment group, a range of RNA concentrations (10–40 μg) was analyzed. Autoradiography and scanning densitometry (Bio-Rad Model 620 scanning densitometer, Bio-Rad Laboratories, Rockville Centre, NY) revealed a linear relationship between

[1] Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; LDL, low-density lipoprotein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.

optical density of the hybridization signal and the amount of RNA analyzed. The data have been normalized for actin content and are expressed as the percent of control values (i.e., cells incubated in the presence of 10% delipidated serum plus vehicle).

Southern Blot Analysis. Ten micrograms of human genomic DNA (Clontech Laboratories, Palo Alto, CA) was digested over a 24-h period with two additions of the appropriate restriction endonuclease (Boehringer Mannheim Biochemicals, Indianapolis, IN). The first addition of enzyme contained 30 units of activity followed by a second addition of 15 units 20 h later. The digested DNA samples were resolved by electrophoresis in 0.7% agarose gels and transferred by capillary action to Zeta-Probe nylon membranes as described by the manufacturer (Bio-Rad Laboratories, Rockville Centre, NY). Hybridizations were carried out in the presence of 5× SSC, 1× Denhardt's reagent, 0.02 M sodium phosphate, pH 6.7, 10% (w/v) dextran sulfate, 100 μg/mL salmon sperm DNA, and 50% (v/v) formamide at 42 °C. Blots were washed twice for 15 min at room temperature in 2× SSC/0.1% (w/v) SDS and once in 0.5× SSC/0.1% SDS followed by 15-min washes in 0.1× SSC/0.1% SDS at room temperature and 50 °C.

Expression in Escherichia coli. The 1115-bp hpt807 cDNA was isolated from pTZ18 after *Eco*RI restriction endonuclease digestion, and the fragment was ligated into the *Eco*RI site of the bacterial expression vector pUC18. The cDNA was cloned in both orientations relative to the direction of *E. coli* β-galactosidase transcription. One orientation, placz-hpt(+), encodes a fusion protein containing the initiator methionine and the first six amino acid residues of β-galactosidase followed in frame by 346 amino acids of human farnesyl pyrophosphate synthetase. The other orientation, placz-hpt(−), has the 1115-bp cDNA cloned into pUC18 in the opposite orientation relative to placz-hpt(+). *E. coli* strain DH5αF'IQ control cells (not transformed) or cells transformed with the placz-hpt constructs were grown in 20 mL of LB medium to an OD_{660} of 0.7. Isopropyl β-D-thiogalactopyranoside was added to a final concentration of 1 mM, and after 4 h the cells were pelleted, washed with phosphate-buffered saline, and resuspended in 0.5 mL of the same buffer. The cell suspensions were subjected to five rounds of freezing (−70 °C) and thawing prior to centrifugation at 12000*g* to remove unbroken cells. The supernatants were frozen in aliquots for protein determination (Lowry et al., 1951), SDS–PAGE (Laemmli, 1970), and farnesyl pyrophosphate synthetase assays as described below.

Western Blot Analysis. Twenty five micrograms of protein from *E. coli* cell extracts was resolved by SDS–PAGE and transferred to Biotrace nitrocellulose (Gelman Sciences Inc., Ann Arbor, MI) by using a Trans-Blot Cell apparatus according to the manufacturer's instructions (Bio-Rad Laboratories, Rockville Centre, NY). Filters were incubated sequentially with a polyclonal antibody preparation (1:200 dilution) raised against purified chicken liver farnesyl pyrophosphate synthetase (V. Ding, unpublished results) and goat anti-rabbit IgG alkaline phosphatase conjugate. The protein was visualized by the addition of substrate as described by the supplier (Bio-Rad Laboratories).

Farnesyl Pyrophosphate Synthetase Assay. Farnesyl pyrophosphate synthetase activity was measured by a modification of the method described by Reed and Rilling (1975). Assays were performed in 20 mM Tris-HCl, pH 7.6, containing 1 mM $MgCl_2$, 1 mM dithiothreitol, 100 μM geranyl pyrophosphate, and 100 μM isopentenyl pyrophosphate (^{14}C, 10 μCi/μmol). Reaction mixtures (45 μL) were preincubated at 37 °C for 2 min prior to addition of bacterial cell extracts (5 μL containing 2.5–5.0 μg of protein). After 5 min the reactions were terminated by addition of 500 μL of saturated NaCl, and radiolabeled products were extracted into 500 μL of 1-butanol and quantitated by liquid scintillation counting. Farnesyl pyrophosphate synthetase activities are expressed as nanomoles of product formed per minute per milligram of protein.

HPLC Analysis. Farnesyl pyrophosphate synthetase assays were performed as described above except reactions were terminated by quick freezing samples in liquid nitrogen. The samples were later thawed, and the radiolabeled species present in the reaction mixtures were resolved by anion-exchange HPLC on a Supelcosil-LC-SAX column (Supelco Inc., Bellefonte, PA). Chromatography was carried out in buffer A (0.1 M potassium phosphate, pH 3.7, 20% acetonitrile, 2% tetrahydrofuran) for 11 min, followed by a gradient from 100% buffer A to 100% buffer B (0.3 M potassium phosphate, 20% acetonitrile, 5% tetrahydrofuran) over a 1-min period followed by chromatography for 13 min in buffer B. Products were identified by comparison of their retention times with those of authentic standards.

Results and Discussion

Isolation and Characterization of Human Farnesyl Pyrophosphate Synthetase cDNA Clones. Approximately 1.2×10^6 plaques of a human fetal liver cDNA library in bacteriophage λgt11 were screened with a mixture of two partial length rat liver farnesyl pyrophosphate synthetase cDNAs, pPRT118 and pPRT241. These cDNAs contain sequences virtually identical with CR39 (Clarke et al., 1987), a cholesterol-repressible rat liver cDNA recently identified as encoding farnesyl pyrophosphate synthetase (Ashby & Edwards, 1989). Nine hybridization-positive bacteriophage plaques were purified. The cDNA clone that contained the most coding sequence information, hpt807, was isolated, and its characterization is described below.

The restriction map of hpt807 and the sequencing strategy employed are shown in Figure 1. Analysis of the complete nucleotide sequence of the 1115-bp cloned cDNA revealed that it contained one open reading frame encoding 346 amino acids. The other potential reading frames were interrupted by multiple termination codons. As shown in Figure 2, the 3'-untranslated region of the human cDNA contains a polyadenylation addition sequence (AATAAA) located 15 nucleotides upstream from a poly (A+) track. The 5'-end of hpt807 does not contain an in-frame methionine residue, indicating that the cDNA does not contain the entire coding sequence of farnesyl pyrophosphate synthetase.

There is considerable nucleotide sequence homology between hpt807 and the rat cDNAs used to isolate it. The cDNA probe pPRT118 spans the 5'-end of hpt807 to nucleotide 421, while pPRT241 extends from nucleotide 505 to the 3'-end of the human cDNA. There is 85% DNA sequence homology between hpt807 and CR39 starting at the 5'-end of the human cDNA and extending 1008 bp (Figure 2, arrow). The recently identified yeast farnesyl pyrophosphate synthetase gene also has regions of homology with hpt807 (Anderson et al., 1989). The homology between the amino acid sequences deduced from hpt807 and CR39 cDNAs is shown in Figure 3. Greater than 88% identity was found between the two sequences beginning at the N-terminal aspartate residue of the human sequence extending 314 amino acids to a serine residue (Figure 3, arrow). Since the rat and human liver farnesyl pyrophosphate synthetases are about the same molecular weight, and the

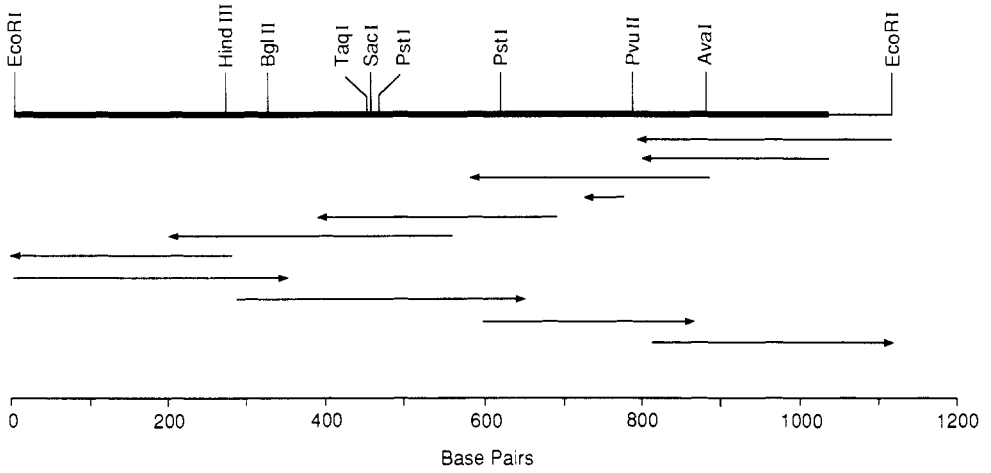


FIGURE 1: Restriction map of farnesyl pyrophosphate synthetase cDNA and the strategy for nucleotide sequence determination. Human hpt807 cDNA is shown schematically with the protein coding sequence indicated by the thick solid line and the 3′-untranslated sequences represented by the thin solid line. The arrows beneath the cDNA show the direction and length of each sequence determined by independent experiments.

```
                                                    28                                                  55
A  GAT GTT TAT GCC CAA GAA AAG CAG GAT TTC GTT CAG CAC TTC TCC CAG ATC GTT
   Asp Val Tyr Ala Gln Glu Lys Gln Asp Phe Val Gln His Phe Ser Gln Ile Val

                                                    82                                                  109
   AGG GTG CTG ACT GAG GAT GAG ATG GGG CAC CCA GAG ATA GGA GAT GCT ATT GCC
   Arg Val Leu Thr Glu Asp Glu MET Gly His Pro Glu Ile Gly Asp Ala Ile Ala

                                                   136                                                  163
   CGG CTC AAG GAG GTC CTG GAG TAC AAT GCC ATT GGA GGC AAG TAT AAC CGG GGT
   Arg Leu Lys Glu Val Leu Glu Tyr Asn Ala Ile Gly Gly Lys Tyr Asn Arg Gly

                                                   190                                                  217
   TTG ACG GTG GTA GTA GCA TTC CGG GAG CTG GTG GAG CCA AGG AAA CAG GAT GCT
   Leu Thr Val Val Val Ala Phe Arg Glu Leu Val Glu Pro Arg Lys Gln Asp Ala

                                                   244                                                  271
   GAT AGT CTC CAG CGG GCC TGG ACT GTG GGC TGG TGT GTG GAA CTG CTG CAA GCT
   Asp Ser Leu Gln Arg Ala Trp Thr Val Gly Trp Cys Val Glu Leu Leu Gln Ala

                                                   298                                                  325
   TTC TTC CTG GTG GCA GAT GAC ATC ATG GAT TCA TCC CTT ACC CGC CGG GGA CAG
   Phe Phe Leu Val Ala Asp Asp Ile MET Asp Ser Ser Leu Thr Arg Arg Gly Gln

                                                   352                                                  379
   ATC TGC TGG TAT CAG AAG CCG GGC GTG GGT TTG GAT GCC ATC AAT GAT GCT AAC
   Ile Cys Trp Tyr Gln Lys Pro Gly Val Gly Leu Asp Ala Ile Asn Asp Ala Asn

                                                   406                                                  433
   CTC CTG GAA GCA TGT ATC TAC CGC CTG CTG AAG CTC TAT TGC CGG GAG CAG CCC
   Leu Leu Glu Ala Cys Ile Tyr Arg Leu Leu Lys Leu Tyr Cys Arg Glu Gln Pro

                                                   460                                                  487
   TAT TAC CTG AAC CTG ATC GAG CTC TTC CTG CAG AGT TCC TAT CAG ACT GAG ATT
   Tyr Tyr Leu Asn Leu Ile Glu Leu Phe Leu Gln Ser Ser Tyr Gln Thr Glu Ile

                                                   514                                                  541
   GGG CAG ACC CTG GAC CTC CTC ACA GCC CCC CAG GGC AAT GTG GAT CTT GTC AGA
   Gly Gln Thr Leu Asp Leu Leu Thr Ala Pro Gln Gly Asn Val Asp Leu Val Arg

                                                   568                                                  595
   TTC ACT GAA AAG AGG TAC AAA TCT ATT GTC AAG TAC AAG ACA GCT TTC TAC TCC
   Phe Thr Glu Lys Arg Tyr Lys Ser Ile Val Lys Tyr Lys Thr Ala Phe Tyr Ser

                                                   622                                                  649
   TTC TAC CTT CCT ATA GCT GCA GCC ATG TAC ATG GCA GGA ATT GAT GGC GAG AAG
   Phe Tyr Leu Pro Ile Ala Ala Ala MET Tyr MET Ala Gly Ile Asp Gly Glu Lys

                                                   676                                                  703
   GAG CAC GCC AAT GCC AAG AAG ATC CTG CTG GAG ATG GGG GAG TTC TTT CAG ATT
   Glu His Ala Asn Ala Lys Lys Ile Leu Leu Glu MET Gly Glu Phe Phe Gln Ile

                                                   730                                                  757
   CAG GAT GAT TAC CTT GAC CTC TTT GGG GAC CCC AGT GTG ACC GGC AAA ATT GGC
   Gln Asp Asp Tyr Leu Asp Leu Phe Gly Asp Pro Ser Val Thr Gly Lys Ile Gly

                                                   784                                                  811
   ACT GAC ATC CAG GAC AAC AAA TGC AGC TGG CTG GTG GTT CAG TGT CTG CAA CGG
   Thr Asp Ile Gln Asp Asn Lys Cys Ser Trp Leu Val Val Gln Cys Leu Gln Arg

                                                   838                                                  865
   GCC ACT CCA GAA CAG TAC CAG ATC CTG AAG GAA AAT TAC GGG CAG AAG GAG GCT
   Ala Thr Pro Glu Gln Tyr Gln Ile Leu Lys Glu Asn Tyr Gly Gln Lys Glu Ala

                                                   892                                                  919
   GAG AAA GTG GCC CGG GTG AAG GCG CTA TAT GAG GAG CTG GAT CTG CCA GCA GTG
   Glu Lys Val Ala Arg Val Lys Ala Leu Tyr Glu Glu Leu Asp Leu Pro Ala Val

                                                   946                                                  973
   TTC TTG CAA TAT GAG GAA GAC AGT TAC AGC CAC ATT ATG GCT CTC ATT GAA CAG
   Phe Leu Gln Tyr Glu Glu Asp Ser Tyr Ser His Ile MET Ala Leu Ile Glu Gln

                                                  1000                                                 1027
   TAC GCA GCA CCC CTG CCC CCA GCC GTC TTT CTG GGG CTT GCG CGC AAA ATC TAC
   Tyr Ala Ala Pro Leu Pro Pro Ala Val Phe Leu Gly Leu Ala Arg Lys Ile Tyr

                                                  1054                                                 1081
   AAG CGG AGA AAG TGA CCT AGA GAT TGC AAG GGC GGG GAG AGG AGG CTC TCA ATA
   Lys Arg Arg Lys

                                                  1108
   AAT AAT CGT GTA ACC TTA AAA AAA AAA AAA ACC C
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FIGURE 2: DNA sequence of hpt807 and deduced amino acid sequence of human fetal liver farnesyl pyrophosphate synthetase. The stop codon (TGA), the polyadenylation addition sequence (AATAAA), and the first 14 nucleotides of a poly(A+) track are underlined. The arrow denotes the position in the nucleotide sequence of hpt807 where homology with CR39 (Clarke et al., 1987) ends.

```
1     DVYAQEKQDFVQHFSQIVRVLTEDEMGHPEIGDAIARLKEVLEYNAIGGKYNRGLTVVVAF
1   MNGDQKLDVHNQEKQNFIQHFSQIVKVLTEDELGHPEKGDAITRIKEVLEYNTVGGKYNRGLTVVQTF
    -------DV--QEKQ-F-QHFSQIV-VLTEDE-GHPE-GDAI-R-KEVLEYN--GGKYNRGLTVV--F

62  RELVEPRKQDADSLQRAWTVGWCVELLQAFFLVADDIMDSSLTRRGQICWYQKPGVGLDAINDANLLE
69  QELVEPRKQDAESLQRALTVGWCVELLQAFFLVLDDIMDSSYTRRGQICWYQKPGIGLDAINDALLLE
    -ELVEPRKQDA-SLQRA-TVGWCVELLQAFFLV-DDIMDSS-TRRGQICWYQKPG-GLDAINDA-LLE

130 ACIYRLLKLYCREQPYYLNLIELFLQSSYQTEIGQTLDLLTAPQGNVDLVRFTEKRYKSIVKYKTAFY
137 AAIYRLLKFYCREQPYYLNLLELFLQSSYQTEIGQTLDLITAPQGQVDLQRYTEKRYKSIVKYKTAFY
    A-IYRLLK-YCREQPYYLNL-ELFLQSSYQTEIGQTLDL-TAPQG-VDL-R-TEKRYKSIVKYKTAFY

198 SFYLPIAAAMYMAGIDGEKEHANAKKILLEMGEFFQIQDDYLDLFGDPSVTGKIGTDIQDNKCSWLVV
205 SFYLPIAAAMYMAGIDGEKEHANALKILLEMGEFFQIQDDYLDLFGDPSVTGKVGTDIQDNKCSWLVV
    SFYLPIAAAMYMAGIDGEKEHANA-KILLEMGEFFQIQDDYLDLFGDPSVTGK-GTDIQDNKCSWLVV

266 QCLQRATPEQYQILKENYGQKEAEKVARVKALYEELDLPAVFLQYEEDSYSHIMALIEQYAAPLPPAV
273 QCLLRATPQQRQILEENYGQKDPEKVARVKALYEELDLRSVFFKYEEDS        LQPPQESHRAV
    QCL-RATP-Q-QIL-ENYGQK--EKVARVKALYEELDL--VF--YEEDS---------Q-------AV
                                                    ↑
334 FLGLARKIYKRRK
333 VARPCPHPSSWN
    ------------
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FIGURE 3: Comparison of human fetal liver and rat liver farnesyl pyrophosphate synthetases. The top line in each set represents the amino acid sequence derived from human hpt807 cDNA, the middle line is the amino acid sequence deduced from rat CR39 cDNA (Clarke et al., 1987), and the third line indicates amino acids common to both sequences. The amino acid sequence homologous to the putative active site of chicken liver farnesyl pyrophosphate synthetase (Brems et al., 1981) is underlined, and the arrow denotes the position of amino acid sequence divergence between the enzymes from the two species.

human cDNA encodes nine additional C-terminal amino acids than does the rat cDNA (Figure 3), we believe hpt807 is nearly full length as its coding sequence starts 20 bp (seven amino acids) after the ATG initiation codon of the rat cDNA.

Both nucleotide and deduced amino acid sequence homology between the rat and human cDNAs is almost nonexistent at the 3′-end of the genes and the C-terminal end of the encoded proteins, respectively. This is not surprising since the amino acid sequence corresponding to the putative active-site peptide of chicken liver farnesyl pyrophosphate synthetase (Brems et al., 1981) is located in the middle of the protein (Figure 3, underlined). Comparison of the deduced amino acid composition of hpt807 with that of the purified human liver farnesyl pyrophosphate synthetase (Barnard & Popjak, 1981) shows general agreement (not shown).

Expression of an Active Fusion Protein. To further characterize hpt807, expression plasmids were constructed with hpt807 cDNA inserted in both orientations into the *Eco*RI

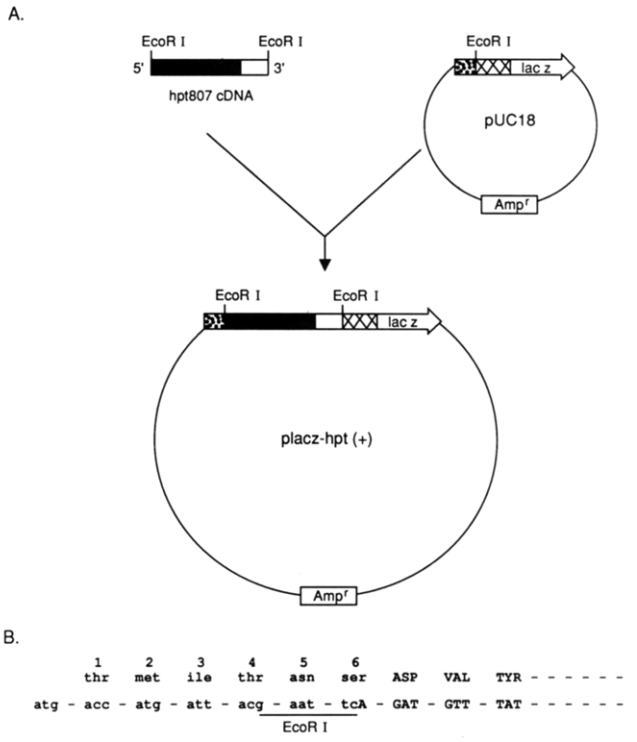

FIGURE 4: Construction of placz-hpt(+) for synthesis of chimeric farnesyl pyrophosphate synthetase in *E. coli*. (A) An *Eco*RI fragment containing the entire human cDNA sequence was cloned into the *Eco*RI site in the polylinker region of pUC18. The orientation of the plasmid construct schematically represented in this figure, placz-hpt(+), encodes a fusion protein under the control of the lac promoter. The junction between β-galactosidase and farnesyl pyrophosphate synthetase encoded by placz-hpt(+) is shown in (B). β-Galactosidase sequences are shown in lower case letters while farnesyl pyrophosphate synthetase sequences are represented by upper case letters. The solid black box indicates the coding region of hpt807, and the open box represents the 3′-untranslated region. The dotted area indicates the lac promoter, the crosshatched area represents the multiple cloning site of pUC18, and the arrow enclosing lac z shows the sequence and direction of translation of β-galactosidase.

site of pUC18. One orientation of the construct, placz-hpt(+) (Figure 4), encodes a hybrid protein containing the initiator methionine and the first six N-terminal amino acid residues of *E. coli* β-galactosidase, followed in frame by 346 amino acids encoded by the human farnesyl pyrophosphate synthetase cDNA. The opposite orientation, placz-hpt(−), encodes a fusion protein 12 amino acids in length whose synthesis is terminated by an in-frame stop codon. Bacteria were transformed with the plasmids, and cell extracts were subjected to SDS–PAGE (Figure 5A). Extracts from cells transformed with placz-hpt(+) but not parental control cells or cells transformed with placz-hpt(−) contained an induced protein of M_r 39 500. This molecular weight is consistent with that calculated for the fusion protein (M_r 40 000) encoded by placz-hpt(+). Western blot analysis shows that the induced fusion protein is specifically recognized by antibodies raised against purified chicken liver farnesyl pyrophosphate synthetase (Figure 5B).

Although homology data and specific recognition of the expressed chimeric protein by an antibody suggest that hpt807 encodes farnesyl pyrophosphate synthetase, measurement of enzyme activity in *E. coli* extracts would unequivocally identify the cDNA. To determine whether the induced fusion protein is active, bacterial cell extracts were used as the enzyme source in farnesyl pyrophosphate synthetase assays. Extracts from control cells and cells transformed with placz-hpt(−) possess marginal farnesyl pyrophosphate synthetase activity while

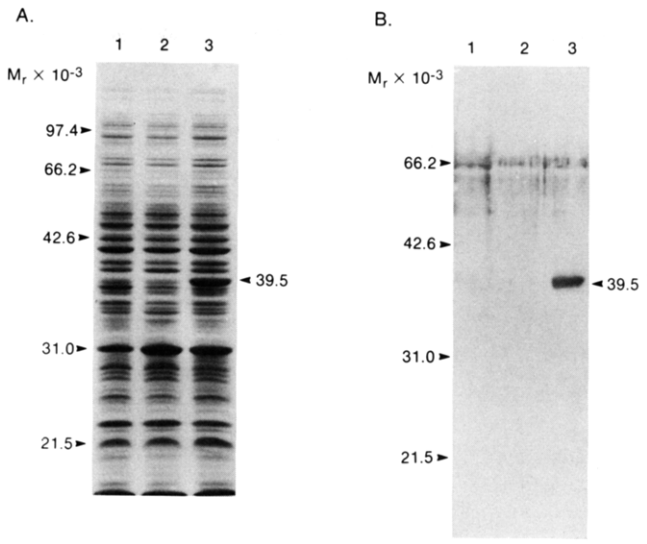

FIGURE 5: (A) SDS–PAGE analysis of chimeric farnesyl pyrophosphate synthetase in *E. coli*. Extracts from the following cells were prepared and electrophoresed on SDS–polyacrylamide gels. Lanes: 1, *E. coli* DH5αF′IQ; 2, *E. coli* DH5αF′IQ containing placz-hpt(−); 3, *E. coli* DH5αF′IQ containing placz-hpt(+). (B) Western blot analysis of chimeric farnesyl pyrophosphate synthetase production in *E. coli*. Proteins from a gel similar to those in (A) were transferred to a nitrocellulose membrane and probed with an antibody raised against purified chicken liver farnesyl pyrophosphate synthetase. Molecular weight standards are phosphorylase *b* (97 200), bovine serum albumin (66 200), ovalbumin (42 600), carbonic anhydrase (31 000), and soybean trypsin inhibitor (21 500).

Table I: Farnesyl Pyrophosphate Synthetase Activity in *E. coli* Extracts[a]

cells	plasmid	GPP	sp act. [nmol min^{-1} (mg of protein)$^{-1}$]	rel sp act.
DH5αF′IQ		+	0.95	1.00
DH5αF′IQ	placz-hpt(−)	+	0.80	0.68
DH5αF′IQ	placz-hpt(+)	+	157.23	165.50
DH5αF′IQ	placz-hpt(+)	−	0.78	0.82

[a] Farnesyl pyrophosphate synthetase activities were measured in bacterial cell extracts from control cells and cells transformed with plasmids as indicated. Assays were carried out in the presence (+) and absence (−) of geranyl pyrophosphate (GPP) as described under Experimental Procedures.

extracts from cells transformed with placz-hpt(+) contain considerable activity (Table I). Relative to control cells, placz-hpt(+)-transformed cells have a 165-fold higher specific activity of farnesyl pyrophosphate synthetase. The observed activity is dependent on the addition of geranyl pyrophosphate to the incubations since assays carried out in the absence of this allylic pyrophosphate show little activity (Table I). This result demonstrates that there is essentially no interfering isopentenyl pyrophosphate isomerase activity in the cell extracts. Farnesyl pyrophosphate synthetase reactions performed with extracts from *E. coli* transformed with placz-hpt(+) were further analyzed by anion-exchange HPLC. Reactions carried out in the absence of geranyl pyrophosphate contain only unreacted isopentenyl pyrophosphate, whereas a second radiolabeled species identified as farnesyl pyrophosphate is present in reactions performed with geranyl pyrophosphate added to the incubations (not shown). These data confirm the identify of hpt807 cDNA as encoding human fetal liver farnesyl pyrophosphate synthetase.

RNA Analysis. In order to determine the size of a full-length mRNA encoding farnesyl pyrophosphate synthetase, Northern blots of poly(A+) RNA from human liver and Hep G2 cells were probed with hpt807 cDNA. A transcript of

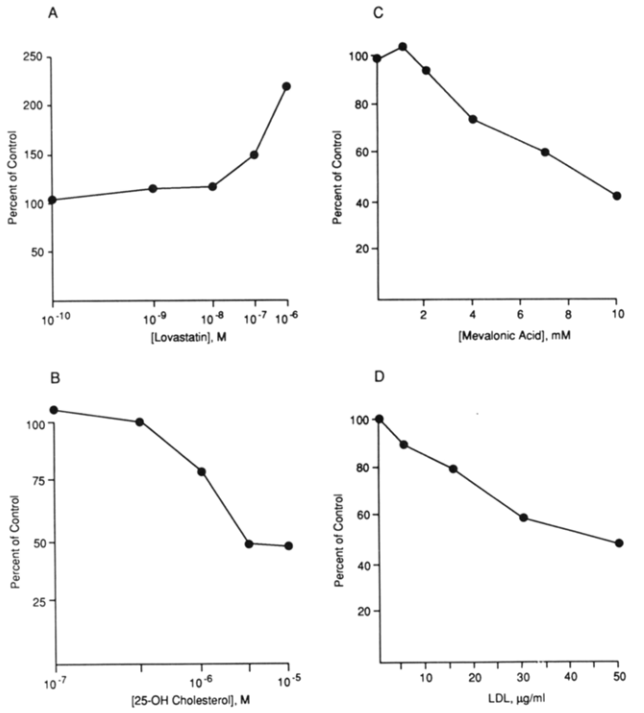

FIGURE 6: Farnesyl pyrophosphate synthetase mRNA levels in Hep G2 cells. Hep G2 cells were cultured in the presence of (A) lovastatin, (B) 25-hydroxycholesterol, (C) mevalonic acid, and (D) low-density lipoprotein at the indicated concentrations for 24 h prior to isolation of total RNA. Samples were applied to nitrocellulose filters and hybridized to ^{32}P-labeled hpt807 cDNA. Data are expressed relative to control cells incubated in the absence of the test compound.

approximately 1.4 kb was evident in RNA from both sources although Hep G2 cells contained 5-fold more farnesyl pyrophosphate synthetase mRNA than human liver (not shown). This is likely due to the fact that cells dividing in culture probably have a greater need for isoprene-derived products, i.e., cholesterol and dolichol. The size of the mRNA is similar to that of the rat liver message (1.2 kb) and is reasonable assuming the addition of a poly(A+) tail (200 bp) and 5′-coding and noncoding sequences to our 1115-bp human cDNA.

To examine whether the expression of farnesyl pyrophosphate synthetase mRNA is regulated, Hep G2 cells were cultured in the presence of agents known to modulate the rate of cholesterol synthesis. Lovastatin, a potent inhibitor of HMG-CoA reductase, increased the level of mRNA encoding farnesyl pyrophosphate synthetase approximately 2.5-fold (Figure 6A) above control values. This dose-dependent effect is most pronounced at high levels of the drug. Cholesterol in its 25-hydroxylated form (Figure 6B) or in low-density lipoprotein particles (Figure 6D) reduces the amount of farnesyl pyrophosphate synthetase mRNA to 50% of control values. Mevalonic acid also decreased mRNA levels in a dose–dependent manner to 40% of control values (Figure 6C). These data demonstrate that the expression of farnesyl pyrophosphate synthetase mRNA is regulated in Hep G2 cells over a 6-fold range. This value is similar to changes observed in mRNA levels for HMG-CoA reductase (Cohen & Griffioen, 1988), HMG-CoA synthase, and LDL receptor in Hep G2 cells (Molowa & Cimis, 1989), but it is substantially lower than the 36-fold change in rat liver CR39 mRNA levels in animals fed diets of cholesterol or hypocholesterolemic drugs (Clarke et al., 1987). The reason for this difference is unknown, but Hep G2 cells appear to be generally less responsive to agents that modulate gene expression of proteins involved in cholesterol homeostasis than other cell lines (Luskey et al., 1982, 1983; Chin et al., 1982).

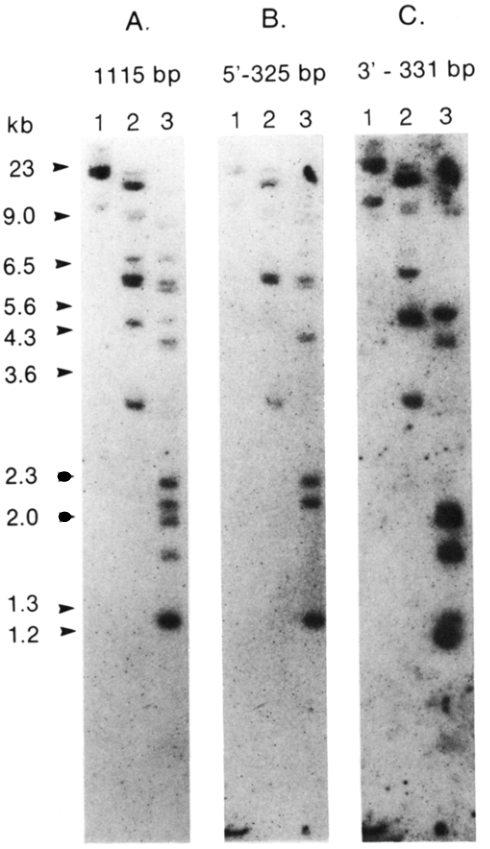

FIGURE 7: Genomic Southern blot analysis. Human genomic DNA was digested with *Bam*HI (lanes 1), *Eco*RI (lanes 2), and *Pst*I (lanes 3), separated on an agarose gel, and transferred to a nylon membrane. Blots were hybridized to ^{32}P-labeled (A) hpt807 cDNA and (B) and 5′ 325-bp or (C) 3′ 331-bp fragments of hpt807 cDNA. *Hin*dIII and *Bst*EII digested λ DNA size markers are indicated by the arrows.

The similarities observed in the regulation of farnesyl pyrophosphate synthetase, HMG-CoA reductase, HMG-CoA synthase, and low-density lipoprotein receptor in vivo and in vitro suggest a common control mechanism. Isolation of the gene for farnesyl pyrophosphate synthetase should be enlightening as the other genes have a common regulatory motif in their promoters which seems to be required for sterol-mediated suppression of transcription (Osborne et al., 1988; Smith et al., 1988; Sudhoff et al., 1987a,b). At present it is not known if variations observed in farnesyl pyrophosphate synthetase mRNA levels are the result of changes in the transcriptional rate of the message or are due to changes in the stability of the RNA. Likewise, the role of protein synthesis and turnover in controlling the level of farnesyl pyrophosphate synthetase in cells is not known.

Genomic Southern Blot Analysis. Human genomic DNA was digested with restriction endonucleases, and Southern blots were probed with the 1115-bp human cDNA. *Bam*HI digestion yielded hybridizing bands of large molecular weights while *Eco*RI and *Pst*I digests yielded approximately 8 and 11 bands, respectively (Figure 7A). For further analysis, the genomic blot was stripped and reprobed with nonoverlapping 5′ (325 bp, Figure 7B) and 3′ (331 bp, Figure 7C) DNA fragments. Both probes hybridized to the large *Bam*HI fragments, but differential hybridization was observed between the two probes when DNA was digested with *Eco*RI or *Pst*I. The two probes hybridized to five common species in *Eco*RI-digested samples and three common species in samples digested with *Pst*I. These results suggest that there are multiple genes (perhaps five) that encode divergent farnesyl

pyrophosphate synthetases or that there is cross hybridization to genes related to farnesyl pyrophosphate synthetase. This observation is consistent with that of Clarke et al. (1987), who reported similar results from rat genomic blots probed with CR39 and concluded that multiple genes encode rat farnesyl pyrophosphate synthetase. These results are interesting because, where investigated, HMG-CoA reductase (Lindgren et al., 1985), HMG-CoA synthase (Mehrabian et al., 1986), and LDL receptor (Lindgren et al., 1985) are normally encoded by single genes in higher eukaryotes. While speculative, it may be that different isozymes of farnesyl pyrophosphate synthetase exist in various tissues, i.e., liver versus peripheral tissues, or that different forms of the enzyme are expressed during development or in diverse metabolic states in order to maintain the proper balance between cholesterol synthesis and the synthesis of other important molecules, i.e., dolichol, ubiquinone, or other isoprenylated compounds. It is also possible that other prenyltransferases in human tissues, i.e., dolichol phosphate synthetase, are related to farnesyl pyrophosphate synthetase. Current work is ongoing in our laboratory to address these possibilities.

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Registry No. Farnesyl pyrophosphate synthetase, 50812-36-7.

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