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Testis-Specific Transcription Initiation Sites of Rat Farnesyl Pyrophosphate Synthetase mRNA

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A variety of rat tissues were screened at low stringency with a rat farnesyl pyrophosphate (FPP) synthetase cDNA. In testis, an FPP synthetase-related RNA was detected that was larger than the liver FPP synthetase mRNA and was present at very high levels comparable with liver FPP synthetase RNA levels obtained from rats fed diets supplemented with cholestyramine and mevinolin. Sequence analysis of testis cDNA clones, together with primer extension and S1 nuclease experiments, indicated that testis FPP synthetase transcripts contain an extended 5′ untranslated region. The 5′ extension contained one or two out-of-frame upstream ATGs, depending on the site of transcription initiation. Protein in vitro translation studies indicated that the extended 5′ untranslated region may play a role in regulating the translation of the FPP synthetase polypeptide in rat testis. Southern blot analysis with a probe containing both testis and liver 5′ untranslated sequences provided evidence that both liver and testis transcripts derive from the same gene. The data suggest that an upstream testis-specific promoter results in the abundant production of FPP synthetase transcripts that are translated at low efficiency; another promoter functions in liver and other somatic tissues and directs the regulated synthesis of shorter discrete transcripts.

Farnesyl pyrophosphate (FPP) synthetase is a member of the prenyltransferase family involved in the synthesis of isoprenoid compounds (46). A diverse array of end products derive from the isoprene biosynthetic pathway including cholesterol, dolichol, ubiquinone, cytochrome aa_3, isoprenylated proteins, and isopentenyl tRNAs. FPP synthetase catalyzes the sequential condensation of three five-carbon isoprene units to form FPP (43).

Regulation is exerted at many steps of the isoprene biosynthetic pathway to provide control over the rates of endogenous cholesterol synthesis and the synthesis of nonsterol products (6, 11). In rat liver, FPP synthetase activity is decreased in response to cholesterol feeding and is increased by the hypocholesterolemic drugs cholestyramine and mevinolin (1). Similar changes in activity are also exhibited by two other enzymes of the isoprene biosynthetic pathway, 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and HMG CoA synthase (13, 36).

Both nonsterol and sterol molecules are involved in the feedback regulation of the isoprene biosynthetic pathway, but the identity of the molecules involved in such regulation is poorly understood (6, 25, 41, 48). With the exception of tRNA isopentenyl transferase (38), the prenyltransferases catalyzing the formation of nonsterol products have not been characterized. Thus, very little is known about the *cis*-prenyltransferase that synthesizes dolichol (16) or the prenyltransferases that catalyze the formation of the farnesyl moiety on cytochrome aa_3 (43), the polyprenyl moiety on ubiquinone (40), or the isoprene derivatives covalently associated with cellular polypeptides such as ras (18, 49), lamins A and B (4, 54), and other polypeptides of unknown identity (33). The prenyl moieties on cellular polypeptides, on heme a, and on ubiquinone very likely allow a stable interaction of these components with the membrane. The prenyltransferases catalyzing the formation of nonsterol products may represent potential sites for toxic side effects of mevinolin, a drug that inhibits the activity of HMG CoA reductase and hence the biosynthesis of both sterol and nonsterol compounds.

Previous analysis of mouse, rat, and human genomic DNAs with the FPP synthetase cDNA has indicated the presence of at least five and as many as seven gene copies with high sequence identity to FPP synthetase (14, 19). In an attempt to isolate other prenyltransferases, a rat liver FPP synthetase cDNA clone was used to screen various rat tissues at low stringency for detection of different-sized mRNAs. The experiments reported here showed that the testis contains an FPP synthetase-related RNA that is larger than the liver FPP synthetase mRNA. Characterization of this testis mRNA indicated that it is likely to be derived from the same gene encoding the liver form of FPP synthetase and that it contains an extended 5′ untranslated region that may play a unique role in regulating the translation of the FPP synthetase polypeptide in rat testis.

MATERIALS AND METHODS

Materials. Adult male Sprague-Dawley rats were obtained from Simonsen Laboratories. Mevinolin (Lovastatin) was a gift from A. Alberts (Merck Sharpe & Dohme, Rahway, N.J.). Cholestyramine (Questran) was purchased from Johnson and Mead. [^{35}S]methionine (specific activity of >1,000 Ci/mmol), Biodyne nylon membranes, and ^{32}P-labeled nucleotides [γ-^{32}P]ATP (specific activity of >7,000 Ci/mmol) and [α-^{32}P]dCTP (specific activity of ~3,000 Ci/mmol) were obtained from ICN Pharmaceuticals, Inc. (Irvine, Calif.). ^{14}C-methylated rainbow protein molecular weight markers were obtained from Amersham Corp. (Arlington Heights, Ill.). Oligonucleotides were synthesized by the phosphoramidite method (22) by Dohn Glitz (Department of Biological Chemistry, University of California at Los Angeles) on a Du Pont/Vega Coder 300 DNA synthe-

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sizer or were obtained from Research Genetics (Huntsville, Ala.). SP6 RNA polymerase, RNasin, pGEM-2, and nuclease-treated rabbit reticulocyte lysate were obtained from Promega Biotec (Madison, Wis.). Avian myeloblastosis virus reverse transcriptase was purchased from Life Sciences, Inc. (St. Petersburg, Fla.). Restriction endonucleases, Klenow polymerase, S1 nuclease, and T4 polynucleotide kinase were obtained from Bethesda Research Laboratories, Inc. (Gaithersburg, Md.). Dactinomycin and glycogen were purchased from Boehringer Mannheim Biochemicals (Indianapolis, Ind.). *N,N*-Diallyltartardiamide was obtained from Bio-Rad Laboratories (Richmond, Calif.). Deoxynucleoside triphosphates, dideoxynucleoside triphosphates, protein A-Sepharose, leupeptin, pepstatin, and bovine serum albumin were purchased from Sigma Chemical Co. (St. Louis, Mo.). cDNAs used in this study included a full-length rat FPP synthetase cDNA, CR39-1.2 (14), a full-length hamster HMG CoA reductase cDNA, λDS11 (51), a partial rat HMG CoA synthase cDNA, LA10 (36), a partial rat low-density lipoprotein (LDL) receptor cDNA (obtained as a gift from Douglas Wilkin, Department of Biological Chemistry, University of California at Los Angeles), and human α-tubulin cDNA (a gift courtesy of Winston Salser, Department of Biology, University of California at Los Angeles).

Screening of rat testis cDNA library. A Sprague-Dawley rat testis cDNA library in λgt11 (Clonetech) was plated and screened with the 1.2-kilobase (kb) *Eco*RI insert of CR39 according to the instructions of the manufacturer. Approximately 600,000 plaques were plated (60,000 plaques per 150-mm-diameter petri dish), and 25 bacteriophage recombinants were taken through three rounds of plaque purification. Preliminary dot-blot analysis of clones was performed to ascertain the extent of homology with liver FPP synthetase cDNA. Three synthetic oligonucleotides derived from the 5′ end (nucleotide positions 49 to 70), the active site region (nucleotide positions 517 to 608), and the 3′ end (nucleotide positions 1073 to 1113) of the liver FPP synthetase cDNA (see Fig. 3) were radiolabeled with [γ-^{32}P]ATP (34). The FPP synthetase cDNA and λ DNA provided positive control probes which would hybridize to the insert or vector DNA of each clone, respectively. Dot blots were prepared according to the instructions of the manufacturer; 2.5-μl portions of DNA samples were directly spotted onto Biodyne nylon membranes and baked for 2 h at 80°C. Dot blots were hybridized at 55°C for 18 h with 10 to 20 ng of ^{32}P-labeled synthetic oligonucleotide probes per ml of hybridization buffer (0.75 M NaCl, 0.15 M Tris hydrochloride [pH 8.0], 10 mM EDTA, 0.10% sodium dodecyl sulfate [SDS], 0.10% sodium PP_i, 5× Denhardt solution [0.1% each Ficoll, polyvinylpyrrolidone, and bovine serum albumin], 100 μg of calf thymus DNA per ml). The blots were washed once for 30 min at room temperature with 2× SSC (0.30 M NaCl, 30 mM sodium citrate, pH 7.0)–0.1% SDS, once for 30 min at 50°C with 1× SSC–0.1% SDS, and four times for 15 min each at room temperature with 0.1× SSC–0.1% SDS. Blots were then exposed to Kodak XAR-5 film at −70°C with a Cronex Hi-Plus intensifying screen. cDNA inserts were subcloned into either M13mp9 (37) or the plasmid pGEM-2 (Promega Biotec).

DNA sequencing. DNA was sequenced by the dideoxy-chain termination method (47) with the Pharmacia-LKB ^{T7}Sequencing kit with an M13mp19 standard, the M13 universal primer or specific oligonucleotides, and T7 DNA polymerase.

Northern (RNA) blot analysis. RNA was isolated from the testis and other tissues of adult male Sprague-Dawley rats as described by Chomzynski and Sacchi (8). Liver RNA was isolated as described by Chirgwin et al. (7). Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography as described by Chirgwin et al. (7). Rats were maintained in 12-h light-dark-cycled rooms and were fed diets supplemented with 5% cholesterol or with 5% cholestyramine and 0.1% mevinolin as described previously (13). RNA was analyzed by electrophoresis on formaldehyde gels as described by Lehrach et al. (30), transferred to Biodyne nylon membranes, and baked for 2 h at 80°C. Northern blots were hybridized at 53°C for 16 h with ^{32}P-labeled cDNA probes by the method of Church and Gilbert (10). DNA probes were labeled with [α-^{32}P]dCTP as described by Feinberg and Vogelstein (17). Hybridization mixtures contained 10 to 20 ng of ^{32}P-labeled probe per ml of hybridization buffer. Blots were washed two times for 30 min each at room temperature with 2× SSC–0.1% SDS and three times for 20 min each at 42°C with 0.1× SSC–0.1% SDS. Blots were then exposed to Kodak XAR-5 film at −70°C with a Cronex Hi-Plus intensifying screen. Blots were stripped free of probe as recommended by ICN.

Southern blot analysis. Rat testis genomic DNA was isolated by the method of Herrmann and Frischauf (20). A 10-μg sample was digested with various restriction endonucleases, analyzed by electrophoresis on a 1% Tris-acetate agarose gel, transferred to Biodyne nylon membranes, and baked for 2 h at 80°C. A 327-base-pair *Eco*RI-*Sma*I rat genomic DNA fragment extending from nucleotide positions −47 to −364 containing sequence encoding the 5′ untranslated segment of the FPP synthetase gene was subcloned into a Bluescript plasmid, isolated, and used as a probe (D. H. Spear, S. Kutsunai, C. Correll, and P. A. Edwards, manuscript in preparation). Radiolabeling of the probe and hybridization conditions were the same as described above for Northern blots. Blots were washed twice for 30 min each at room temperature with 2× SSC–0.1% SDS and once for 30 min at 65°C with 1× SSC–0.1% SDS and then exposed to film as described above.

Primer extension analysis. A synthetic liver RNA was used as a control of known size in the primer extension analyses. Construction of pGEM-CR39 and synthesis of sense RNA with SP6 polymerase has been previously described (14). A synthetic oligonucleotide complementary to nucleotide positions +30 to −9 of FPP synthetase cDNA (M60; see Fig. 7) was purified on a 20% polyacrylamide–8 M urea gel (35) and desalted on NACS columns (Bethesda Research Laboratories). The purified oligonucleotide (200 ng) was end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase as described previously (34) except that bovine serum albumin was omitted. Primer-RNA hybridizations and extension reactions were performed as described by Kingston (26) with modifications. An 8-μg sample of poly(A)$^+$ RNA, 4 ng of ^{32}P-labeled oligonucleotide primer, 1.35 M KCl, 0.05 M Tris (pH 7.5), and 0.005 M EDTA were mixed in a final volume of 12 μl, denatured at 95°C for 3 min, and allowed to hybridize for 15 min at 68°C. The reaction mixture was centrifuged briefly (12,000 × *g*) at room temperature. A reaction mixture (24 μl) was added to give a final concentration of 20 mM Tris hydrochloride (pH 8.7), 10 mM $MgCl_2$, 10 mM dithiothreitol, 0.4 mM deoxynucleoside triphosphates, 0.4 μg of dactinomycin per ml, 50 U of RNasin, and 54 U of reverse transcriptase. The reaction mixture was incubated for 60 min at 45°C, and the reaction was stopped with 200 μl of 10 mM Tris hydrochloride (pH 8.0), 12.5 mM EDTA, and 6 μg of glycogen.

The reaction mixture was extracted once with an equal volume of phenol-chloroform (1:1, vol/vol) and precipitated with 0.5 volume of 8 M ammonium acetate and 2.5 volumes of ethanol. Pellets were then suspended in 8 μl of TE (10 mM Tris hydrochloride, 1 mM EDTA, pH 8.0) and 12 μl of sequencing loading buffer (35). The samples were heated for 8 min at 95°C, chilled on ice, and then subjected to electrophoresis on a sequencing gel.

S1 nuclease analysis. The S1 probe was synthesized as described by Reynolds et al. (45) with a few modifications. For mapping the multiple testis transcripts and longest liver transcript, a synthetic oligonucleotide complementary to nucleotide positions −27 to −51 of the liver FPP synthetase cDNA (AA112; see Fig. 7) was purified and 200 ng was end labeled as described above. A 2.2-kb *Eco*RI rat genomic DNA fragment containing sequence encoding the 5′ untranslated segment of the FPP synthetase gene was subcloned into M13mp9, and 1.5 μg was used as the template for probe synthesis (Spear et al., in preparation). For mapping the shorter liver transcripts, a synthetic oligonucleotide complementary to nucleotide positions +51 to +70 of the FPP synthetase cDNA (M378; see Fig. 7) was purified, 200 ng was end labeled as described above, and 1.5 μg of the testis cDNA (TF1.4) subcloned into M13mp9 provided the template for probe synthesis. Klenow fragment of DNA polymerase I (5.9 U) was used, and restriction digestion was performed with 10 U of *Eco*RI. The reaction was stopped with 12 μl of 80% formamide–0.2× Tris-borate–0.1% bromphenol blue–0.1% xylene cyanol, followed by denaturation at 95°C for 5 min and the addition of 10 μl of 1 M NaOH. The products were analyzed by electrophoresis on a 5% polyacrylamide–8 M urea gel along with molecular size standards consisting of *Hpa*II-digested fragments of pBR322. The appropriate-sized ^{32}P-labeled probe was removed from the gel by electroelution, precipitated with 6 μg of glycogen, 0.5 volume of ammonium acetate, and 2.5 volumes of ethanol, and suspended in TE. Hybridizations were performed under conditions of probe excess, and RNA-DNA hybrids were treated with various amounts of S1 nuclease (100 to 1,000 U/ml) for 1 h in a 24°C water bath. Digested products were then analyzed next to a sequencing reaction by electrophoresis on a 5% polyacrylamide–8 M urea gel.

In vitro translation of poly(A)$^+$ RNA and immunoprecipitation of [^{35}S]methionine-labeled FPP synthetase. In vitro translations were done as described by Clarke et al. (12) with some modifications. In vitro translation reaction mixtures contained 35 μl of nuclease-treated rabbit reticulocyte lysate, 4 μl of [^{35}S]methionine (specific activity of >1,000 Ci/mmol), 1 μl of RNasin (40 U/ml), 1 μl of a 1 mM amino acid mixture (minus methionine), and rat liver poly(A)$^+$ RNA or rat testis poly(A)$^+$ RNA at concentrations ranging from 7.2 to 60 μg/ml. RNA samples were heated at 67°C for 10 min, and translation reactions were performed at 37°C for 60 min. In vitro translations were stopped by adding 450 μl of ice-cold RIPA buffer (1% Triton X-100, 0.5% deoxycholate, 0.1% SDS, 0.01% NaN_3, 5 mM EDTA, 0.1 M NaCl, 0.01 M Na_2HPO_4) containing 0.2 mM phenylmethylsulfonyl fluoride and 5 μg each of pepstatin and leupeptin per ml. Reaction mixtures were vortexed and then centrifuged at 12,000 × *g* for 30 min at 4°C. Total incorporation of [^{35}S] methionine into trichloroacetic acid-precipitable material was determined as described by Beale et al. (3).

Optimal conditions for immunoprecipitating the FPP synthetase polypeptide were determined by the procedure described by Clarke et al. (12) except that in vitro translation mixtures were treated with 30 μl of protein A-Sepharose (30%, vol/vol, in RIPA buffer and 1% bovine serum albumin). All incubations with protein A-Sepharose were done at room temperature for 1 h followed by centrifugation at 12,500 × *g* for 2 to 3 min. Preimmune rabbit serum immunoglobulin G (IgG) (2.7 μg) or anti-FPP synthetase IgG (1.28 μg) (1) was added to the supernatant. Incubations with preimmune rabbit serum IgG were done at room temperature for 1 h, and incubations with anti-FPP synthetase IgG were done at 4°C overnight. Protein A-Sepharose was added (30 μl/4.0 μl of IgG) and after 1 h was removed by centrifugation. The pellets were washed four times with RIPA buffer, stored at −20°C, or processed for electrophoresis. Immunoprecipitates were analyzed as described by Clarke et al. (12) on 10% polyacrylamide gels without urea.

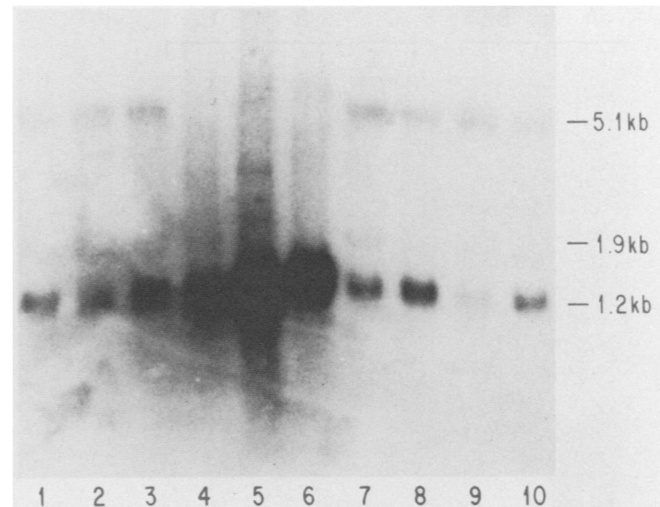

FIG. 1. Northern analysis of FPP synthetase-related RNAs in different rat tissues. Total RNA was isolated from the following tissues: lane 1, brain; lane 2, heart; lane 3, lung; lane 4, liver isolated from rats fed a normal diet; lane 5, liver isolated from rats fed a diet supplemented with 5% cholestyramine and 0.1% mevinolin; lane 6, testis; lane 7, kidney; lane 8, adrenal gland; lane 9, spleen; lane 10, small intestine. Total RNA (15 μg) was analyzed on each lane of a 1.0% agarose-formaldehyde gel. Subsequent Northern blotting analysis was performed as described in Materials and Methods with the CR39 1.2-kb liver FPP synthetase cDNA as a probe (4.5 × 10^7 cpm/μg). The blot was washed under nonstringent conditions: 42°C, 0.1× SSC–0.1% SDS. The rat rRNA size standards (1.9 and 5.1 kb) are indicated.

RESULTS

Expression of rat FPP synthetase mRNA in different tissues. A rat liver cDNA encoding FPP synthetase was used as a probe to determine the levels of FPP synthetase mRNA in a variety of rat tissues. The hybridization and washing were performed at low stringency to favor detection of any related mRNAs that might be expressed in a tissue-specific manner. Figures 1 and 2 show that testis FPP synthetase RNA is larger than the 1.2-kb message present in liver and all other somatic tissues assayed, such as brain, heart, lung, kidney, adrenal gland, spleen, and small intestine. In other analyses, tissues such as skeletal muscle, epididymis, and ovary were examined and also showed the 1.2-kb RNA (data not shown). FPP synthetase RNA in the testis was present at very high levels comparable to liver FPP synthetase RNA levels in rats fed diets supplemented with cholestyramine

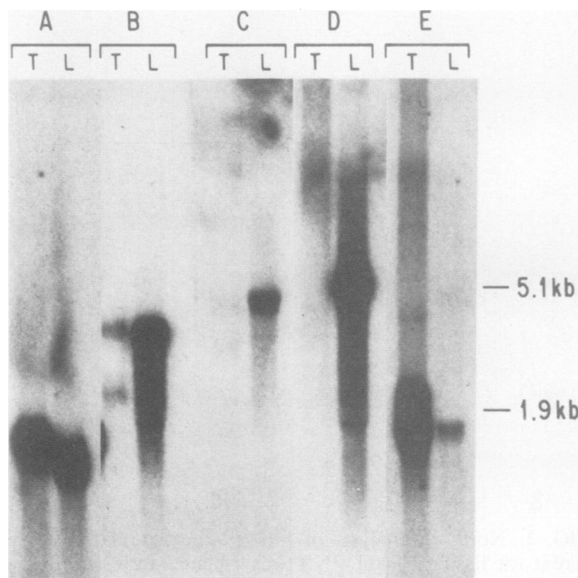

FIG. 2. Differential hybridization of FPP synthetase, HMG CoA synthase, HMG CoA reductase, and LDL receptor cDNA probes to adult rat testis and liver RNA. Testis poly(A)$^+$ RNA was isolated from rats fed a normal diet, and liver poly(A)$^+$ RNA was isolated from rats fed a diet supplemented with 5% cholestyramine and 0.1% mevinolin. Testis (T) or liver (L) RNA (10 μg) was analyzed on a 1.0% agarose-formaldehyde gel. Pairs of lanes were analyzed by Northern blotting with the following ^{32}P-labeled probes: (A) FPP synthetase (1.63×10^8 cpm/μg); (B) HMG CoA synthase cDNA (1.66×10^8 cpm/μg); (C) HMG CoA reductase cDNA (1.52×10^8 cpm/μg); (D) LDL receptor cDNA (2.18×10^8 cpm/μg); (E) human α-tubulin cDNA (2.18×10^8 cpm/μg). RNA isolation and Northern blot analysis are described in Materials and Methods. Autoradiographic exposure time was 18 h. The blots in panels A to D were washed free of probe and hybridized to human α-tubulin cDNA. Panel E shows a representative example (the result obtained from the blot in panel C).

and mevinolin. The latter drugs are known to induce liver FPP synthetase mRNA approximately ninefold (14).

To determine whether other mRNAs encoding cholesterol biosynthetic enzymes were also present at high levels in the testis or exhibited different-sized mRNAs, a Northern blot with panels of lanes containing testis or liver RNA was probed with rat liver FPP synthetase, HMG CoA synthase, HMG CoA reductase, and LDL receptor cDNA probes (Fig. 2). Unlike FPP synthetase mRNA levels in the testis, RNA levels for HMG CoA synthase, HMG CoA reductase, and LDL receptor in the testis were very low compared with liver RNA levels in rats fed diets supplemented with cholestyramine and mevinolin. The presence of a larger testis mRNA was observed only in the panel probed with the FPP synthetase cDNA. The smaller band in the testis RNA in Fig. 2B that hybridized to a HMG CoA synthase probe did not appear reproducibly in testis RNA preparations and may be artifactual or a polyadenylation variant. To normalize the mRNA levels detected by each of the cDNA probes, the Northern blots in Fig. 2 were washed free of probe and hybridized to human α-tubulin cDNA. Figure 2E shows a representative example. In data not shown, analysis of the reprobed blots (A to D) indicated that the quality and quantity of RNA in all testis lanes for all panels were the same. The α-tubulin mRNA levels were very high in the testis and of different size compared with α-tubulin mRNA in the liver. This observation is consistent with previous reports showing heterogeneity in testis α-tubulin mRNA (52, 53).

Isolation of rat testis FPP synthetase cDNA. To isolate the different FPP synthetase mRNA species in the testis, we screened a rat testis cDNA library as described in Materials and Methods. Twenty-five separate clones were isolated and analyzed. Insert sizes ranged from 0.6 to 1.35 kb. Six of the testis cDNA clones contained inserts between 1.25 and 1.35 kb, which were larger than the published liver FPP synthetase cDNA of 1.2 kb (14). Three synthetic oligonucleotides derived from the 5′ end, the active site region, or the 3′ end of the liver FPP synthetase cDNA were radiolabeled with [γ-^{32}P]ATP and used in a dot-blot analysis to determine the extent of homology of the testis clones to the liver FPP synthetase cDNA (see Materials and Methods). By this analysis, none of the testis clones isolated showed evidence of containing sequence differences over the regions encompassed by the synthetic oligonucleotide probes (data not shown). The six largest testis cDNA inserts were subcloned into M13 sequencing vectors, and sequence determination from the 3′ and 5′ ends indicated that they represented isolates containing variable amounts of 5′ region.

The complete nucleotide sequence of the largest cDNA insert, clone TF1.4, is shown in Fig. 3. The sequence showed identity with the 5′ end of liver FPP synthetase cDNA but contained 129 base pairs of additional 5′ sequence. Thus, the FPP synthetase mRNA in testis contains additional information not present in the FPP synthetase liver mRNA, and the longer 5′ untranslated region accounts for the larger size. Four nucleotide polymorphisms between liver and testis sequence are noted in Fig. 3. All changes are either C-to-T or T-to-C transitions. Three of the four changes (positions 237, 495, and 618) are also present in a FPP synthetase rat genomic clone (Spear et al., in preparation). One of the four changes (at position 328) is not present in the rat genomic clone and specifies an amino acid change from tyrosine to histidine, encoded by liver and testis cDNA clones, respectively. This sequence polymorphism was observed in two separate cDNA clonal isolates.

Identification of transcription start sites. Since the testis FPP synthetase mRNA contains an extended 5′ untranslated region, further analysis of the transcription initiation sites and the exact lengths of the testis and liver FPP synthetase mRNAs were determined by primer extension and S1 nucle-

FIG. 3. Nucleic acid sequence and predicted amino acid sequence of rat testis FPP synthetase cDNA compared with rat liver cDNA. The initiator methionine codon is designated +1, and the 5′ untranslated region of the testis cDNA (TF1.4) extends from −1 to −157. The testis sequence is compared with the sequence of the liver cDNA (CR39-1.2). Identities between rat testis and liver sequences are indicated by the dashed line. The liver FPP synthetase cDNA clone initiates at −27, as indicated by the dashed line. Four nucleotide differences occur at positions 237, 328, 495, and 618; one of these (328) results in an amino acid change (His to Tyr). The sequence from nucleotides 964 to 1063 in the liver sequence represents a correction of a previously published sequence (14). The 5′ sequence of TF1.4 from −157 to 190 was determined on both strands; the remaining sequence comparison was determined from one strand. The area of difference between the testis and liver sequences at position 328 was verified by resequencing this region in another isolated clone as described in Materials and Methods.

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 -157  TTATATTTGGGTTCTGCCTACTGAGCCGGGAGTCTGGGAACTACAACTCCCAGAGTGCTG  -98

  -97  AGCGGATGCACGCTCTGCTTTTAGGTGTAAGCCGCAAACATCTTGGACCCCGGGAGAATC  -38

       CGCGTTGAAGCACAGAGCATTTAGCTCCTCTGTCAGAATGAATGGGGACCAGAAACTGGA
  -37            ------+---------+---------+---------+---------+---  23
                                             M  N  G  D  Q  K  L  D

       TGTTCATAACCAAGAAAAGCAGAATTTCATCCAGCACTTCTCCCAGATTGTCAAGGTGCT
   24  ------+---------+---------+---------+---------+---------+---  83
        V  H  N  Q  E  K  Q  N  F  I  Q  H  F  S  Q  I  V  K  V  L

       GACTGAGGATGAACTGGGACACCCAGAGAAGGGAGATGCTATTACCCGGATCAAAGAGGT
   84  ------+---------+---------+---------+---------+---------+---  143
        T  E  D  E  L  G  H  P  E  K  G  D  A  I  T  R  I  K  E  V

       CCTGGAGTACAACACTGTAGGAGGCAAGTACAATCGGGGTCTGACGGTGGTACAGACCTT
  144  ------+---------+---------+---------+---------+---------+---  203
        L  E  Y  N  T  V  G  G  K  Y  N  R  G  L  T  V  V  Q  T  F

       CCAGGAACTGGTGGAACCAAGGAAACAGGATGCTGAGAGCCTACAGCGGGCCCTGACGGT
  204  ------+---------+---------+------C--+---------+---------+---  263
        Q  E  L  V  E  P  R  K  Q  D  A  E  S  L  Q  R  A  L  T  V

       GGGCTGGTGTGTAGAACTGCTCCAGGCTTTCTTCCTCGTGTTAGATGACATCATGGACTC
  264  ------+---------+---------+---------+---------+---------+---  323
        G  W  C  V  E  L  L  Q  A  F  F  L  V  L  D  D  I  M  D  S

       TTCCCACACTCGCCGGGGGCAGATCTGCTGGTATCAGAAGCCGGGCATAGGCTTGGATGC
  324  ----T-+---------+---------+---------+---------+---------+---  383
        S  H  T  R  R  G  Q  I  C  W  Y  Q  K  P  G  I  G  L  D  A
          (Y)
       CATCAACGATGCTCTGCTTCTGGAAGCCGCTATCTACCGCCTGCTTAAGTTCTACTGCAG
  384  ------+---------+---------+---------+---------+---------+---  443
        I  N  D  A  L  L  L  E  A  A  I  Y  R  L  L  K  F  Y  C  R

       GGAGCAGCCCTACTACCTCAACCTGCTGGAGCTCTTTCTACAGAGTTCCTATCAGACTGA
  444  ------+---------+---------+---------+---------+----C----+---  503
        E  Q  P  Y  Y  L  N  L  L  E  L  F  L  Q  S  S  Y  Q  T  E

       GATCGGGCAGACTCTCGACCTCATCACAGCACCCCAGGGCCAAGTGGATCTTGGTAGATA
  504  ------+---------+---------+---------+---------+---------+---  563
        I  G  Q  T  L  D  L  I  T  A  P  Q  G  Q  V  D  L  G  R  Y

       CACTGAAAAGAGGTACAAATCTATCGTCAAGTACAAGACAGCTTTCTACTCTTTCTACCT
  564  ------+---------+---------+---------+---------+-------T-+---  623
        T  E  K  R  Y  K  S  I  V  K  Y  K  T  A  F  Y  S  F  Y  L

       GCCTATCGCGGCTGCCATGTACATGGCTGGAATTGATGGGGAGAAGGAACACGCTAATGC
  624  ------+---------+---------+---------+---------+---------+---  683
        P  I  A  A  A  M  Y  M  A  G  I  D  G  E  K  E  H  A  N  A

       CCTGAAGATCCTGCTGGAGATGGGCGAGTTCTTCCAGATCCAGGACGACTACCTTGATCT
  684  ------+---------+---------+---------+---------+---------+---  743
        L  K  I  L  L  E  M  G  E  F  F  Q  I  Q  D  D  Y  L  D  L

       CTTTGGAGACCCCAGTGTGACCGGAAAGGTCGGCACTGACATCCAGGACAACAAATGCAG
  744  ------+---------+---------+---------+---------+---------+---  803
        F  G  D  P  S  V  T  G  K  V  G  T  D  I  Q  D  N  K  C  S

       CTGGCTGGTGGTTCAGTGTCTGCTACGAGCCACTCCTCAGCAGCGCCAGATCTTAGAGGA
  804  ------+---------+---------+---------+---------+---------+---  863
        W  L  V  V  Q  C  L  L  R  A  T  P  Q  Q  R  Q  I  L  E  E

       GAATTATGGGCAGAAGGACCCAGAAAAAGTGGCGCGGGTGAAAGCACTGTACGAGGAGCT
  864  ------+---------+---------+---------+---------+---------+---  923
        N  Y  G  Q  K  D  P  E  K  V  A  R  V  K  A  L  Y  E  E  L

       GGATCTGCGGAGTGTGTTCTTCAAGTACGAGGAAGACAGTTACAACCGCCTCAAGAGTCT
  924  ------+---------+---------+---------+---------+---------+---  983
        D  L  R  S  V  F  F  K  Y  E  E  D  S  Y  N  R  L  K  S  L

       CATAGAGCAGTGCTCCGCGCCCCTGCCCCCATCCATCTTCCTGGAACTAGCAAACAAGAT
  984  ------+---------+---------+---------+---------+---------+---  1043
        I  E  Q  C  S  A  P  L  P  P  S  I  F  L  E  L  A  N  K  I

       CTACAAGCGGAGAAAGTAACCTCGAATTGTAGAGGCTGCGAGGGAGGGGTCTCAATAAAT
 1044  ------+---------+---------+---------+---------+---------+---  1103
        Y  K  R  R  K  *

       TATTGTTCAACAAAAAAAAAAAAAA
 1104  ------+---------+--------  1128
```

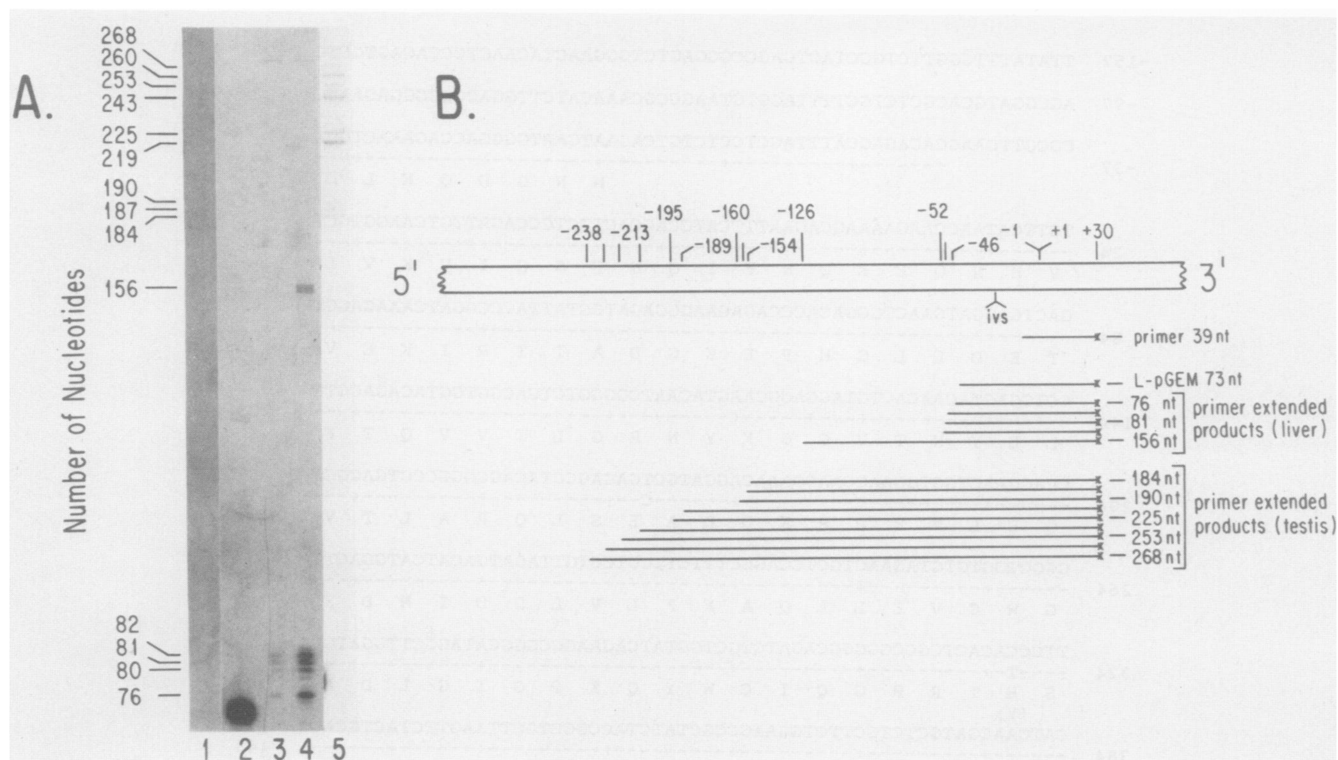

FIG. 4. Comparison of transcription initiation sites in rat testis and liver tissue at the 5′ end of the FPP synthetase gene mapped by primer extension analysis. (A) Poly(A)$^+$ RNA (8 μg) was hybridized with a 5′-^{32}P-end-labeled synthetic oligonucleotide (3.78×10^5 cpm/pmol) complementary to positions +30 to −9 of FPP synthetase cDNA. Primer-extended products were synthesized as described in Materials and Methods and analyzed on a 7 M urea–5% acrylamide sequencing gel. The gel was exposed to film for 7 days at room temperature. Lane 1, No RNA; lane 2, 8 ng of L-pGEM control (a transcript synthesized by SP6 polymerase with CR39 liver cDNA as the template); lane 3, 8 μg of liver poly(A)$^+$ RNA isolated from rats fed a diet supplemented with 5% cholesterol; lane 4, 8 μg of liver poly(A)$^+$ RNA isolated from rats fed a diet supplemented with 5% cholestyramine and 0.1% mevinolin; lane 5, 8 μg of testis poly(A)$^+$ RNA isolated from rats fed a normal chow diet. Primer-extended product sizes (indicated on the left) were determined with a DNA-sequencing reaction as a standard. (B) Structure of the corresponding region of the gene is shown. The position of the primer (+30 to −9) is indicated; +1 designates the A of the initiator methionine codon. An intron (ivs) interrupts the 5′ untranslated region of the gene between nucleotides −23 and −22. Beneath the primer, the extended products are shown as lines and the lengths (in nucleotides) correspond to the liver and testis primer extension products in panel A. The transcription initiation sites predicted from primer-extended products are identified by the numbered lines above the gene (from −46 to −238).

ase analyses. A complex pattern of transcription initiation sites was detected by primer extension (Fig. 4). A ^{32}P-labeled synthetic oligonucleotide complementary to positions +30 to −9 of FPP synthetase cDNA was hybridized with poly(A)$^+$ RNA and extended with reverse transcriptase. Analysis of liver RNA isolated from rats fed diets supplemented with either cholesterol or cholestyramine and mevinolin showed four shorter primer-extended products of 76 and 80 to 82 nucleotides (Fig. 4, lanes 3 and 4). A single longer extension product of 156 nucleotides was also observed in liver RNA isolated from rats fed the inducing diet (cholestyramine and mevinolin). This extension product was not detectable in RNA isolated from rats fed a diet supplemented with cholesterol. Two faint primer extension products of equal intensity are detectable at the top of the gel in lanes 3 and 4. Since the other primer extension products in lanes 3 and 4 reflect the different abundance of FPP synthetase mRNA levels, it seems likely that these long products from liver are artifacts. In the testis, nine major extended products were observed that ranged from 184 to 268 nucleotides (Fig. 4, lane 5). The data in Fig. 4 and from four other primer extension analyses (data not shown) indicated that the sites for transcription initiation in the liver occur within two discrete areas of the region from −46 to −126, while transcription initiation sites in the testis are dispersed over the region from −154 to −238.

To confirm these results, we mapped the 5′ ends of the FPP synthetase transcripts in liver and testis by S1 nuclease analysis. The same complex pattern of transcription initiation was observed from the data indicated in Fig. 5 and from five other S1 nuclease analyses (data not shown). S1 nuclease analysis was performed with a single-stranded end-labeled probe complementary to positions −27 to −352 of the FPP synthetase gene. In the testis, 12 major protected fragments with lengths that ranged from 124 to 214 nucleotides were observed (Fig. 5, lane 3), and the sites of transcription initiation spanned the region from −150 to −240 (Fig. 5B). This pattern was similar to those seen in primer extension assays. In the liver, four major protected fragments of 100, 101, 102, and 103 nucleotides were observed (Fig. 5, lane 5), and the sites of transcription initiation spanned the region from −126 to −129 (Fig. 5B). These fragments corresponded to the longest transcript observed in primer extension assays with liver RNA isolated from rats fed the the inducing diet (cholestyramine and mevinolin). The intervening sequence in the genomic clone (Fig. 5)

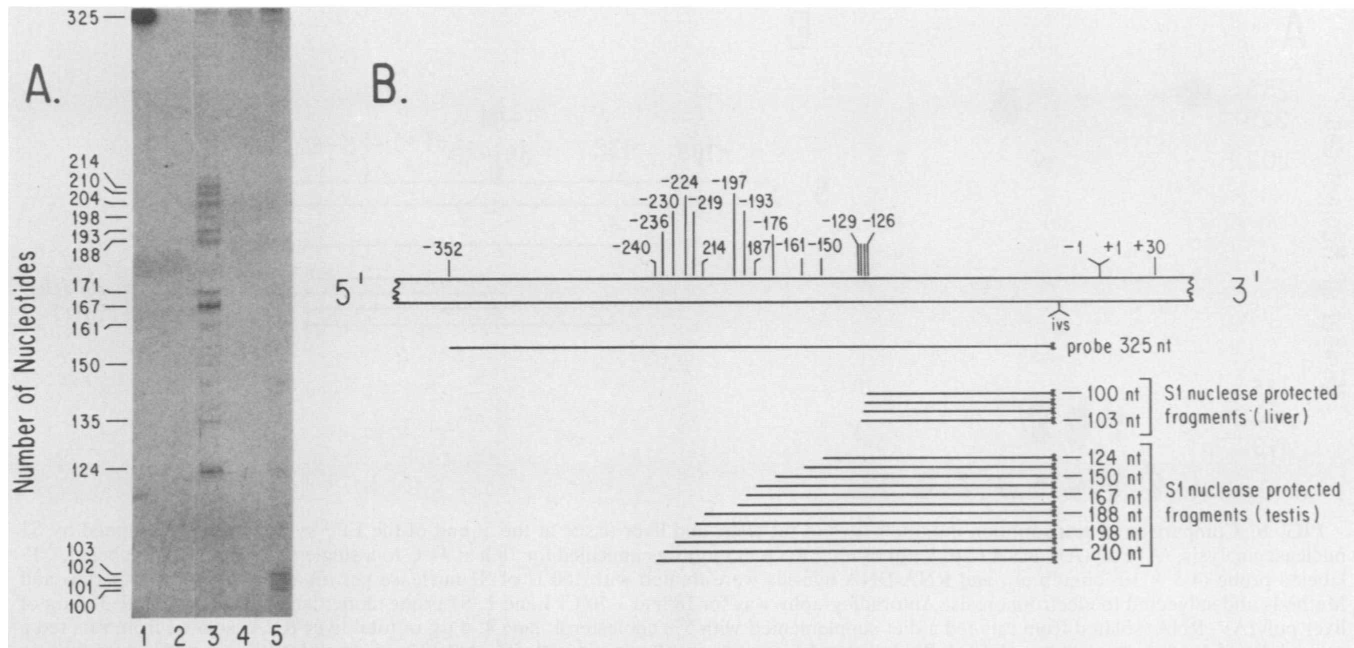

FIG. 5. Comparison of transcription initiation sites in rat testis and liver tissue at the 5' end of the FPP synthetase gene mapped by S1 nuclease analysis. (A) Poly(A)$^+$ RNA (2 to 16 μg) was annealed for 18 h at 54°C to a single-stranded, 5'-end-labeled, ^{32}P-labeled probe (8.99 × 10^3 cpm/fmol); RNA-DNA hybrids were treated with 1,000 U of S1 nuclease per ml as described in Materials and Methods and subjected to electrophoresis. Autoradiography was for 24 h at room temperature. Lane 1, S1 probe alone; lane 2, no RNA; lane 3, 2 μg of rat poly(A)$^+$ testis RNA isolated from rats fed a normal chow diet; lane 4, 16 μg of liver poly(A)$^+$ RNA isolated from rats fed a diet supplemented with 5% cholesterol; lane 5, 8 μg of liver poly(A)$^+$ RNA isolated from rats supplemented with 5% cholestyramine and 0.1% mevinolin. S1-protected product sizes are indicated and were determined with a DNA-sequencing reaction as a standard. (B) Segment of FPP synthetase genomic DNA encompassed by the S1 probe is shown. The S1 nuclease-protected products are shown as lines that correspond to the bands shown in panel A. The transcription initiation sites predicted from S1 nuclease-protected products are designated by the numbered lines above the gene (from −126 to −240).

(Spear et al., in preparation) precluded S1 protection analysis of the shorter and more common liver RNA species. As in primer extension analyses, the longest liver RNA product was not observed in S1 nuclease assays of RNA isolated from cholesterol-fed animals (Fig. 5).

To analyze the shorter, more abundant liver RNA species, we performed another S1 nuclease analysis using a single-stranded end-labeled probe complementary to positions +70 to −165 of the FPP synthetase gene. For this analysis, the testis cDNA (TF1.4) subcloned into M13mp9 provided the template for probe synthesis. In the liver, major protected fragments of 119, 124, 125, and 202 nucleotides were observed (Fig. 6, lanes 3 to 5). Again, as observed in primer extension and previous S1 nuclease analyses, the longest liver RNA product observed in cholestyramine- and mevinolin-fed animals was not observed in cholesterol-fed animals (Fig. 6, lane 3 versus lane 5) or in animals fed normal chow (lane 4). S1 analysis with testis RNA showed full protection of the probe and a major protected fragment of 229 nucleotides (Fig. 6, lane 6). The S1 probe, measuring 235 nucleotides, included an extra 3 nucleotides of the *Eco*RI linker for the testis cDNA (TF1.4) subcloned into M13mp9. Therefore, these three extra nucleotides are not part of the major testis protected fragment. In addition, some testis transcripts initiated three to seven nucleotides 3' of the TF1.4 cDNA (Fig. 4 and 5). Also included in this assay were kidney, adrenal, brain, and liver RNA isolated from rats fed a normal diet. These RNA samples showed protected fragments similar in size to those in the liver RNA isolated from rats fed a diet supplemented with cholesterol.

Analysis of translation efficiency. The nucleotide sequence of the 5' segment of the rat FPP synthetase gene mapping the rat testis and liver FPP synthetase transcription initiation sites is summarized in Fig. 7. Testis transcripts contain at least one and as many as two upstream initiator methionine codons. None of the ATG codons are in an optimal context, as defined by the consensus sequence (A/GCC AUG G) determined by Kozak (29). These ATG codons result in two upstream small open reading frames (ORFs) in the longest testis transcripts; ORF 1 begins at −214 and extends to the TGA at −136, and ORF 2 begins at −92 and extends to the TGA at −32 (Fig. 7). The start sites for the testis transcripts span positions −150 to −240; thus, all FPP synthetase testis transcripts contain ORF 2 and about 50% also contain ORF 1. The majority of liver transcripts initiate within the region −46 to −55 and thus do not encompass any upstream ATGs. However, the start sites for the less abundant, longer liver transcripts include the start of ORF 2 (Fig. 7). These longer liver transcripts were detectable only in rats fed diets supplemented with cholestyramine and mevinolin (Fig. 4 to 6).

On the basis of Kozak's scanning model of translation initiation (29), it seems likely that the testis FPP synthetase RNA would be translated less efficiently than liver FPP synthetase RNA. Total levels of FPP synthetase RNA in testis were comparable to levels in induced liver RNA (RNA isolated from rats fed diets supplemented with cholestyramine and mevinolin) (Fig. 1 and 2). Thus, we analyzed induced liver and normal testis RNA preparations for levels of functional FPP synthetase mRNA in an in vitro translation system. Both testis and liver RNA preparations yielded

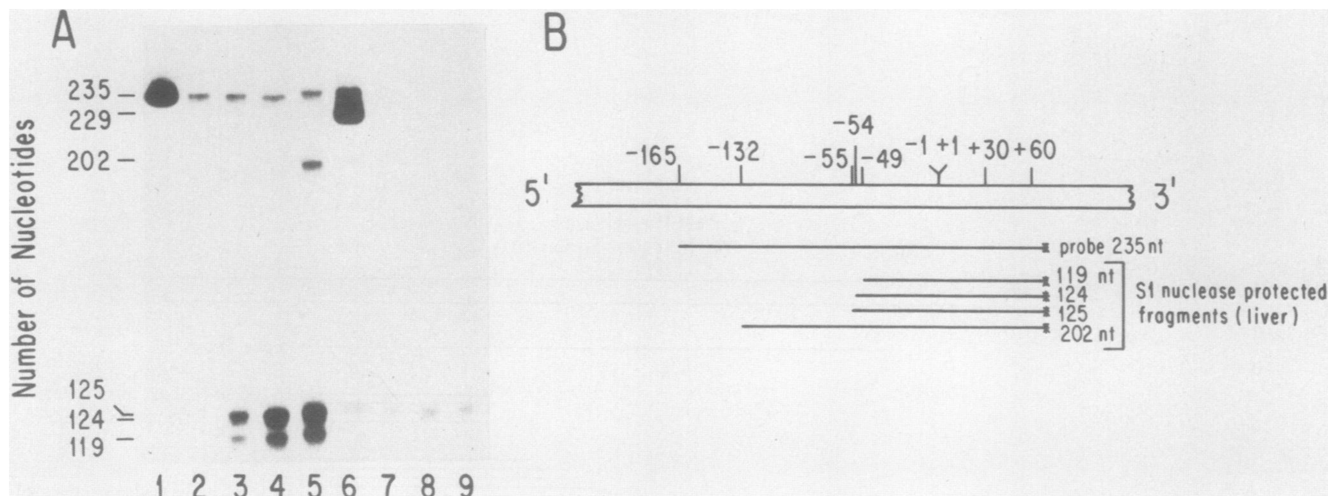

FIG. 6. Comparison of transcription initiation sites in rat testis and liver tissue at the 5′ end of the FPP synthetase gene mapped by S1 nuclease analysis. (A) Poly(A)$^+$ RNA (2 to 8 μg) or total RNA (15 μg) was annealed for 18 h at 44°C to a single-stranded, 5′-end-labeled, ^{32}P-labeled probe (4.5 × 10^3 cpm/fmol), and RNA-DNA hybrids were treated with 100 U of S1 nuclease per ml as described in Materials and Methods and subjected to electrophoresis. Autoradiography was for 18 h at −70°C. Lane 1, S1 probe alone; lane 2, no RNA; lane 3, 8 μg of liver poly(A)$^+$ RNA isolated from rats fed a diet supplemented with 5% cholesterol; lane 4, 4 μg of total liver RNA isolated from rats fed a normal diet; lane 5, 2 μg of liver poly(A)$^+$ RNA isolated from rats supplemented with 5% cholestyramine and 0.1% mevinolin; lane 6, 2 μg of rat poly(A)$^+$ testis RNA isolated from rats fed a normal chow diet; lane 7, 15 μg of total kidney RNA isolated from rats fed a normal diet; lane 8, 15 μg of total adrenal RNA isolated from rats fed a normal diet; lane 9, 15 μg of total brain RNA isolated from rats fed a normal diet. S1-protected product sizes are indicated and were determined with a DNA-sequencing reaction as a standard. (B) Segment of FPP synthetase genomic DNA encompassed by the S1 probe is shown. The testis cDNA (TF1.4) subcloned into M13mp9 provided the template for probe synthesis. The S1 nuclease-protected products are shown as lines that correspond to the bands shown in panel A. The transcription initiation sites predicted for the shorter liver fragments from S1 nuclease-protected products are designated by the numbered lines above the gene (from −49 to −132).

similar amounts of total protein synthesis for a given amount of RNA input as determined by trichloroacetic acid precipitation (data not shown). For purposes of comparison, the same amount of trichloroacetic acid-precipitable radioactivity was subjected to immunoprecipitation and analysis by gel electrophoresis. Figure 8 shows the results of immunoprecipitation of [^{35}S]methionine-labeled FPP synthetase polypeptide from rabbit reticulocyte lysates primed with either liver or testis RNA. The amount of FPP synthetase polypeptide was higher in translations primed with liver RNA than in those primed with testis RNA (compare lane 4 with lane 3 in Fig. 8). Four different in vitro translation experiments with two different testis and liver RNA preparations at various RNA concentrations gave similar results. The level of functional mRNA in testis was approximately four- to fivefold lower than the level of functional mRNA in induced liver. Since the level of total FPP synthetase RNA is very similar, it seems likely that the longer testis FPP synthetase RNA is translated less efficiently than liver FPP synthetase RNA.

Testis FPP synthetase 5′ untranslated sequences are present as a single gene copy. The S1 nuclease and sequence data suggested that the testis and liver transcripts originate from the same gene. However, the existence of at least five genes with high sequence identity to FPP synthetase in the rat genome could implicate two separate genes encoding testis- and liver-specific isoforms. To determine the number of copies of DNA encoding the segment of the 5′ untranslated FPP synthetase gene, we probed a rat genomic Southern blot with a portion of rat genomic DNA extending from nucleotide positions −47 to −364. The 327-base-pair *Eco*RI-*Sma*I genomic probe identified only a single band in each digest of rat DNA (Fig. 9, lanes 1 to 5), indicating that this genomic fragment is present as a single copy in the rat genome. Two other experiments gave similar results. This result is most consistent with the presence of a single gene copy of FPP synthetase capable of encoding both testis and liver transcripts. When the blot in Fig. 9 was stripped of probe and hybridized to the FPP synthetase 1.2-kb cDNA, a complex pattern of bands resulted (data not shown), consistent with previous results (14, 19).

DISCUSSION

In the present study, we identified very high levels of FPP synthetase mRNA in the testis of rats fed normal diets comparable to liver FPP synthetase mRNA levels in rats fed diets supplemented with cholestyramine and mevinolin. The FPP synthetase mRNA in testis was larger than the liver mRNA owing to an additional 5′ sequence not present in the liver FPP synthetase mRNA.

It is clear that in the liver, the production of FPP synthetase transcripts is regulated in response to cholesterol availability. However, RNA levels of FPP synthetase in the testis are not decreased by cholesterol feeding and are not induced by cholestyramine and mevinolin (data not shown). Since testis HMG CoA synthase RNA levels are also not responsive to different dietary regimens (data not shown), the lack of regulation may be due either to the inability of exogenous cholesterol or hypocholesterolemic drugs to exert their influence on the testis or to an altered mechanism of regulation.

The coding regions of the testis and liver mRNAs were identical except for four polymorphisms between the liver and testis FPP synthetase nucleotide sequence (Fig. 3), which probably represent different alleles of one gene. Sprague-Dawley rats are outbred and would be expected to

TTCATTGGCCAGTCGTCACAAGGCCCATAC

-290 -270

ORF1

TCACACGAGGTCATTCAGCCAATCAGCGAACTCGGCCTCGCCCATCATGAGGCGCAAGTG

-250 -230 -210

TCTTACTTGCGGCTTGGTCCGCGGCAGGCTGGGAGTCGTAGTATTTATATTTGGGTTCTGC

-190 -170 -150

ORF2

CTACTGAGCCGGGAGTCTGGGAACTACAACTCCCAGAGTGCTGAGCGGATGCACGCTCTG

-130 -110 -90

CTTTTAGGTGTAAGCCGCAAACATCTTGGACCCCGGGAGAATCCGCGTTGAAGCACAGAG

-70 -50 -30 ivs

AA112

CATTTAGCTCCTCTGTCAGAATGAATGGGGACCAGAAACTGGATGTTCATAACCAAGAAA

-10 +1 10 30

M60

AGCAGAATTTCATCCAGCACTTCTCCCAGATTGTCAAGGTGCTGACTGAGGATGAACTGG

50 70 90

M378

FIG. 7. FPP synthetase transcription initiation sites in rat testis and liver. Nucleotide sequence of the 5′ segment of the rat FPP synthetase gene is shown. The A of the ATG used to encode the initiator methionine codon of the FPP synthetase protein is designated +1, and the 5′ untranslated region and genomic DNA are indicated by negative numbers. The multiple transcription initiation sites determined by primer extension (Fig. 4) and S1 nuclease mapping (Fig. 5 and 6) are indicated by closed circles and squares, respectively, for liver or by open circles and squares, respectively, for testis. The oligonucleotide primer (M60) used in primer extension analyses is indicated by an arrow (below nucleotides) positioned at +30 to −9. The primers AA112 and M378 used for S1 probe synthesis are also indicated by arrows. The asterisks indicate the ends of liver FPP synthetase cDNA (−27) and of testis FPP synthetase cDNA (−157). An intervening sequence (ivs) present in the genomic sequence is indicated between positions −22 and −23 of the cDNA sequence. Putative promoter elements, upstream ATG codons, and the initiator ATG codon are boxed, and respective stop codons are underlined. Upstream ORFs 1 and 2 are indicated.

have allelic heterogeneity (2). The 5′ and 3′ untranslated regions of FPP synthetase testis and liver mRNAs were identical as determined by sequence analysis of testis and liver cDNAs (Fig. 3) and by S1 protection experiments (Fig. 5 and 6). Southern blot analysis of rat genomic DNA with a probe from the promoter region of the FPP synthetase gene was also consistent with one gene copy (Fig. 9). This probe included FPP synthetase genomic DNA from −47 to −364 and thus contained the 5′ untranslated region of the testis transcripts and of the long liver transcripts. Coding region probes encompassing part of the 5′ untranslated segment of the short liver transcripts hybridized to five rat genomic DNA restriction fragments (19). Some of these fragments have been identified as pseudogenes (Spear et al., in preparation). However, it is difficult to exclude the possibility that a separate FPP synthetase gene is responsible for producing the short liver transcripts. Given the sequence identity between the testis and liver transcripts in the 5′ untranslated region and the occurrence of a TATA-like promoter element upstream of the short liver transcript initiation site, this possibility seems unlikely.

The data taken together suggest that FPP synthetase is encoded by two classes of transcripts (Fig. 10). We hypothesize that one promoter is functioning in testis and directs the abundant synthesis of larger heterogeneous transcripts; another promoter functions in liver and other tissues such as

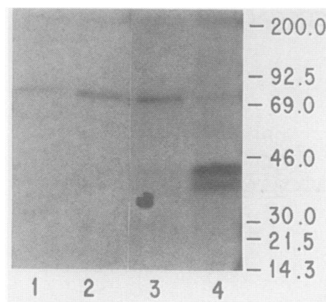

FIG. 8. In vitro translation of testis and liver RNA. Poly(A)$^+$ RNAs (3 μg) from either testis (lanes 1 and 3) or liver (lanes 2 and 4) were added to an in vitro translation system as described in Materials and Methods. Immunoprecipitates with either preimmune (lanes 1 and 2) or immune (lanes 3 and 4) IgG were analyzed on a 10% polyacrylamide gel. Protein size standards are indicated in kilodaltons. The two polypeptides of 39 and 34 kilodaltons in lane 4 may represent limited proteolysis; the smaller product was not routinely observed.

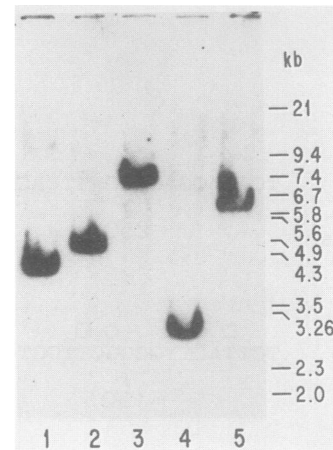

FIG. 9. Genomic Southern blot analysis. Rat genomic DNA (10 μg) was digested with various restriction endonucleases and analyzed on a 1% Tris-acetate agarose gel. Lane 1, *Pst*I; lane 2, *Bgl*II; lane 3, *Sst*I; lane 4, *Eco*RI; lane 5, *Hin*dIII. Southern blot analysis was performed as described in Materials and Methods with a 327-base-pair *Eco*RI-*Sma*I rat genomic fragment as a probe (2.1 × 10^5 cpm/ng). The blot was washed under stringent conditions: 65°C, 1× SSC–0.1% SDS. Autoradiographic exposure time was 9 days. λ DNA digested with *Eco*RI and *Hin*dIII provided size standards. Positions of the bands are indicated.

kidney, brain, and adrenal gland and directs the regulated synthesis of shorter, discrete transcripts.

There are many examples and various mechanisms for multiple mRNAs that arise from one gene (5). Examples of genes with multiple promoters include the mouse α-amylase gene (55), the human and rat aldolase A gene (23), the acetyl-CoA carboxylase gene (24, 31), and the porphobilinogen deaminase gene (9). Other examples include the proto-oncogenes, many of which have two promoters. Transcripts from one promoter have short uncomplicated leader sequences; transcripts from the other promoter are inefficiently translated and have a long, G+C-rich leader sequence, with upstream out-of-frame AUG codons (28). Transcription initiation sites in genes with multiple promoters can be tissue specific such as the mouse α-amylase gene or cell-type specific. The presence of multiple promoters allows additional control and greater flexibility for gene expression and generates diversity. Genes with multiple promoters often exhibit developmental stage-specific expression (50). Further study is required to determine whether the long FPP synthetase transcripts are localized to specific cell types within the testis.

Despite the high FPP synthetase mRNA levels in the testis, polypeptide levels are low (1). This disparity can be explained by Kozak's scanning model for translation initiation (29). In this model, ribosomes scan an mRNA from the 5′ end and initiate translation at the first AUG encountered. Initiation codons in an optimal context tend to be favored over those in other less optimal contexts. There is evidence that the presence of upstream AUGs can dramatically decrease translation at the +1 AUG. For example, insertion of an upstream ORF that terminated 45 nucleotides from the +1 AUG caused an 80% decrease in synthesis compared with a control mRNA that lacked an extra upstream ORF (27). Thus, the efficiency of translation of the longer liver transcripts and, more importantly, the efficiency of translation of the majority of the testis transcripts should be impaired since these transcripts include the start of the second ORF (Fig. 10). Our studies confirmed this prediction; in vitro translation of liver and testis RNA with rabbit reticulocyte lysates showed the efficiency of translation of liver FPP synthetase mRNA to be four- to fivefold greater than testis FPP synthetase mRNA (Fig. 8). The efficiency of translation of the shorter, major liver transcripts is evidently much greater since these transcripts do not include any upstream ORFs.

Another enzyme of the isoprene biosynthetic pathway, HMG CoA reductase, has been shown to be under translational control (39, 42). Hamster HMG CoA reductase mRNAs have heterogeneous 5′ untranslated regions and can contain an extremely long 5′ untranslated region containing eight AUG codons upstream of the start codon and a potential stem-and-loop structure (44). However, the 5′ region of human HMG CoA reductase mRNA is much simpler than that of the hamster and lacks upstream AUG codons (32). It is not known whether these structural factors play a role in translational regulation of HMG CoA reductase. A nonsterol mevalonate-derived product may also be involved in translational control (39, 42).

In summary, it is not clear what function is served by the abundant production of an inefficiently translated, larger FPP synthetase transcript in the testis. An example of a testis-specific mRNA that is translated at low efficiency is an isoform of α-tubulin. In mice, humans, and the macaque monkey, an alternate testis-specific promoter creates a testis-specific exon 1 that has the curious feature of providing a CAG codon in place of an AUG initiator methionine codon (15). Expression of this same α-tubulin isoform in other somatic tissues makes use of an exon 1 that provides an AUG codon. Translational control of the FPP synthetase and the α-tubulin isoform polypeptides in the testis may play a role in regulating gene expression or may be related to the temporal and spatial appearance of message levels and protein levels associated with a specific developmental stage in spermatogenesis.

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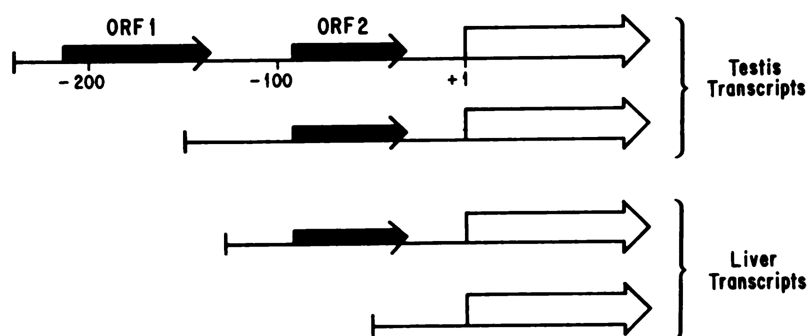

FIG. 10. Translation start sites in FPP synthetase mRNAs in testis and liver. Two classes of transcripts are shown. Testis-specific transcripts contain either one or two upstream ORFs (ORF 1 and ORF 2 [black bars]). Testis transcripts initiate at dispersed sites between the 5' boundaries shown. Liver transcripts initiate at two discrete sites as indicated. The FPP synthetase ORF initiates at +1 and is designated by the large open arrow.

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