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Molecular Cloning and Sequence of a Cholesterol-Repressible Enzyme Related to Prenyltransferase in the Isoprene Biosynthetic Pathway

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Differential hybridization and molecular cloning have been used to isolate CR39, a cDNA which hybridizes to a 1.2-kilobase (kb) mRNA in rat liver. The level of CR39 mRNA was increased seven- to ninefold over normal levels by dietary cholestyramine and mevinolin and decreased about fourfold compared with normal levels by cholesterol feeding or administration of mevalonate. Similar changes in the mRNA levels of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase and HMG-CoA synthase were observed under the various conditions. In vitro translation of either CR39 hybrid selected RNA or 1.2-kb CR39 RNA generated by an SP6 in vitro transcription system produced a polypeptide of 39,000 daltons. As deduced from the nucleotide sequence of a full-length CR39 cDNA, the rat CR39 polypeptide contained 344 amino acids and had a molecular weight of 39,615. The predicted amino acid composition and submit molecular weight of the rat CR39 were very similar to those of prenyltransferases isolated from chicken, pig, and human. The sequence of amino acid residues 173 through 203 in the rat CR39 polypeptide showed that 17 out of 30 matched an active-site peptide of avian liver prenyltransferase. Thus, alterations in the rate of cholesterogenesis resulted in the coordinate regulation of three mRNAs encoding HMG-CoA reductase, HMG-CoA synthase, and CR39, the latter being tentatively identified as prenyltransferase.

Regulation of the isoprene biosynthetic pathway determines the amount of sterol and nonsterol products synthesized by each cell (10). The regulation of this pathway is exemplified by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. Reductase activity levels are rapidly and profoundly changed in response to a variety of conditions, including fasting and refeeding (the diurnal cycle) and cholesterol feeding and also by agents which alter the sterol balance (26; C. F. Clarke, A. M. Fogelman, and P. A. Edwards, *in* A. M. Gotto, ed., *Plasma Lipoproteins*, in press). Feeding of cholestyramine, a bile acid sequestrant, and mevinolin, a competitive inhibitor of reductase, to rats results in increased transcription of the hepatic reductase gene (19), increased rate of reductase polypeptide synthesis, and decreased rate of reductase degradation (27). Administration of mevalonate, the end product of the reaction catalyzed by reductase, causes a rapid increase in the rate of degradation of the reductase polypeptide (28) and decreased transcription of the reductase gene within 30 min (19). Hence reductase transcription, synthesis, and degradation are all modulated and result in dramatic changes in enzyme activity.

While the activity of reductase is considered to be rate controlling in the isoprene biosynthetic pathway (58), the other enzymes leading to the formation of HMG CoA are also regulated. The mRNA levels of HMG-CoA synthase, the enzyme that precedes HMG-CoA reductase in the cholesterogenic path, have recently been shown to change coordinately with reductase mRNA (33, 48). The activities of acetoacetyl-CoA thiolase (20) and acetoacetyl-CoA synthetase (5) also change coordinately with the activity of HMG-CoA reductase in response to agents which alter hepatic cholesterol biosynthesis. Cells in culture coordinately regulate the activity of many of the enzymes mentioned above in response to changes in the cholesterol content of the growth medium (13). Chang and co-workers (12, 13) have provided evidence that this coordinate control is due to a common factor.

Regulation is also exerted on enzymes in the isoprene biosynthetic pathway distal to mevalonate formation. Gould and Swyryd (35) demonstrated that one or more of the five enzymes falling between mevalonate formation and squalene synthetase showed considerably less activity in cholesterol-fed rats than in control rats. Many of these enzymes, including pyrophosphomevalonate decarboxylase, isopentenyl pyrophosphate isomerase, and prenyltransferase, exhibit 8- to 14-fold changes in activity in response to fasting and refeeding (61). The activities of later enzymes, such as squalene synthetase, are also regulated. The activity of squalene synthetase in cells in culture has been shown to be feedback regulated by the low-density lipoprotein content of the growth medium (30). It has been proposed that squalene synthetase provides branchpoint regulation by modulating the flux of farnesyl pyrophosphate into sterols as opposed to the nonsterol products dolichol and ubiquinone (31).

We decided to take advantage of the regulation described above and isolate members of the isoprene biosynthetic pathway based on the differential hybridization of a rat liver cDNA library to [^{32}P]cDNA probes prepared from rat liver mRNA isolated from animals having induced or repressed rates of cholesterogenesis. Genes exhibiting coordinate regulation with HMG-CoA reductase would be identified by

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virtue of the induction of mRNAs by dietary cholestyramine and mevinolin and the repression of mRNAs by dietary cholesterol or mevalonate. The technique of differential hybridization has been used quite successfully to isolate HMG CoA reductase (14) and HMG CoA synthase (44) from the compactin-induced cell lines. This technique has been used to isolate induced genes in a variety of systems, including genes involved in the response to heat shock (7) and DNA damage (47), and in identifying and isolating genes responsive to growth factors (21) or hormones (41).

This work identifies another regulated member of the isoprene biosynthetic pathway, CR39, a gene which encodes a cholesterol-repressible 39,000-dalton polypeptide. The sequence of a full-length cDNA is presented, and its probable function as a prenyltransferase is discussed.

MATERIALS AND METHODS

Materials. ^{32}P-labeled nucleotides and Biodyne nylon membranes were obtained from ICN Pharmaceuticals, Inc. [^{35}S]methionine (specific activity, ≈1,000 Ci/mmol) was purchased from Amersham Corp. Oligonucleotides were synthesized by the phosphoramidite method (39) by Dohn Glitz on a DuPont/Vega Coder 300 DNA synthesizer. Mevinolin was a gift from A. Alberts (Merck, Sharpe and Dohme). Cholestyramine (Questran) was obtained from Johnson and Mead. Male Sprague-Dawley rats were purchased from Simonsen Laboratories. SP6 RNA polymerase, RNasin, and pGEM-2 were purchased from Promega Biotech. Calf intestinal phosphatase was obtained from Boehringer Mannheim. Avian myeloblastosis virus reverse transcriptase was obtained from Life Sciences, Inc. All restriction enzymes and other enzymes used in cDNA cloning and sequencing were obtained from Bethesda Research Laboratories.

cDNAs used in this study include a partial hamster reductase cDNA, pRed-10 (14), a full-length hamster reductase cDNA, λDS11 (60), a partial rat HMG-CoA synthase cDNA (48), and a mouse actin cDNA (obtained from Robert La Polla and Norman Davidson).

General methods. Preparation of phage and plasmid DNA, restriction enzyme digestions, and agarose gel electrophoresis were carried out by standard procedures as described in Maniatis et al. (45). DNA sequencing of CR39 cDNA clones were performed by the dideoxy chain termination method of Sanger et al. (59) with either the M13 universal primer or specific oligonucleotides. When necessary, GC-rich regions of subclones were analyzed on 9% polyacrylamide gels containing 100 mM Tris borate (pH 8.3), 7 M urea, and 38% (vol/vol) formamide (23).

Rat liver RNA isolation and Northern blotting. Male Sprague-Dawley rats (80 to 100 g) were housed under a 12-h light–12-h dark cycle for at least 10 days prior to use. Rats were fed one of several diets: a regular chow diet, chow supplemented with 5% cholesterol, or chow supplemented with 5% cholestyramine and 0.10% mevinolin as described previously (19). Mevalonate (1.0 mg/g of body weight) was administered to some animals as an intragastric dose 3 h prior to RNA isolation. Rats were killed in the middle of the 12-h dark cycle, and total RNA was isolated from the liver by guanidium thiocyanate extraction and centrifugation through a cesium chloride cushion (15). Polyadenylated [poly(A)$^+$] RNA was isolated on oligo(dT)-cellulose as described (15).

RNA was fractionated on 1% agarose gels containing formaldehyde (42, 45), transferred to a Biodyne nylon membrane, and UV-crosslinked by the method of Church and Gilbert (16). Transferred RNA was then prehybridized for 1 h at 65°C in 0.50 M sodium phosphate (pH 7.0)–1.0% bovine serum albumin–7% sodium dodecyl sulfate (SDS)–1 mM EDTA and then hybridized for 10 to 16 h under the same conditions to the designated ^{32}P-labeled cDNA clones. DNA probes were labeled with ^{32}P as described by Feinberg and Vogelstein (32). Incorporation of ^{32}P into the cDNA probes ranged from 5×10^8 to 2×10^9 cpm/μg. Hybridization mixtures contained from 1 to 2 ng of ^{32}P-labeled probe per ml of hybridization buffer. Blots were washed as suggested by the manufacturer and exposed to X-ray film by means of a DuPont Cronex Hi-Plus intensifying screen. Blots were stripped free of probe as recommended by ICN Pharmaceuticals.

Construction and screening of a rat liver cDNA library. Poly(A)$^+$ RNA from rats fed a diet supplemented either with cholesterol or with cholestyramine and mevinolin (see above) was used as a template for cDNA synthesis by the procedure described by Gubler and Hoffman (37). The "induced" cDNA (derived from cholestyramine- and mevinolin-fed rats) was incorporated into the *Eco*RI site of the λgt11 vector by the methods of Young and Davis (66, 67). The induced cDNA was methylated at *Eco*RI sites, blunt-ended with T4 DNA polymerase, and ligated to *Eco*RI linkers. *Eco*RI-digested cDNA was ligated to *Eco*RI-digested λgt11 vector that had been treated with calf intestinal phosphatase. About 22 ng of induced cDNA per μg of λgt11 DNA was used for the ligation. The ligated DNA was packaged by using an extract purchased from Amersham, and the bacteriophage were amplified in *Escherichia coli* γ1088 to yield 1.1×10^6 PFU. Approximately 98% of the phage contained inserts, and about 50% of these inserts were larger than 500 base pairs (bp).

Approximately 40,000 recombinants of the rat liver cDNA library were plated (10,000 PFU per 150-mm-diameter petri dish) and transferred to nylon membranes (4). Recombinants were screened by differential plaque hybridization with ^{32}P-labeled single-stranded cDNAs generated from either cholesterol-fed rats (suppressed) or from rats fed chow supplemented with cholestyramine and mevinolin (induced). Reverse transcription of rat liver poly(A)$^+$ mRNA in the presence of [α-^{32}P]dCTP was performed as described previously (62). Several bacteriophage recombinants that hybridized more intensely to the induced [^{32}P]cDNA than to the suppressed [^{32}P]cDNA were taken through three or more rounds of plaque purification.

RESULTS

Isolation of CR39 by differential hybridization. The conditions used for generating the induced and suppressed cDNAs were chosen to maximize the signal-to-background ratio. Since cholestyramine and mevinolin act synergistically to increase HMG-CoA reductase mRNA (17) and cholesterol feeding suppresses HMG-CoA reductase mRNA levels (43), it seemed likely that other enzymes involved in cholesterogenesis might behave similarly. A screen of 40,000 rat liver cDNA clones by differential hybridization yielded more than 20 plaques which gave stronger hybridization signals when probed with induced [^{32}P]cDNA, made from poly(A)$^+$ RNA isolated from rats fed a cholestyramine- and mevinolin-supplemented diet, than with [^{32}P]cDNA prepared from poly(A)$^+$ RNA isolated from cholesterol-fed rats. Each of these recombinants was taken through several rounds of plaque purification. To be certain that the induction was not due to a phenomenon unrelated to the isoprene or choles-

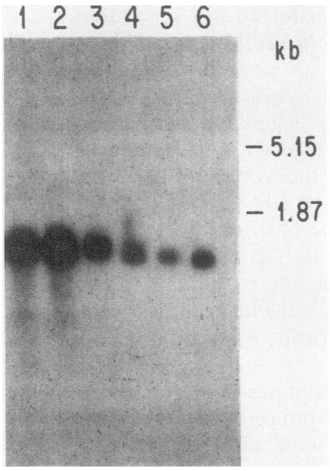

FIG. 1. Regulation of CR39 mRNA levels in rat liver. Liver poly(A)$^+$ RNA was isolated from rats fed the following diets: lanes 1 and 2, chow supplemented with 5% cholestyramine and 0.1% mevinolin; lane 3, normal chow; lane 4, normal chow plus mevalonate; lanes 5 and 6, chow supplemented with 5% cholesterol. Mevalonate (1.0 mg/g of body weight) was administered as an intragastric dose 3 h prior to RNA isolation. The same amount (10 μg) of poly(A)$^+$ mRNA was applied to each lane of an agarose-formaldehyde gel, and subsequent Northern blotting analysis with ^{32}P-labeled CR39 cDNA was performed as described in the text. Autoradiographic exposure time was 6 h.

terol biosynthetic pathway (for example, induction of P450 drug metabolism pathways), a second criterion of regulation was used. Since a response to mevalonate administration is characteristic of the regulation of HMG-CoA reductase (19) and HMG-CoA synthase (48), we limited our study to those cDNA recombinants that hybridized to mevalonate-repressible mRNA species. One of the first three recombinants examined, CR39 (for cholesterol repressible), exhibited such regulation and contained a cDNA insert of 220 bp. Figure 1 shows the regulation of hepatic CR39 mRNA in response to dietary cholestyramine and mevinolin, cholesterol, or mevalonate. The decrease seen in CR39 mRNA in response to mevalonate administration indicates that a product of mevalonate metabolism is involved in the rapid regulation of CR39 mRNA levels. Also evident in Fig. 1 is the cholesterol-mediated decrease in CR39 mRNA levels compared with levels in the liver of a normal chow-fed rat. Based on the mobilities of 28S and 18S rRNAs (11, 64), we estimate the size of the CR39 transcript to be about 1,200 nucleotides.

The Northern blot in Fig. 1 was washed free of CR39 probe and hybridized to cDNAs encoding actin, HMG-CoA reductase, and HMG-CoA synthase. Densitometry of autoradiographs from several different Northern blots was used to quantitate the relative levels of transcripts. Table 1 shows the extent of regulation for CR39, HMG-CoA synthase, and HMG-CoA reductase mRNAs. Levels of actin mRNA were used to normalize for the amount of poly(A)$^+$ RNA analyzed under the various dietary conditions. For purposes of comparison, the induced level (cholestyramine and mevinolin) was set at 100%. The levels of CR39 mRNA were increased seven- to ninefold over normal levels by dietary cholestyramine and mevinolin and decreased fourfold by cholesterol feeding. Administration of mevalonate to a normal chow-fed rat decreased CR39 mRNA levels about fourfold within 3 h, while mevalonate administration to animals previously induced by cholestyramine and mevinolin caused a twofold decrease in the levels of CR39 mRNA. All the observed changes in CR39 mRNA levels were paralleled by similar changes in the hepatic levels of mRNA for both HMG-CoA reductase and HMG-CoA synthase (Table 1) (19, 48).

CR39 mRNA encodes a 39,000-dalton polypeptide. To further characterize the CR39 mRNA, a full-length clone was selected from the cDNA library by using the 220-bp insert of the original isolate. A recombinant (CR39-1.2) which contained an insert of about 1.2 kb was selected. To ascertain whether this clone was in fact full length and to determine the size of the protein encoded by CR39 mRNA, the experiment shown in Fig. 2 was performed. The 1.2-kb insert of CR39-1.2 was isolated and inserted into the *Eco*RI restriction site of pGEM-2, a vector containing a promoter for SP6 RNA polymerase. Transformants containing the CR39 insert in each of the two possible orientations were selected. The pGEM-CR39 plasmids were isolated, linearized with *Hin*dIII, and used as templates with SP6 RNA polymerase to generate radiolabeled CR39 RNA in both the sense and antisense orientations. Sense and antisense CR39 RNAs were identified by testing for their hybridization to CR39 mRNA in a Northern blot analysis (data not shown). Both the sense and antisense pGEM-CR39 plasmids were then used as templates with SP6 RNA polymerase and a cap analog, m^{7}G-(5′)-ppp-(5′)-Gp, to synthesize capped CR39 mRNA. Such in vitro-synthesized capped RNA is able to serve as a translatable mRNA in rabbit reticulocyte in vitro translation systems (52). Capped CR39 mRNA corresponding to antisense (Fig. 2, lane 2) or to sense (lanes 3 and 4) orientation was translated in vitro in a rabbit reticulocyte translation extract. Only the sense RNA directed the synthesis of a unique polypeptide of 39,000 daltons (lanes 3 and 4). The other polypeptides in Fig. 2 were also generated when only water was added to the translation reaction (lane 1). To determine whether the 39,000-dalton polypeptide was actually a full-length product, CR39 RNA was isolated from rat liver poly(A)$^+$ RNA by hybrid selection and added to the rabbit reticulocyte in vitro translation system. The CR39-1.2 hybrid-selected RNA also directed the synthesis of the 39,000-dalton polypeptide (lane 5, Fig. 2), while the RNAs hybrid-selected by the pUC19 vector sequence alone produced only the background products (lane 6). We conclude

TABLE 1. Comparison of CR39 mRNA levels with HMG-CoA synthase and HMG-CoA reductase mRNA levels[a]

Dietary supplement	Relative mRNA level (%)		
	CR39	HMG-CoA synthase	HMG-CoA reductase
Cholestyramine and mevinolin	100	100	100
Cholestyramine and mevinolin + mevalonate (3 h)	51	32	22
Normal diet	11	17	17
Mevalonate (3 h)	2.7	2.6	ND[b]
Cholesterol	2.8	2.2	ND

[a] The blot shown in Fig. 1 was washed free of CR39 probe and hybridized sequentially to ^{32}P-labeled cDNAs encoding hamster HMG-CoA reductase, rat HMG-CoA synthase, and mouse actin. The blot was washed and exposed to X-ray film as described in the legend to Fig. 1. Densitometric scanning of the resulting films and of other Northern blot experiments (data not shown) was used to quantitate relative RNA levels. The values have been normalized for the amount of poly(A)$^+$ RNA by using actin as an invariant mRNA under these dietary conditions. For comparison, the level of mRNA in the maximally induced state (cholestyramine- and mevinolin-supplemented diets) was assigned a value of 100%.

[b] ND, Not detectable.

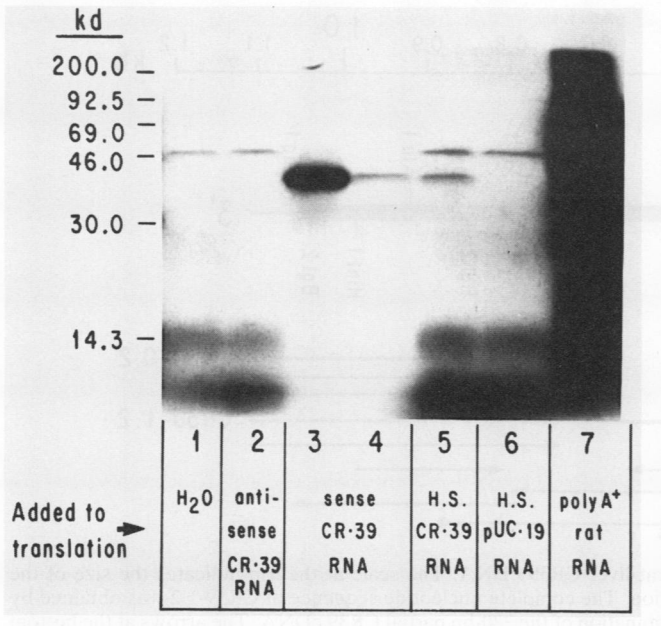

FIG. 2. In vitro translation of CR39 mRNA yields a polypeptide of 39,000 daltons. CR39 mRNA was either synthesized with SP6 RNA polymerase and the pGEM-2 SP6 promoter-containing vector or isolated by hybrid selection. For in vitro synthesis, the *Eco*RI insert of a full-length CR39 cDNA (CR39-1.2) was ligated to the *Eco*RI site of pGEM-2, a vector containing a promoter for SP6 RNA polymerase. pGEM-CR39 clones containing the CR39 1.2-kb insert in both orientations were identified by restriction endonuclease mapping. Both sense and antisense RNAs were synthesized in vitro by using SP6 polymerase, the sense and antisense pGEM-CR39 plasmid DNAs, and the cap analog m^7G-(5')-ppp-(5')-Gp. Synthesis of RNA was done exactly as described by Pelletier and Sonenberg (52). Hybrid selection was performed as described (45, 51) with liver poly(A)$^+$ RNA isolated from rats fed cholestyramine- and mevinolin-supplemented diets. Filters used in the hybrid selection contained either pUC19 vector DNA or the CR39 1.2-kb insert ligated into the pUC19 *Eco*RI site. In vitro translation reactions with rabbit reticulocyte lysate were performed as described (18), and reaction mixtures contained 50 μl of rabbit reticulocyte translation mix, 7 μl of [^{35}S]methionine, and 5.5 μl of the designated RNA sample. Portions (1 μl in lanes 1, 2, and 5 to 7 and 0.1 and 0.01 μl in lanes 3 and 4, respectively) of each translation reaction mixture were subjected to SDS-polyacrylamide gel electrophoresis and fluorography. Protein sizing standards are indicated (in kilodaltons). Additions to the in vitro translation reaction were: lane 1, water; lane 2, antisense (noncoding strand) CR39 RNA; lanes 3 and 4, sense (coding) CR39 RNA; lane 5, RNA hybrid selected (H.S.) with filters containing CR39-1.2 cDNA; lane 6, RNA hybrid selected with filters containing pUC19 DNA; lane 7, rat liver poly(A)$^+$ RNA.

that rat liver CR39 mRNA encodes a polypeptide of 39,000 daltons and that the CR39 1.2-kb cDNA contains the complete amino acid coding region.

Determination of the complete sequence of a full-length CR39 DNA. Figure 3 shows the restriction endonuclease map of CR39-1.2 and the sequencing strategy employed. Also shown is the partial CR39 cDNA initially obtained. The full-length cDNA corresponded to an mRNA 1.2 kb in length, and there were 1,035 bases in the coding region.

Figure 4 shows the complete nucleotide sequence of CR39-1.2 and the predicted amino acid sequence of the 39,615-dalton protein. A computer search for homology with the nucleotide sequence of CR39-1.2 showed no significant homology to sequences in the Genbank data bank (August 1986 version). However, 17 of 30 amino acids between 173 through 203 were identical (underlined residues in Fig. 4) with a 30-amino-acid active-site peptide of chicken liver prenyltransferase (8, 9). The sequence of the active-site peptide from chicken liver prenyltransferase and the corresponding sequence from CR39 are presented in Fig. 5. Identical residues in the two sequences are circled, and conservative substitutions are indicated by asterisks. Arginine has been implicated in the active site of pig liver prenyltransferase (2), and the conservation of arginine between the chicken sequence and the rat sequence is indicated (underlined in Fig. 5). Table 2 lists the amino acid composition of prenyltransferase enzymes from human, pig, and chicken liver, along with the amino acid composition of CR39. The composition of CR39, high in hydrophobic residues and containing few cysteine residues, was similar to that of the other prenyltransferases. The subunit molecular weights of the prenyltransferases (Table 2) were very similar, ranging from 38,400 (human) to 42,500 (chicken). In fact, all eucaryotic prenyltransferases studied so far have been dimeric proteins with molecular weights of about 80,000 (57).

Restriction analysis of CR39 rat genomic DNA. CR39-1.2 was labeled with ^{32}P and hybridized to restricted rat genomic DNA (Fig. 6A). The pattern of bands hybridizing to CR39-1.2 indicated that the rat CR39 gene was either large and contained many introns or was present in more than one copy. Two other experiments gave the same results. Analysis of the same blots with a HMG-CoA reductase probe (B1 [56]) gave the expected simple pattern of one restriction fragment in each lane (data not shown). These results indicate that the restriction pattern in Fig. 6A is not due to incomplete restriction enzyme digestion.

The organization of the CR39 gene was further investigated in the experiments shown in Fig. 6B and C. *Pst*I fragments of the CR39-1.2 cDNA (see Fig. 3) were isolated and used separately to probe filters containing rat genomic DNA that had been subjected to the same restriction analysis described for panel 6A. The 5′ and 3′ *Pst*I fragments did not hybridize to each other as determined by blot hybridization analysis (data not shown). The 5′ and 3′ *Pst*I fragments of CR39-1.2 cDNA both hybridized to five of the same fragments that also hybridized to the full-length probe (lanes 1, Fig. 6). The other two digests also showed four or five fragments hybridizing to both the 5′ and 3′ probes of CR39-1.2. In fact, there were very few restriction fragments which hybridized uniquely to either the 5′ or the 3′ probe of CR39-1.2. Since the genomic DNA was restricted with enzymes that did not have sites within the cDNA, each gene copy of CR39 could generate at most one fragment which would hybridize with both the 5′ and 3′ probes. From this analysis, we conclude that there are a minimum of five gene copies hybridizing to the CR39-1.2 cDNA. When the blots in Fig. 6 were analyzed under less stringent washing conditions, the data indicated the presence of one to two additional gene copies (data not shown).

DISCUSSION

The current study has identified an additional regulated gene in the isoprene biosynthetic pathway. We refer to this gene as CR39, since cholesterol feeding repressed its mRNA levels and the protein encoded had a molecular weight of 39,000. This molecular weight was determined by in vitro transcription-translation (Fig. 2) and from analysis of the sequence of CR39 cDNA (Fig. 4). However, no stop codon was present upstream of the initiator methionine (Fig. 4).

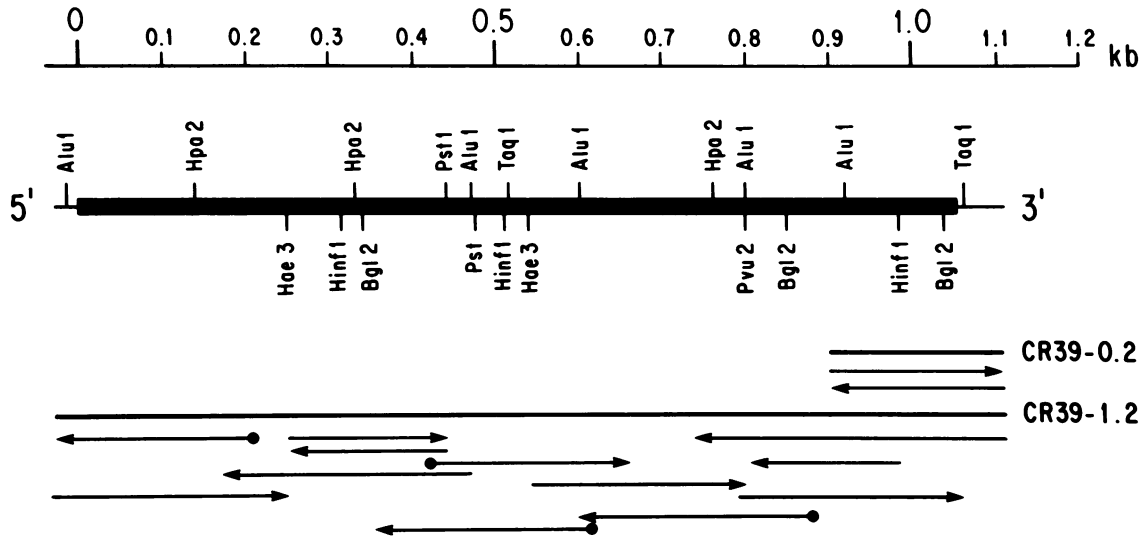

FIG. 3. Restriction endonuclease map and sequencing strategy for rat liver CR39 cDNA. The scale at the top indicates the size of the cDNA in kilobases, starting with the beginning of the protein-coding region. The complete nucleotide sequence of CR39-1.2 was obtained by the dideoxy chain termination method. Also shown is the sequence determination of the 220-bp partial CR39 cDNA. The arrows at the bottom indicate the portion of sequence determined in a given experiment. Arrows with circles indicate the use of a synthetic oligonucleotide as a primer. The protein-coding region for CR39 (amino acids) is indicated by the thick black line, and selected restriction endonuclease sites are indicated.

Hence, we cannot exclude the possibility that there may be a few additional amino acids at the amino terminus of the protein. The repression of CR39 mRNA levels by both cholesterol and mevalonate and the induction of CR39 mRNA levels by cholestyramine and mevinolin suggest that the CR39 gene may be responding to the same agents that alter HMG-CoA reductase and HMG-CoA synthase mRNA levels. We have not yet determined whether the changes in CR39 mRNA levels are due to transcriptional or posttranscriptional controls. However, the rapid decrease of CR39 mRNA levels that occurred within 3 h of mevalonate administration was consistent with coordinate regulation of CR39 with HMG-CoA reductase and HMG-CoA synthase, since the mRNAs for these two enzymes also decreased rapidly under the same conditions (Table 1). CR39, HMG-CoA reductase, and HMG-CoA synthase mRNA levels all decline rapidly after addition of 25-hydroxycholesterol to cultured cells (D. Rosser and P. Edwards, unpublished data). Those preliminary results indicate that hydroxysterols also coordinately regulate the levels of mRNA for the three proteins and provide further support for a common regulatory mechanism.

The 39,615-dalton protein encoded by CR39 contained a sequence from amino acids 173 through 203 that showed 17 of 30 residues identical with the active-site peptide of chicken liver prenyltransferase. The liver prenyltransferase catalyzes two condensation reactions: (i) dimethylallyl pyrophosphate is transferred to isopentenyl pyrophosphate to form geranyl pyrophosphate, and (ii) geranyl pyrophosphate is condensed with another isopentenyl pyrophosphate to form farnesyl pyrophosphate (for a review of prenyltransferase chemistry, see reference 53). The extent of amino acid homology suggests that CR39 is functioning as a prenyltransferase. The coordinate regulation of CR39 mRNA levels with those of HMG-CoA reductase and HMG-CoA synthase implies that it may serve as the prenyltransferase in the isoprene biosynthetic pathway. However, we cannot rule out the possibility that CR39 may function in another capacity, such as catalyzing the transfer of prenyl groups to nonterpene acceptors. Examples of this latter activity are the transferases which form N^6-(Δ^2-isopentenyl) adenosine in tRNAs (24, 38) and the 4-hydroxybenzoate-polyprenyltransferases which form ubiquinone, a respiratory coenzyme (53). Antisera raised to CR39 synthetic peptides (or to a fusion protein) may allow us to determine the precise prenyltransferase function of CR39.

DNA hybridization experiments showed that there were a minimum of five CR39 gene copies in the rat. This number increased to six or seven copies when the stringency of the hybridization and washing conditions was decreased. Some of these other gene copies may be due either to genes encoding enzymes with different functions (e.g., catalyzing the prenylation of different substrates) or to isozymes. So far there is no indication of isoenzymes for prenyltransferase (1), and two-dimensional analysis of in vitro translation products directed by CR39 hybrid-selected RNA indicates one polypeptide, based on size and charge (Clarke et al., unpublished observation). It seems likely, based on the analysis of other eucaryotic genes, that some of these gene copies may be due to pseudogenes (63). It is also possible that some of these copies represent genes that are expressed differentially over the course of development, analogous to globin (22) and histone genes (46).

The conversion of acetate to cholesterol and nonsterol products involves many enzymes, and several points along the pathway have been shown to exhibit regulation. The differential hybridization approach used here should allow any member of this pathway whose mRNA levels are regulated to be cloned. Regulated genes in this pathway probably fall into at least two classes: coordinately regulated genes, in which transcriptional changes occur quickly and are presumably effected by common *trans*-acting factors, and genes that have secondary regulation, in which changes occur with a slower response time and the magnitude of change may be less. However, the magnitude of the change in mRNA levels will be determined by both mRNA stability

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          10                  30                  50                  70                  90
CAC AGA GCA TTT AGC TCC TCT GTC AGA ATG AAT GGG GAC CAG AAA CTG GAT GTT CAT AAC CAA GAA AAG CAG AAT TTC ATC CAG CAC TTC TCC CAG ATT
                                Met Asn Gly Asp Gln Lys Leu Asp Val His Asn Gln Glu Lys Gln Asn Phe Ile Gln His Phe Ser Gln Ile

         110                 130                 150                 170                 190
GTC AAG GTG CTG ACT GAG GAT GAA CTG GGA CAC CCA GAG AAG GGA GAT GCT ATT ACC CGG ATC AAA GAG GTC CTG GAG TAC AAC ACT GTA GGA GGC AAG
Val Lys Val Leu Thr Glu Asp Glu Leu Gly His Pro Glu Lys Gly Asp Ala Ile Thr Arg Ile Lys Glu Val Leu Glu Tyr Asn Thr Val Gly Gly Lys

         210                 230                 250                 270                 290
TAC AAT CGG GGT CTG ACG GTG GTA CAG ACC TTC CAG GAA CTG GTG GAA CCA AGG AAA CAG GAT GCC GAG AGC CTA CAG CGG GCC CTG ACG GTG GGC TGG
Tyr Asn Arg Gly Leu Thr Val Val Gln Thr Phe Gln Glu Leu Val Glu Pro Arg Lys Gln Asp Ala Glu Ser Leu Gln Arg Ala Leu Thr Val Gly Trp

         310                 330                 350                 370                 390
TGT GTA GAA CTG CTC CAG GCT TTC TTC CTC GTG TTA GAT GAC ATC ATG GAC TCT TCC TAC ACT CGC CGG GGG CAG ATC TGC TGG TAT CAG AAG CCG GGC
Cys Val Glu Leu Leu Gln Ala Phe Phe Leu Val Leu Asp Asp Ile Met Asp Ser Ser Tyr Thr Arg Arg Gly Gln Ile Cys Trp Tyr Gln Lys Pro Gly

         410                 430                 450                 470                 490
ATA GGC TTG GAT GCC ATC AAC GAT GCT CTG CTT CTG GAA GCC GCT ATC TAC CGC CTG CTT AAG TTC TAC TGC AGG GAG CAG CCC TAC TAC CTC AAC CTG
Ile Gly Leu Asp Ala Ile Asn Asp Ala Leu Leu Leu Glu Ala Ala Ile Tyr Arg Leu Leu Lys Phe Tyr Cys Arg Glu Gln Pro Tyr Tyr Leu Asn Leu

         510                 530                 550                 570                 590
CTG GAG CTC TTT CTA CAG AGT TCC TAC CAG ACT GAG ATC GGG CAG ACT CTC GAC CTC ATC ACA GCA CCC CAG GGC CAA GTG GAT CTT GGT AGA TAC ACT
Leu Glu Leu Phe Leu Gln Ser Ser Tyr Gln Thr Glu Ile Gly Gln Thr Leu Asp Leu Ile Thr Ala Pro Gln Gly Gln Val Asp Leu Gly Arg Tyr Thr

         610                 630                 650                 670                 690
GAA AAG AGG TAC AAA TCT ATC GTC AAG TAC AAG ACA GCT TTC TAC TCT TTT TAC CTG CCT ATC GCG GCT GCC ATG TAC ATG GCT GGA ATT GAT GGG GAG
Glu Lys Arg Tyr Lys Ser Ile Val Lys Tyr Lys Thr Ala Phe Tyr Ser Phe Tyr Leu Pro Ile Ala Ala Ala Met Tyr Met Ala Gly Ile Asp Gly Glu

         710                 730                 750                 770                 790
AAG GAA CAC GCT AAT GCC CTG AAG ATC CTG CTG GAG ATG GGC GAG TTC TTC CAG ATC CAG GAC GAC TAC CTT GAT CTC TTT GGA GAC CCC AGT GTG ACC
Lys Glu His Ala Asn Ala Leu Lys Ile Leu Leu Glu Met Gly Glu Phe Phe Gln Ile Gln Asp Asp Tyr Leu Asp Leu Phe Gly Asp Pro Ser Val Thr

         810                 830                 850                 870                 890
GGA AAG GTC GGC ACT GAC ATC CAG GAC AAC AAA TGC AGC TGG CTG GTG GTT CAG TGT CTG CTA CGA GCC ACT CCT CAG CAG CGC CAG ATC TTA GAG GAG
Gly Lys Val Gly Thr Asp Ile Gln Asp Asn Lys Cys Ser Trp Leu Val Val Gln Cys Leu Leu Arg Ala Thr Pro Gln Gln Arg Gln Ile Leu Glu Glu

         910                 930                 950                 970                 990
AAT TAT GGG CAG AAG GAC CCA GAA AAA GTG GCG CGG GTG AAA GCA CTG TAC GAG GAG CTG GAT CTG CGG AGT GTG TTC TTC AAG TAC GAG GAA GAC AGT
Asn Tyr Gly Gln Lys Asp Pro Glu Lys Val Ala Arg Val Lys Ala Leu Tyr Glu Glu Leu Asp Leu Arg Ser Val Phe Phe Lys Tyr Glu Glu Asp Ser

        1010                1030                1050                1070
TTA CAA CCG CCT CAA GAG TCT CAT AGA GCA GTC GTC GCG CGC CCC TGC CCC CAT CCA TCT TCC TGG AAC TAG CAA ACA AGA TCT ACA AGC GGA GAA AGT
Leu Gln Pro Pro Gln Glu Ser His Arg Ala Val Val Ala Arg Pro Cys Pro His Pro Ser Ser Trp Asn End

        1090                1110                1130                1150
AAC CTC GAA TTG TAG AGG CTG CGA GGG AGG GGT CTC AAT AAA TTA TTG TTC AAC AAA AAA AAA AAA A
```

FIG. 4. Complete nucleotide sequence of CR39-1.2 sense strand and the predicted amino acid sequence of the protein. The nucleotide of the initiator methionine codon is designated +1, and the 5′ untranslated region of the cDNA extends from −1 to −28. The amino acid sequence is shown underneath the nucleotide sequence, and residue number 1 designates the initiator methionine. The amino acid sequence homologous to the active-site sequence of pig liver prenyltransferase (8) is underlined. The entire sequence of CR39-1.2 was determined on both strands.

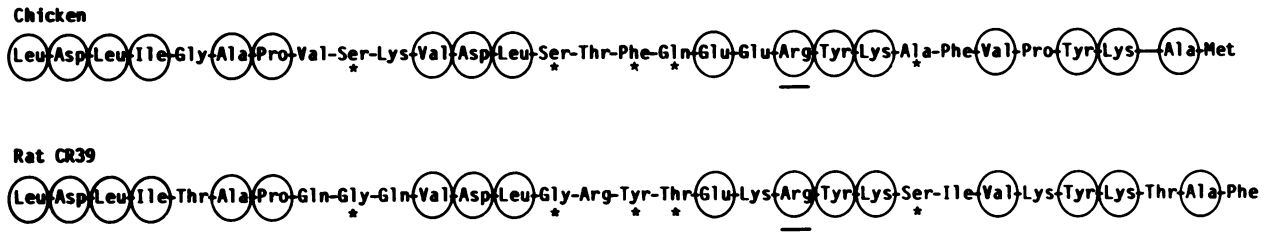

FIG. 5. Amino acid sequence of the active-site peptide of chicken liver prenyltransferase: comparison with amino acid sequence of rat liver CR39. The amino acid sequence for the chicken liver prenyltransferase was determined by Edman degradation by Brems et al. (8). The rat liver CR39 sequence was predicted from the nucleotide sequence of the cDNA shown in Fig. 4. Identical residues are circled, conservative substitutions (25) are designated by asterisks, and the implicated active-site arginine (2) is indicated by an underline.

and the rate of transcription. No information on mRNA stability of the cholesterogenic enzymes is available. Candidates for coordinate regulation in this pathway include many of the enzymes that synthesize mevalonate from acetate. Work by Chang et al. (12, 13) has implicated a common controlling factor that exerts coordinate regulation over the activities of acetyl-CoA thiolase, HMG-CoA synthase, HMG-CoA reductase, and mevalonate kinase. The regulation of HMG-CoA synthase and HMG-CoA reductase mRNA levels accounts for much of the regulation of enzyme activity (33, 34, 48). Changes in transcription of the HMG-CoA reductase gene in rat liver can fully account for the altered mRNA levels (19). The gene structures of both HMG-CoA reductase and HMG-CoA synthase have been characterized (34, 55, 56), and the promoter region of HMG-CoA reductase has been partially defined (50). Further work is required to determine whether the promoters controlling HMG-CoA reductase, HMG-CoA synthase, and CR39 transcription have common *cis*-acting sequences that are responsive to common factors.

Prenyltransferase, like squalene synthetase, may serve a branchpoint regulatory function (10). As shown in Fig. 7, prenyltransferase can be considered a branchpoint regulator controlling the flux of dimethylallyl pyrophosphate to farnesyl pyrophosphate, compared with the dimethylallyl pyrophosphate that is used to generate N^6-(Δ^2-isopentenyl)adenosine, a modified base found 3′ and adjacent to the anticodon sequence in tRNAs recognizing codons starting with uridine (e.g., $tRNA^{Leu}$, $tRNA^{Phe}$, $tRNA^{Ser}$, $tRNA^{Trp}$, and $tRNA^{Tyr}$) (for reviews, see references 38 and 40). The isopentenyladenine plays a role in stabilizing the codon-anticodon base-pairing and may serve to increase the stability of U-A base pairs to the level of G-C base pairs (36).

Faust et al. (29) have shown that human fibroblasts incorporate [^{3}H]mevalonate into tRNA. When de novo cholesterol synthesis is repressed by addition of exogenous low-density lipoprotein cholesterol to the growth medium, the fibroblasts continue to incorporate [^{3}H]mevalonate into the nonsterol products isopentenyl tRNA and ubiquinone. Thus, the regulation of prenyltransferase observed in the current study may be considered to control the amount of

TABLE 2. Amino acid composition of prenyltransferase isolated from chicken, pig, and human liver compared with the predicted amino acid composition of rat liver CR39

Amino acid	Residues per subunit			
	Human[a]	Pig[b]	Chicken[c]	Rat CR39[d]
Cys	5.8	6.0	6.45	6
Asx	32.8	30.9	30.05	33
Thr	10.0	11.7	12.05	15
Ser	10.3	14.3	17.05	15
Glx	45.2	45.9	58.05	55
Pro	12.0	15.2	14.25	14
Gly	23.7	22.8	26.15	20
Ala	27.2	26.5	31.40	21
Val	20.9	21.2	27.80	22
Met	6.4	7.3	6.85	5
Ile	16.5	19.0	14.10	18
Leu	37.4	36.7	41.40	40
Tyr	17.4	17.3	20.85	19
Phe	14.2	16.0	15.75	14
Trp	2.0	4.6	ND	4
Lys	21.8	21.1	27.65	21
His	4.4	5.5	6.00	6
Arg	17.5	17.5	22.25	16

[a] Composition determined by Barnard and Popjak (3). Residues per subunit based on a subunit molecular weight of 38,500.

[b] Composition determined by Barnard and Popjak (2). Residues per subunit based on a subunit molecular weight of 38,500.

[c] Composition determined by Reed and Rilling (54). Residues per subunit based on a subunit molecular weight 42,500. ND, Not determined.

[d] Composition deduced from the cDNA sequence in Fig. 4. Rat subunit molecular weight is 39,615.

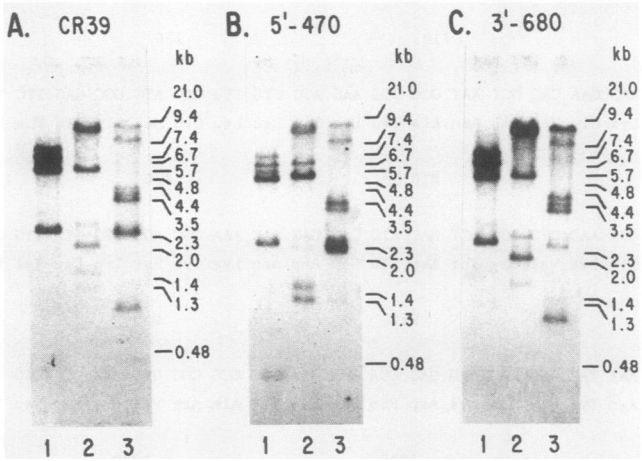

FIG. 6. Restriction analysis of CR39 rat genomic DNA. Rat liver genomic DNA was isolated by the method of Blin and Stafford (6) and digested with restriction endonuclease *Hin*dIII (lanes 1), *Bam*HI (lanes 2), or *Eco*RI (lanes 3). Size fractionation of the DNA transfer to nylon membrane and blot hybridization was performed as described in the text. Three different [^{32}P]DNA probes were prepared: CR39-1.2, the full-length cDNA (A); 5′-470, from a *Pst* digest of CR39-1.2 (B); and 3′-680, the other restriction fragment from the *Pst* digest of CR39-1.2 (C). The amount of probe used in each hybridization was 1 ng/ml. Filters were hybridized and washed at 65°C as described in the legend to Fig. 1. The blots were exposed to X-ray film as described in the text for 16 days. λ DNA digested with *Eco*RI and *Hin*dIII and pBR322 DNA digested with *Taq*I provided size standards; the positions of selected bands are indicated.

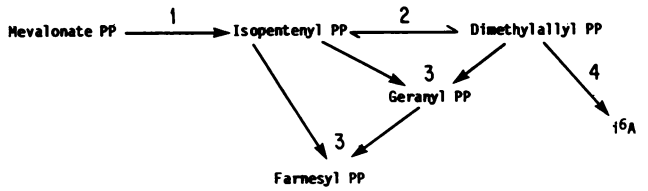

FIG. 7. Prenyltransferase catalyzes a branchpoint reaction of the isoprene biosynthetic pathway. The steps of the pathway from mevalonate pyrophosphate to farnesyl pyrophosphate are shown. Enzymes participating in this segment of the pathway are numbered as follows: 1, mevalonate pyrophosphate decarboxylase; 2, isopentenyl pyrophosphate isomerase; 3, prenyltransferase; 4, N^6-(Δ^2-isopentenyl)adenine prenyltransferases. The pyrophosphate moiety is designated by PP, and N^6-(Δ^2-isopentenyl)adenosine is shown as i^6A.

isoprene units available for RNA or terpene biosynthesis. In addition, the possibility exists that other prenyltransferases may be responsible for generating farnesyl pyrophosphate specifically for dolichol or ubiquinone synthesis (57).

In summary, the approach used in the current study should permit the isolation and identification of other mRNAs and proteins that are involved in regulating the biosynthesis of both sterols and nonsterol products from mevalonate.

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