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Molecular cloning of mevalonate kinase and regulation of its mRNA levels in rat liver

(cholesterol synthesis/mevalonic aciduria/pravastatin/ATP-binding site)

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ABSTRACT **Mevalonate kinase [ATP:(*R*)-mevalonate 5-phosphotransferase, EC 2.7.1.36] may be a regulatory site in the cholesterol biosynthetic pathway, and a mutation in the gene coding for this enzyme is thought to cause the genetic disease mevalonic aciduria. To characterize this enzyme, a rat liver cDNA library was screened with a monospecific antibody, and a 1.7-kilobase cDNA clone coding for mevalonate kinase was isolated. The complete DNA sequence was determined, and the longest open reading frame coded for a protein containing 395 amino acids with a deduced molecular weight of 41,990. Identification of the cDNA clone was confirmed by expression of enzyme activity in yeast and by protein sequence data obtained from sequencing purified rat mevalonate kinase. The deduced amino acid sequence of mevalonate kinase contained a motif for the ATP-binding site found in protein kinases, and it also showed sequence homology to the yeast RAR1 protein. The size of mevalonate kinase mRNA in rat liver was ≈2 kilobases. Treatment with diets containing cholesterol-lowering agents caused an increase in both mevalonate kinase activity and mRNA levels, whereas diets containing 5% cholesterol lowered the levels of both enzyme activity and mRNA. These data indicate that long-term regulation of enzyme activity in rat liver is controlled by changes in the levels of mevalonate kinase mRNA.**

Mevalonate kinase [ATP:(*R*)-mevalonate 5-phosphotransferase, EC 2.7.1.36] is a cytosolic enzyme in the cholesterol biosynthetic pathway and catalyzes the phosphorylation of mevalonate to form mevalonate 5-phosphate (1). Enzyme activity is inhibited by farnesyl pyrophosphate and geranyl pyrophosphate, which are intermediates in the pathway and inhibit mevalonate kinase activity by binding competitively at the ATP-binding site on the enzyme (2, 3). Based on these data, Dorsey and Porter (2) postulated that mevalonate kinase activity in the cell may be controlled by feedback inhibition from these intermediates. Feedback regulation of mevalonate kinase activity would theoretically regulate the synthesis of farnesyl pyrophosphate, which is a key intermediate at a branch point in the pathway leading to the biosynthesis of cholesterol, ubiquinone, or dolichol. Therefore, mevalonate kinase may play an important role in regulating cholesterol biosynthesis.

A mutation in the gene coding for mevalonate kinase is presumed to be the cause of the recently discovered genetic disease mevalonic aciduria (4, 5). The genetic disease is transmitted as an autosomal recessive trait, and there are six reported cases (6). Subjects with mevalonic aciduria have extremely high levels of mevalonate in their plasma and urine, and cells from these subjects have <10% of the normal levels of mevalonate kinase activity (4-6). As a first step in identifying the molecular defect causing mevalonic aciduria and to study the regulation of mevalonate kinase activity, we have isolated and characterized a cDNA clone coding for rat mevalonate kinase.* Data are also presented to show that long-term regulation of enzyme activity in rat liver is controlled by changes in the levels of mevalonate kinase mRNA.

MATERIALS AND METHODS

Preparation of the Antiserum. Monospecific antisera to rat mevalonate kinase was prepared in rabbits by using the purified enzyme (3), and the IgG fraction was collected by affinity chromatography (7).

Isolation of a cDNA Clone Coding for Mevalonate Kinase. A λgt11 cDNA library (8) derived from mRNA purified from the livers of rats treated with diets containing 5% cholestyramine and 0.1% lovastatin was kindly provided by Peter Edwards (UCLA). Positive clones were identified by immunoscreening (9). To obtain a full-length cDNA clone, the cDNA insert from one of the clones was isolated and radiolabeled by random priming (10) to a specific activity of 10^9 cpm per μg of DNA. The radiolabeled probe was used to screen $\approx 2 \times 10^5$ recombinants. Plaque hybridization was performed as described (8), after which the filters were washed at 68°C with 2× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7) containing 0.1% SDS, followed by washes with 0.1× SSC containing 0.1% SDS. Fifteen cDNA clones were isolated. The cDNA inserts were subcloned into the pGEM-3Z vector (Promega) for restriction enzyme mapping or into M13 vectors for DNA sequencing.

DNA Sequencing. The DNA sequence of the cDNA was determined by the dideoxynucleotide chain-termination method (11). Sequencing reactions using the Klenow fragment of DNA polymerase I (New England Biolabs) or the modified T7 DNA polymerase (Sequenase, United States Biochemicals) were performed following the manufacturer's protocol. The DNA and protein sequences were aligned using the Intelligenetics computer program, and the EMBL/GenBank and PIR data bases were searched for homologies to the DNA sequence and protein sequence of mevalonate kinase.

Expression of the Mevalonate Kinase cDNA in *Saccharomyces cerevisiae*. A 1.3-kilobase (kb) *Hin*fI fragment that contained the mevalonate kinase coding sequence and 14 base pairs (bp) of the 5′ untranslated region was ligated into the *Sma* I site of the yeast plasmid pMH101 (12) to form the expression vector pGR50. The cDNA was inserted between the yeast GAL1 promoter and CYC1 terminator elements in the vector. Yeast cultures of strain Y294 were transformed with either pHM101 or pGR50 and grown at 30°C in minimal medium (13) supplemented with either 2% glucose or 2%

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Abbreviation: HMG-CoA, hydroxymethylglutaryl-CoA.
*The sequence reported in this paper has been deposited in the GenBank data base (accession no. M29472).

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-81                                    GAATTCCATGGAGAAGACCGGGAGCTACTGCCGGAGTTTCGGCTCACCTGGGCGCTGGCCGGGGCAGGAGTCTCAGGAGCC

1     ATG TTG TCA GAA GTC CTG CTG GTG TCT GCT CCA GGG AAA GTC ATT CTC CAT GGA GAA CAT GCT GTG GTC CAT GGC AAG GTA
      MET Leu Ser Glu Val Leu Leu Val Ser Ala Pro Gly Lys Val Ile Leu His Gly Glu His Ala Val Val His Gly Lys Val  27

8     GCT CTG GCG GTG GCC TTG AAC TTG AGA ACA TTT CTC GTG CTG CGA CCG CAG AGC AAT GGG AAA GTG AGC CTC AAT TTA CCA
      Ala Leu Ala Val Ala Leu Asn Leu Arg Thr Phe Leu Val Leu Arg Pro Gln Ser Asn Gly Lys Val Ser Leu Asn Leu Pro  54

163   AAC GTC GGT ATT AAG CAG GTC TGG GAT GTG GCC ACA CTT CAG CTG CTG GAC ACA GGC TTT CTT GAG CAA GGC GAT GTC CCG
      Asn Val Gly Ile Lys Gln Val Trp Asp Val Ala Thr Leu Gln Leu Leu Asp Thr Gly Phe Leu Glu Gln Gly Asp Val Pro  81

244   GCA CCC ACC TTG GAG CAA CTG GAG AAG CTG AAG AAG GTG GCG GGC CTC CCC CGA GAC TGT GTA GGC AAC GAA GGC CTG TCT
      Ala Pro Thr Leu Glu Gln Leu Glu Lys Leu Lys Lys Val Ala Gly Leu Pro Arg Asp Cys Val Gly Asn Glu Gly Leu Ser 108

325   CTG CTT GCC TTT CTG TAC CTG TAC CTG GCT ATC TGC CGG AAA CAG AGG ACA CTC CCA AGC CTG GAC ATC ATG GTG TGG TCG
      Leu Leu Ala Phe Leu Tyr Leu Tyr Leu Ala Ile Cys Arg Lys Gln Arg Thr Leu Pro Ser Leu Asp Ile MET Val Trp Ser 135

406   GAA CTG CCC CCT GGG GCG GGC TTG GGC TCC AGT GCA GCC TAC TCG GTG TGT GTG GCA GCC GCC CTC CTG ACT GCC TGT GAG
      Glu Leu Pro Pro Gly Ala Gly Leu Gly Ser Ser Ala Ala Tyr Ser Val Cys Val Ala Ala Ala Leu Leu Thr Ala Cys Glu 162

487   GAG GTC ACC AAC CCG CTC AAG GAC AGG GGC TCC ATT GGC AGT TGG CCC GAG GAG GAC CTG AAG TCA ATT AAC AAG TGG GCC
      Glu Val Thr Asn Pro Leu Lys Asp Arg Gly Ser Ile Gly Ser Trp Pro Glu Glu Asp Leu Lys Ser Ile Asn Lys Trp Ala 189

568   TAC GAG GGG GAG AGA GTG ATC CAT GGG AAC CCC TCT GGC GTG GAC AAT TCC GTC AGC ACC TGG GGA GGA GCC CTG CGC TAC
      Tyr Glu Gly Glu Arg Val Ile His Gly Asn Pro Ser Gly Val Asp Asn Ser Val Ser Thr Trp Gly Gly Ala Leu Arg Tyr 216

649   CAG CAA GGG AAG ATG TCA TCC TTG AAG AGG CTC CCA GCT CTG CAG ATC CTG CTC ACC AAC ACC AAG GTC CCA CGA AGC ACC
      Gln Gln Gly Lys MET Ser Ser Leu Lys Arg Leu Pro Ala Leu Gln Ile Leu Leu Thr Asn Thr Lys Val Pro Arg Ser Thr 243

730   AAG GCC CTC GTG GCT GGC GTC AGA AGC AGG CTA ATC AAG TTC CCT GAG ATC ATG GCC CCG CTC CTG ACA TCA ATT GAC GCA
      Lys Ala Leu Val Ala Gly Val Arg Ser Arg Leu Ile Lys Phe Pro Glu Ile MET Ala Pro Leu Leu Thr Ser Ile Asp Ala 270

811   ATC TCC CTG GAG TGT GAG CGC GTG CTG GGA GAG ATG GCG GCC GCA CCA GTC CCA GAA CAG TAC CTT GTC CTA GAA GAG CTA
      Ile Ser Leu Glu Cys Glu Arg Val Leu Gly Glu MET Ala Ala Ala Pro Val Pro Glu Gln Tyr Leu Val Leu Glu Glu Leu 297

892   ATG GAC ATG AAC CAG CAC CAT CTG AAT GCC CTT GGT GTG GGC CAC GCC TCC CTG GAC CAG CTC TGT CAG GTA ACA GCA GCA
      MET Asp MET Asn Gln His His Leu Asn Ala Leu Gly Val Gly His Ala Ser Leu Asp Gln Leu Cys Gln Val Thr Ala Ala 324

973   CAT GGA CTG CAC AGC AAG CTG ACT GGC GCA GGC GGC GGC GGC TGT GGC ATC ACC CTC CTG AAG CCA GGT CTA GAG CGA GCA
      His Gly Leu His Ser Lys Leu Thr Gly Ala Gly Gly Gly Gly Cys Gly Ile Thr Leu Leu Lys Pro Gly Leu Glu Arg Ala 351

1054  AAA GTG GAG GCC GCC AAG CAG GCC CTG ACC GGC TGC GGG TTT GAC TGC TGG GAG ACC AGC ATT GGA GCG CCT GGG GTC TCC
      Lys Val Glu Ala Ala Lys Gln Ala Leu Thr Gly Cys Gly Phe Asp Cys Trp Glu Thr Ser Ile Gly Ala Pro Gly Val Ser 378

1135  ATG CAC TCA GCC ACC TCC ATA GAG GAC CCT GTC CGA CAA GCC CTG GGC CTC TGA CATGTCATCCTGCCAAGAAGCTTCCACGTATCTGG
      MET His Ser Ala Thr Ser Ile Glu Asp Pro Val Arg Gln Ala Leu Gly Leu  .                                     395

1224  GGCTGGAGTTGGCCCCTGTGCTGGCCACTAACACATTTCCTGCTGGTAGTGCCTTGTAGACTCTGAGAGGCACCACCTCCATGGCCCATCTGTATCCTGGCACTGCT

1331  GAAGCCAGACAGGCATCAGTTGCCGGGTCTTCTGAGACAGCAGGCTGACACAGTAGGCCTTCCCCCTGGGTGATTCTGCCTGGGCCTGGCCTCTGTCGGCCTCACAC

1438  AAACTCTCCGGCTCCTAGCCACGCAACCCTTCTGTCCTCAGATGCCGGCCCCTTTGGAACCGCCTAGCCAGTGGCTCATGTCTCCTGTCCTCTCTCGGGGATCATCG

1545  GAGGCAAGGCATGCCGACTGCTTTATCCCCGTGTGTACCTGTGGACAAGCTGCCCATGGTGGGGCGAGGGAAGGGGGAATAAAATGAGCTCGTAC (A)n
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FIG. 1. Nucleotide sequence and deduced amino acid sequence of mevalonate kinase. Nucleotides in the DNA sequence are numbered starting at the translational start site, and the nucleotides in the 5′ untranslated region are represented by negative numbers. The nucleotides and amino acids are numbered in the left and right margins, respectively. The amino acid sequence enclosed in the box is identical to the amino acid sequence determined by protein sequencing mevalonate kinase purified from rat liver. A consensus polyadenylylation signal (AATAAA) is underlined.

galactose. Cells were disrupted with glass beads (12), and mevalonate kinase activity was measured in the 100,000 × *g* supernatant fraction.

Mevalonate Kinase mRNA. Female Sprague–Dawley rats (body wt, 80 g; Camm Research Lab, Wayne, NJ) were maintained on a 12-hr light/12-hr dark cycle. Control animals were fed powdered rat chow for 4 days, while cholesterol-fed animals were treated for the same time period with powdered chow containing 5% cholesterol. Drug-treated animals were fed for 2 days with a diet containing powdered chow and 5% cholestyramine (8.8 g per kg body wt per day) and then they were treated for an additional 2 days with chow containing both 5% cholestyramine and 1% pravastatin (1.8 g per kg body wt per day). Animals were sacrificed at the middle of the dark cycle, the livers were removed, and samples were processed for either mevalonate kinase assays (3) or RNA isolation (14).

The levels of mevalonate kinase mRNA were measured by a ribonuclease protection assay (15, 16). cDNAs coding for either rat mevalonate kinase or mouse actin (8) were subcloned into the pGEM-3Z vector and RNA radiolabeled with [γ-^{32}P]UTP was transcribed *in vitro* following the manufacturer's protocol. Various amounts of total cellular RNA were mixed with the radiolabeled RNA probe in a solution containing 0.4 M NaCl, 40 mM Pipes (pH 6.4), 1 mM EDTA, and 80% (vol/vol) formamide. Samples were denatured by heat-

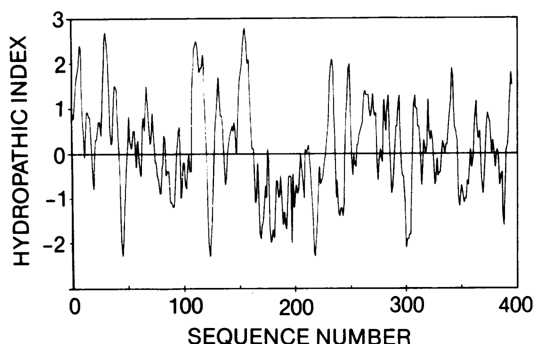

FIG. 2. Hydropathy profile of mevalonate kinase. The deduced amino acid sequence of mevalonate kinase was analyzed by using the algorithm described by Kyte and Doolittle (17). The amino acid sequence is represented on the abscissa, and each point represents the average hydropathy of six successive amino acids. Positive values on the ordinate represent hydrophobic regions in the protein.

ing at 90°C for 10 min and were then hybridized overnight at 50°C. The remaining unhybridized, radiolabeled probe and the unprotected sample RNA were digested by treatment with ribonucleases A and T1, followed by inactivation of the ribonuclease by addition of proteinase K and SDS. Salmon sperm DNA (20 μg) was added as carrier, and then the samples were precipitated with ice-cold 10% (vol/vol) trichloroacetic acid containing 0.2 M tetrasodium pyrophosphate. Precipitates were collected and washed on Whatman GF/F glass fiber filters, and the radioactivity was measured with a scintillation counter. The levels of mevalonate kinase mRNA were normalized to the levels of actin mRNA in normal animals.

Mevalonate Kinase Activity and Protein Sequence. Liver samples were homogenized and 100,000 × *g* supernatant fractions were prepared (3). Mevalonate kinase activity in the supernatant fraction was measured by a spectrophotometric assay (1).

Mevalonate kinase was purified to homogeneity from the livers of rats treated with diets containing 5% cholestyramine and 1% pravastatin (3). The protein sequence of the amino-terminal portion of the pure mevalonate kinase was determined by using an Applied Biosystems 4070A gas-phase sequencer equipped with an on-line Applied Biosystems 120A HPLC. Protein sequencing was performed at the Biological Laboratories, Harvard University.

RESULTS

Isolation and DNA Sequence Analysis of a cDNA Clone Coding for Mevalonate Kinase. Three cDNA clones for rat mevalonate kinase (pMK-2, -9, and -10) were isolated after immunoscreening 4 × 10^5 recombinants from a cDNA expression library. The sizes of the cDNAs in these clones ranged from 0.6 to 1.1 kb. Fifteen additional clones were isolated after screening the cDNA library using the radiolabeled pMK-9 as a probe. Seven of these clones contained a cDNA insert of ≈1.7 kb, which was the largest cDNA isolated.

The DNA sequence coding for rat mevalonate kinase was determined by sequencing two different clones (pMK-101 and pMK-102). The longest open reading frame was 1185 bp and coded for a 395-amino acid polypeptide with a deduced molecular weight of 41,990 (Fig. 1). Translation initiated at the second methionine codon. The open reading frame starting from the first methionine codon (nucleotide −72) coded for only a short polypeptide of 51 amino acids. Positive identification of the cDNA clones and the location of the translational start site were confirmed by sequencing the amino terminus of purified rat mevalonate kinase. The amino acid sequence determined from protein sequencing was identical to the first 20 amino acids of the protein sequence deduced from the cDNA (Fig. 1). The sizes of the 5′ and 3′ untranslated regions were 81 and 467 nucleotides, respectively. A canonical polyadenylylation signal (AATAAA) was present at nucleotide 1622 and a poly(A)$^+$ tail was located 12 nucleotides downstream from this site (Fig. 1).

Analysis of the Deduced Amino Acid Sequence of Mevalonate Kinase. The deduced molecular weight of rat mevalonate kinase (M_r, 41,990) is slightly larger than the subunit molecular weight determined for mevalonate kinase purified from rat liver (M_r, 39,900) (3). This variation in molecular weight may result from anomalous migration of the purified mevalonate kinase on SDS/polyacrylamide gels due to hydrophobic regions in the enzyme. Based on the hydropathy profile (Fig. 2), mevalonate kinase is a very hydrophobic protein. The overall index of hydropathy is +0.15 (17), and hydrophobic amino acids comprise 47% of the total amino acids in the enzyme.

An ATP-binding site motif present in the deduced protein sequence of mevalonate kinase (Fig. 3) is similar to the one found in protein kinases (18). The protein sequence contained 5 of the 6 conserved residues present in the ATP-binding site, and the spatial arrangement between the conserved glycine and lysine residues was also present.

A search of the PIR and Swiss Protein data bases revealed protein sequence homology between mevalonate kinase and the yeast RAR1 protein. The *RAR1* (regulation of autonomous replication) gene codes for a yeast protein with unknown function whose activity increases the mitotic stability of plasmids (19). Allowing for gaps in the alignment, there were 159 matches (36%) of the total of 443 amino acids in the RAR1 protein, and there were three regions in the sequence of mevalonate kinase (amino acids 9–15, 140–147, and 330–339) that were identical in the RAR1 protein (Fig. 4). Also, the motif (18) for the ATP-binding site and the relative position of this site were conserved in both proteins.

Expression of Rat Mevalonate Kinase Activity in Yeast Cells. The functional integrity of the rat mevalonate kinase cDNA was confirmed by expression of the DNA in *S. cerevisiae*. Under growth conditions (with galactose) known to induce expression from the GAL1 promoter, the level of mevalonate kinase activity in cells containing the cDNA for rat mevalonate kinase was induced at least 25-fold when compared to the enzyme activity in cells transformed with the plasmid (pMH101) alone (Table 1). The specific activity of mevalonate kinase in extracts from normal yeast is ≈1.3 nmol per min per mg of protein (21), and this activity was below the reliable detection limit of our spectrophotometric assay.

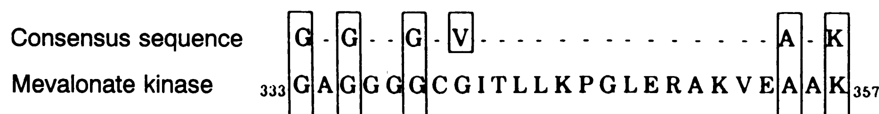

FIG. 3. Identification of the motif for the ATP-binding site of protein kinases. The consensus sequence (18) for the ATP-binding site present in protein kinases is aligned over a portion of the deduced amino acid sequence of mevalonate kinase. Amino acids are identified by the single-letter code, and the dashes represent residues where any amino acid can be inserted. The position of the amino acid sequence is indicated by the subscript numbers.

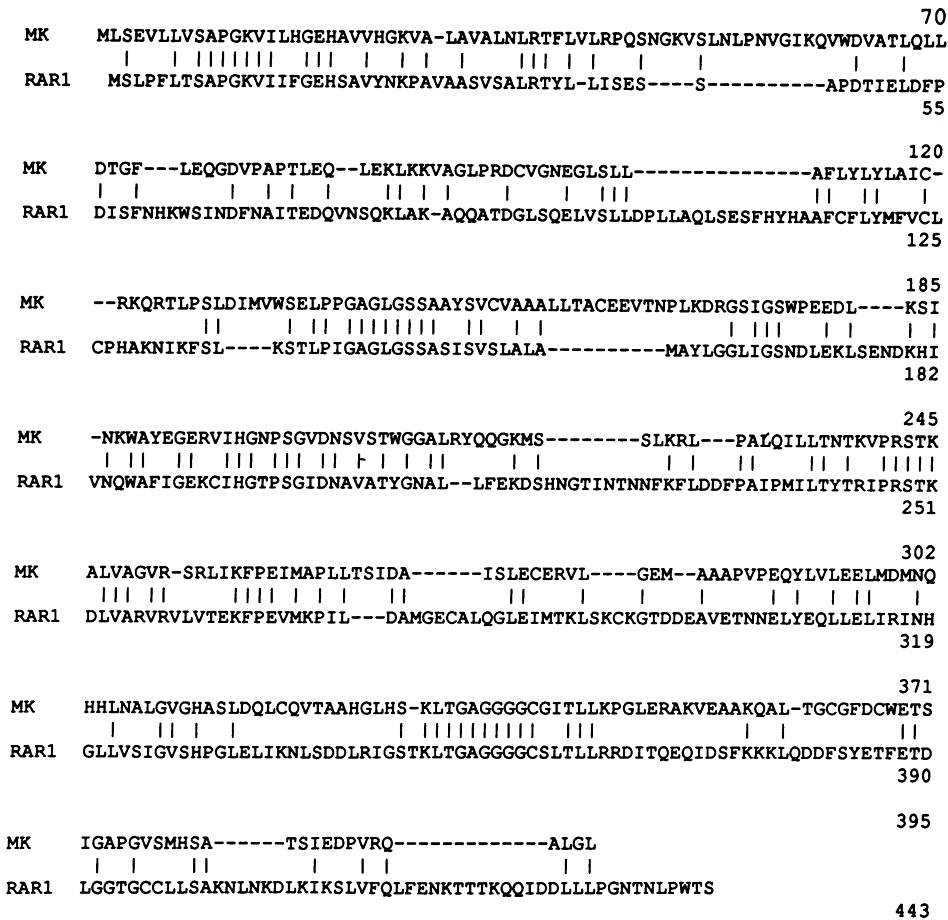

FIG. 4. Alignment of the protein sequence of rat mevalonate kinase (MK) and the yeast RAR1 protein. Amino acids are represented by the single-letter code. The sequences were aligned by using the algorithm of Myers and Miller (20). The dashes represent spaces in the alignment, and numbers on the right indicate residue number.

Mevalonate Kinase mRNA. Rat liver mRNA was analyzed on RNA blots, and the size of mevalonate kinase mRNA is 2 kb (unpublished data). The levels of mevalonate kinase activity and mRNA increased after treatment with cholestyramine and pravastatin (Table 2). Cholestyramine is a bile acid sequestrant (22) and pravastatin (23) is an inhibitor of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis (24). In contrast, diets containing 5% cholesterol lowered both mevalonate kinase activity and mRNA levels (Table 2).

DISCUSSION

Based on the deduced amino acid sequence and the overall index of hydropathy of +0.15, mevalonate kinase is remarkably hydrophobic for a soluble, cytosolic protein. Mevalonate kinase is even more hydrophobic than cholesterol ester transfer protein, which is an extremely hydrophobic protein with an overall index of hydropathy of +0.1 (25). Why mevalonate kinase contains so many hydrophobic regions is not understood. A partial explanation for the unusual hydrophobicity of this soluble enzyme may be the presence of a hydrophobic pocket for binding farnesyl pyrophosphate and geranyl pyrophosphate, which inhibit enzyme activity by binding competitively at the ATP-binding site on the enzyme (2).

The deduced protein sequence of mevalonate kinase contains the Gly-Xaa-Gly-Xaa-Xaa-Gly motif, which is present in the nucleotide binding site of protein kinases and many nucleotide binding proteins (18, 26). Based on crystallographic studies of the NAD binding region of glyceraldehyde-3-phosphate dehydrogenase, the glycines are required for binding to the ribose moiety in the nucleotide cofactor and for forming a tight turn in the secondary structure between the β-strand and α-helical portions of the molecule (27). The ATP-binding site in protein kinases is also characterized by having an invariant lysine residue approximately 14–23 residues downstream from the last glycine in the motif (18, 26). An alanine residue is usually found two positions upstream of this lysine (18). Mevalonate kinase contains the sequence Ala-Xaa-Lys, and the lysine is located 19 residues downstream from the last glycine in the motif. A valine residue frequently located two positions downstream from the last glycine in the motif (18) is not conserved in the sequence of mevalonate kinase. Overall, mevalonate kinase has all of the invariant residues present in the ATP-binding site of protein kinases, and further studies are needed to determine whether this binding site is involved in phosphorylation of mevalonate or possibly phosphorylation of cellular proteins. The latter possibility would imply that the enzyme may have both a biosynthetic and a regulatory function. If mevalonate kinase is also a protein kinase, its structure is not similar to the currently known protein kinases because the location of the ATP-binding site on mevalonate kinase is near the enzyme's

Table 1. Expression of the rat mevalonate kinase activity in *S. cerevisiae*

Yeast plasmid	Growth condition	Mevalonate kinase activity, nmol per min per mg of protein
pMH101	Glucose, 20 hr	<2
	Galactose, 20 hr	<2
pGR50	Glucose, 20 hr	<2
	Galactose, 7 hr	5.0 ± 1.7
	Galactose, 20 hr	54.3 ± 8.6

Mevalonate kinase activity is the average of duplicate assays. The endogenous yeast mevalonate kinase activity of strain Y294 (*MATα ura3-52 leu2-3,112 his3Δ trp1 GAL$^+$ cir$^+$*; ref. 12) was below the detection limit of the assay ($\approx$2 nmol·min^{-1}·mg^{-1}).

Table 2. Levels of mevalonate kinase activity and mRNA in the livers of rats treated with diets containing either normal chow, 5% cholesterol, or 5% cholestyramine and 1% pravastatin

Treatment	Experimental/normal ratio, mean ± SEM: Mevalonate kinase activity	Experimental/normal ratio, mean ± SEM: Mevalonate kinase mRNA
Normal chow	1.00 ± 0.18	1.00 ± 0.20
5% cholesterol	0.54 ± 0.05 (P = 0.038)	0.75 ± 0.05 (P = 0.042)
5% cholestyramine and 1% pravastatin	7.24 ± 1.19 (P = 0.001)	8.36 ± 1.17 (P = 0.001)

Rats were treated as described. All animals were sacrificed at the middle of the dark cycle, and mevalonate kinase activity and mRNA levels were measured. Each group contained four or five animals. The results were analyzed by Student's t test, and the P values are noted in parentheses.

carboxyl terminus, whereas in most protein kinases the site is near the amino-terminal end of the enzyme (18). Moreover, other regions that are conserved in protein kinases are not found in mevalonate kinase. Therefore, mevalonate kinase does not appear to be a member of the currently known protein kinase gene family. Studies using site-directed mutagenesis will be necessary to determine whether the ATP-binding site on mevalonate kinase is functional and required for phosphorylation of either mevalonate or cellular proteins.

The sequence homology between mevalonate kinase and the yeast RAR1 protein suggests that the two proteins may be related. The *RAR1* gene was isolated (19) by screening for mutations that increase the mitotic stability of plasmids whose replication is dependent on weak origins of DNA replication (ARS elements). If the RAR1 protein is involved in sterol biosynthesis, then products derived from the sterol biosynthetic pathway may affect the replication and mitotic stability of plasmids in yeast.

Very little is known about the regulation of mevalonate kinase activity. Recent studies in our laboratory have shown that mevalonate kinase activity in rat liver is regulated in part by changes in enzyme mass (3). In this report, we have extended these studies and examined the regulation of mevalonate kinase mRNA. When rats were fed a diet containing 5% cholestyramine and 1% pravastatin, the levels of mevalonate kinase activity and mRNA increased ≈8-fold and, by comparison, HMG-CoA reductase activity increased ≈89-fold (3). These data suggest that the increase in the levels of mevalonate activity and mRNA may result from induction of the entire cholesterol biosynthetic pathway. For example, treating rats with diets containing cholestyramine and another inhibitor of HMG-CoA reductase (lovastatin) also caused an increase in the enzyme activity and mRNA level of several enzymes (HMG-CoA reductase, HMG-CoA synthase, and farnesyl pyrophosphate synthetase) involved in cholesterol biosynthesis (8, 28–31). In contrast, treating rats with diets containing 5% cholesterol caused down-regulation of cholesterol biosynthesis and lowered both mevalonate kinase activity and mRNA levels. However, the relative decrease in mevalonate kinase activity after cholesterol feeding was not as great as that reported for HMG-CoA reductase, HMG-CoA synthase, and farnesyl pyrophosphate synthetase (3, 27, 29). Overall, changes in mevalonate kinase activity were correlated with similar changes in the levels of its mRNA under the conditions tested in our study, and these results demonstrate that long-term regulation of mevalonate kinase activity in rat liver is controlled primarily by changes in the levels of its mRNA.

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