Coordinate Regulation of 3-Hydroxy-3-methylglutaryl-coenzyme A Synthase, 3-Hydroxy-3-methylglutaryl-coenzyme A Reductase, and Prenyltransferase Synthesis but Not Degradation in HepG2 Cells*

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Human hepatoma HepG2 cells were used to demonstrate coordinate regulation of three enzymes of cholesterol synthesis under a variety of conditions. Addition of either delipidized serum or mevinolin or low density lipoprotein, 25-hydroxycholesterol, or mevalonic acid to HepG2 cells resulted in rapid changes both in the levels of the mRNAs and in the rates of synthesis of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) synthase, HMG-CoA reductase, and farnesyl pyrophosphate synthetase (prenyltransferase). In all cases, the changes in mRNA levels were paralleled by changes in the rates of specific protein synthesis. Pulse-chase techniques were used to determine the half-lives of all three proteins. Addition of low density lipoprotein to the media during the chase increased the rate of degradation of HMG-CoA reductase 4.6-fold but had no affect on the half-lives of HMG-CoA synthase or prenyltransferase. Therefore, we conclude that the coordinate regulation of these three enzymes under a variety of conditions occurs at the level of enzyme synthesis and not at the level of protein stability.

A plus-minus screening procedure was recently used to isolate a rat liver cDNA termed CR39 (1). The open reading frame of the CR39 1.2-kb cDNA codes for 344 amino acids (1). We hypothesized (1) that CR39 may encode a prenyltransferase based on a 17 out of 30 amino acid homology between an internal sequence of CR39 and an active site peptide isolated by Brems et al. from chicken prenyltransferase (2). Moreover, antiserum generated to a fusion protein encoding CR39 and anthranilate synthetase inactivated farnesyl pyrophosphate synthetase, the prenyltransferase involved in cholesterol biosynthesis (3).

The levels of prenyltransferase mRNA, protein, and enzymic activity are regulated 15-35-fold in rat liver by dietary cholesterol, mevalonic acid, or the hypocholesterolemic drugs cholestyramine and mevinolin (1, 3). In the intact rat, the mRNA levels of prenyltransferase, HMG-CoA synthase, and HMG-CoA reductase change coordinately in response to these same diets and drugs (1). It is not currently known whether these changes in mRNA levels result from similar alterations in the rates of transcription of the three genes.

Considerable information is available on the regulation of HMG-CoA reductase. HMG-CoA reductase is known to be regulated by changes of up to 30-fold in the rates of transcription of the gene (4, 6), mRNA levels (7), and in the apparent rate of enzyme synthesis (8, 9). In addition the half-life of the protein can vary over 30-fold with t1/2 values of 17 min to 600 min (8). These changes in the rates of enzyme synthesis and stability can account for the more than 450-fold change in HMG-CoA reductase activity that has been reported to occur in rat liver (10).

Three reports indicate that, under certain conditions, HMG-CoA reductase of Chinese hamster ovary fibroblasts or chicken myeloblasts is also under translational control (11-13). The mechanisms involved in controlling this process are unknown.

The 5' end of both the hamster and human HMG-CoA reductase mRNAs have recently been compared and shown to share limited identity (14). Other differences in the sequences of hamster and human mRNA were also noted (14). It is not known whether the translational control of hamster HMG-CoA reductase (11, 12) is dependent on specific sequences at the 5' end of the mRNA nor is it known whether there is translational control of HMG-CoA reductase in human cells.

In order to further define the coordinate regulation of prenyltransferase, HMG-CoA synthase, and HMG-CoA reductase previously observed in rat liver, human hepatoma HepG2 cells were used to test whether other agents (such as low density lipoprotein and 25-hydroxycholesterol) that are known to affect the expression of HMG-CoA reductase (9, 15, 16) also affect the expression of prenyltransferase and HMG-CoA synthase in a similar manner. HepG2 cells were incubated under a variety of conditions, and monospecific antibodies raised to all three proteins were used to measure the rates of synthesis of prenyltransferase, HMG-CoA synthase, and HMG-CoA reductase. mRNA levels were also determined and compared with the rates of synthesis in order to ascertain both whether coordinate regulation occurs and whether there is any evidence for translational control in the expression of these three proteins in human cells. In addition, the stabilities of the prenyltransferase and HMG-CoA synthase proteins were measured under conditions known to affect the stability of HMG-CoA reductase so as to assess possible coordinate regulation at the level of protein stability.

EXPERIMENTAL PROCEDURES

Materials—Mevinolin was a generous gift from A. Alberts (Merck Sharpe and Dohme). The sources of all other materials have been given previously (1, 3).

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† The abbreviations used are: kb, kilobase(s); HMG, 3-hydroxy-3-methylglutaryl; DMEM, Dulbecco's modified Eagle's medium; LPDS, lipoprotein-depleted human serum; LDL, low density lipoprotein.

‡ P. A. Edwards, unpublished data.
Immunoprecipitation and RNA Analysis—HepG2 cells were grown in Dulbecco's modified Eagle's medium (DMEM), supplemented with 100 units of penicillin/ml, 100 μg of streptomycin/ml, 0.25 μg of Fungizone/ml, and 10% fetal calf serum. To initiate an experiment, unless otherwise stated, cells were washed three times with phosphate-buffered saline and then incubated for 24 h in medium in which the fetal calf serum was replaced with 10% LPDS (lipoprotein-depleted human serum) (17) and mevinolin (2 μg/ml). This latter medium was then supplemented with human low density lipoprotein, 25-hydroxycholesterol (1 μg/μl ethanol; 1 μg/ml medium), mevalonic acid (10 mM), or ethanol (1 μl/ml medium) as indicated in the text, and the incubation was continued. At the indicated time, cells were either washed three times with phosphate-buffered saline and then incubated for 60 min in methionine-free medium supplemented with [35S]methionine (50–100 μCi/ml; ≥1000 Ci/mmol) or were used to prepare mRNA by the method of Chomczynski and Sacchi (18). Poly(A)+ RNA was isolated as described (6). Pulse and pulse-chase experiments were performed essentially as reported previously (8). At the end of the pulse or pulse-chase the radiolabeled cells were lysed and the antigens (HMG-CoA reductase, HMG-CoA synthase, and prenyltransferase) immunoprecipitated sequentially with specific antibodies or antisera and analyzed and quantitated on denaturing gels as reported previously (8). Affinity-purified antibodies to prenyltransferase were raised against a fusion protein of antranilic synthetase and prenyltransferase (3).

Equal amounts (20 μg) of either total or poly(A)+ mRNA were electrophoresed on agarose gels and analyzed and quantitated after transfer to Biorna membranes as reported previously (7). Each gel also contained two members of a control mRNA obtained from cells that had been incubated with LPDS and mevinolin at a concentration of 1 and 2 μg RNA/lane. These latter samples served as internal controls to check for decreases in 80–90% in the concentrations of the experimental mRNAs. CDNA probes for HMG-CoA reductase (ADS11) (19), a gift from Dr. Simoni (Stanford University, Stanford, CA), HMG-CoA synthase (XLA10) (20), and prenyltransferase (3) were radiolabeled and hybridized as described previously (1).

RESULTS

Coordinate Regulation of HMG-CoA Synthase, HMG-CoA Reductase, and Prenyltransferase—Fig. 1, lanes 1 and 2, show that HepG2 cells express a single species of mRNA that hybridizes to the rat prenyltransferase cDNA. The size of the human prenyltransferase mRNA was estimated to be 1.2 kb. On Northern blots the rat HMG-CoA synthase cDNA XLA10 (20) hybridizes to two human mRNA species estimated from DNA size standards to be approximately 3.1 and 2.2 kb (Fig. 1, lanes 3 and 4). Both of these synthase mRNAs are coordinately regulated under every condition tested (see below). On Northern blots the ratio of the intensity of the 3.1- to 2.2-kb bands approximates 8 (Fig. 1 and data not shown). Recently Gil et al. (21) have shown that differential splicing in the 5′-untranslated region of the hamster and human HMG-CoA synthase mRNAs results in a difference of 59 nucleotides in the size of the mRNA. Separation of these two mRNA species on a Northern blot is not expected. However, different polyadenylation sites separated by 1.3 kb are known to occur in the hamster (22). It is possible that the human HMG-CoA synthase mRNA also has different polyadenylation sites that produce the two mRNA species seen in Fig. 1. As expected (7), the hamster HMG-CoA reductase cDNA probe ADS11 (19) hybridizes to two major reductase mRNAs (data not shown).

In initial experiments, HepG2 cells were preincubated for different times in the presence of LPDS supplemented with mevinolin and then pulsed with [35S]methionine for 60 min. Fig. 2 shows that under these conditions the relative rates of synthesis of immunoprecipitated prenyltransferase, HMG-CoA synthase, and HMG-CoA reductase were all increased 3.5–6-fold over a 24-h period.

In order to study the effect of various known inhibitors of cholesterogenesis on the coordinate expression of these three proteins, HepG2 cells were preincubated for 24 h in the presence of LPDS and mevinolin, and then the medium was supplemented with either LDL, 25-hydroxycholesterol, or 25-hydroxycholesterol plus mevalonic acid. After 9.25-h there was a decline in the mRNA levels of prenyltransferase, HMG-CoA synthase, and HMG-CoA reductase (Table I). Maximal inhibition was observed in cells incubated with 25-hydroxycholesterol plus mevalonic acid (Table I). In order to better define the coordinate regulation of the three proteins, mRNA levels and the apparent rates of synthesis of the three proteins...
TABLE I

Comparison of the effect of different sterol-containing media on mRNA levels of HMG-CoA synthase, HMG-CoA reductase, and prenyltransferase

HepG2 cells were preincubated in LPDS plus mevinolin for 24 h. At this time, LDL (100 μg/ml), 25-hydroxycholesterol (1 μg/ml), or 25-hydroxycholesterol plus mevalonic acid (10 mM) were added to dishes. Ethanol (1 μg/ml medium) was added to controls. After 9.25 h mRNA was isolated and quantitated as described under "Experimental Procedures."

| Addition to media | HMG-CoA synthase | HMG-CoA reductase | Prenyltransferase % |
|------------------|------------------|------------------|------------------|
| Ethanol          | 100              | 100              | 100              |
| LDL              | 59               | 41               | 68               |
| 25-Hydroxycholesterol | 21         | 41               | 63               |
| 25-Hydroxycholesterol + mevalonic acid | 20       | 32               | 31               |

Fig. 3. Coordinate regulation of HMG-CoA synthase, HMG-CoA reductase, and prenyltransferase mRNAs and protein synthesis by 25-hydroxycholesterol plus mevalonic acid. HepG2 cells were preincubated for 24 h in medium supplemented with LPDS plus mevinolin. 25-Hydroxycholesterol (1 μg/ml) plus mevalonic acid (10 mM) was then added to experimental cells (●, ◦), and ethanol was added to control cells (○, Δ). At the times indicated, mRNA was isolated and quantitated on Northern blots (Δ, ◦), or cells were pulsed with [35S]methionine for 60 min and the antigens immunoprecipitated and quantitated (O, ●) as described in the legend to Fig. 2. Values shown are the means of values obtained from two or three dishes. Similar results were observed in two other experiments.

Fig. 4. Comparison of the temporal effect of 25-hydroxycholesterol or 25-hydroxycholesterol plus mevalonic acid on the mRNA levels of HMG-CoA synthase, HMG-CoA reductase, and prenyltransferase. Cells were preincubated in medium containing LPDS plus mevinolin as described in the legend to Fig. 3. 25-Hydroxycholesterol (1 μg/ml; ○), 25-hydroxycholesterol plus mevalonic acid (10 mM; ●), or ethanol (Δ) was then added to the cells, and mRNA was isolated at various times and quantitated as described under "Experimental Procedures." Panels A, B, and C show the results for HMG-CoA synthase, HMG-CoA reductase, and prenyltransferase, respectively.

were determined at various times after the addition of 25-hydroxycholesterol plus mevalonic acid to the cells. Fig. 3 shows that the decline in mRNA levels for HMG-CoA synthase, HMG-CoA reductase, and prenyltransferase were paralleled by a similar decline in the apparent rates of specific protein synthesis. 14 h after the addition of the inhibitors, the mRNA levels for HMG-CoA synthase, HMG-CoA reductase, and prenyltransferase declined 75–90% (Fig. 3). However, the initial rates of decline of HMG-CoA synthase and HMG-CoA reductase mRNA levels were more rapid than that of prenyltransferase (Fig. 3). The decline in mRNA levels will depend on a number of processes including the degree and rate of inhibition of transcription of the specific genes and the stability of the specific mRNAs.

The rate of decline of the three mRNAs was similar when cells were incubated with 25-hydroxycholesterol or 25-hydroxycholesterol plus mevalonic acid (Fig. 4). Therefore, under these conditions, the approximate 90% decline in mRNA levels does not appear to require the presence of nonsterols that may be synthesized endogenously from the added mevalonic acid.

Protein Stability—Post-translational control mechanisms are known to affect the stability and hence activity of HMG-CoA reductase (8, 12, 23). Fig. 5 shows that in HepG2 cells incubated in the presence of LPDS and mevinolin the half-lives of HMG-CoA synthase, HMG-CoA reductase, and prenyltransferase were 7.5, 12.1, and 66 h, respectively. Addition of low density lipoprotein to the cells during the chase period decreased the half-life of HMG-CoA reductase 4.6-fold to 2.6 h (Fig. 5B) but had no effect on the half-life of HMG-CoA synthase (Fig. 5A) or prenyltransferase (Fig. 5C). These data do not support a common mechanism that controls the stability of the three proteins.

DISCUSSION

The data presented demonstrate that the biosynthesis of three enzymes, HMG-CoA synthase, HMG-CoA reductase, and prenyltransferase, is coordinately regulated in human hepatoma HepG2 cells. In rat liver, both prenyltransferase mRNA levels and protein concentrations are known to change coordinately with those of HMG-CoA synthase and HMG-CoA reductase. This coordinated regulation is likely to be relevant to the control of cholesterol synthesis, and the half-life of these enzymes is likely to be shorter in the presence of LDL.
CoA reductase following intragastric administration of mevalonic acid or after addition to the diet of cholesterol or of the two drugs cholestyramine and mevinolin (1, 3). Previously, Luskey et al. (9) have reported the coordinate regulation of HMG-CoA reductase and a 53-kDa protein in UT-1 Chinese hamster ovary cells that contain multiple copies of the HMG-CoA reductase gene. The current study demonstrates the coordinate regulation of three mRNAs in human cells under conditions known to either induce cholesterol synthesis (LPDS plus mevinolin; Fig. 2) or inhibit cholesterol synthesis (LDL and 25-hydroxycholesterol in the absence or presence of mevalonic acid; Table I and Figs. 1, 3, and 4). Furthermore, the changes in mRNA levels for all three proteins are paralleled by similar changes in the relative rates of protein synthesis as measured by immunoprecipitation of radiolabeled proteins (Fig. 3). Hence, we find no evidence for translational control of HMG-CoA synthase, HMG-CoA reductase, or prenyltransferase in human hepatoma HepG2 cells.

Recently Peffley and Sinensky (11) and Nakashishi et al. (12) reported that in Chinese hamster ovary cells incubated in the presence of 25-hydroxycholesterol and mevalonic acid there was a greater decrease in the rate of synthesis of HMG-CoA reductase than in the HMG-CoA reductase mRNA levels. Tanaka et al. (13) had made a similar observation in studies using chicken myeloblasts. All these authors propose that HMG-CoA reductase is therefore, in part, under translational control. The absence of such a control mechanism in the present study may be related to the different cell types, differences in the nucleotide sequence of HMG-CoA reductase in chicken, hamster, and human mRNAs, or to different experimental conditions.

Other studies have shown that the promoters for HMG-CoA synthase, HMG-CoA reductase, and the LDL receptor contain an octanucleotide that may play an important role in control of transcription of these three genes (4, 24). It seems likely that the prenyltransferase promoter might also contain a similar sequence that might bind to a common trans-acting factor and result in coordinate regulation of all four genes. Isolation of the prenyltransferase promoter is underway in order to test this proposal.

The present report demonstrates that in the presence of LPDS and mevinolin human HMG-CoA reductase has a half-life of approximately 12 h. This is similar to the value reported for HMG-CoA reductase in rat liver (8) and Chinese hamster ovary fibroblasts (23) when the experiments were carried out in the presence of mevinolin. As expected, the reductase half-life was decreased (4.6-fold) when LDL was included in the media during the chase (Fig. 5). The current studies demonstrate that HMG-CoA synthase, like HMG-CoA reductase, has a relatively short half-life (7.5 h). The half-life of prenyltransferase was comparably much longer and approximated 66 h (Fig. 5). In contrast to HMG-CoA reductase, the stabilities of HMG-CoA synthase and prenyltransferase proteins were not affected by the addition of LDL to the incubation media (Fig. 5). Hence, the coordinate regulation of these three enzymes is at the level of enzyme synthesis and not at the level of enzyme stability.

Why is prenyltransferase coordinately regulated with HMG-CoA reductase and HMG-CoA synthase? One attractive hypothesis is that precursors of prenyltransferase, for example, isopentenyl pyrophosphate, are substrates for other important pathways that diverge from the cholesterogenic pathway before the formation of farnesyl pyrophosphate. Therefore, under conditions that result in low levels of HMG-CoA reductase and a decreased rate in the biosynthesis of mevalonic acid (e.g. cholesterol or 25-hydroxycholesterol administration), the reduced levels of prenyltransferase will allow precursors of this latter enzyme to be shunted into these other pathways. Conversely, if high levels of farnesyl pyrophosphate synthetase persisted under conditions known to inhibit HMG-CoA reductase, then farnesyl pyrophosphate synthetase would be expected to compete for these limited precursors and preferentially shunt them to farnesyl pyrophosphate. Such shunting might be deleterious to the cell. Experiments to test this hypothesis are currently being performed.

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