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Biochemistry

Biogenesis of 3-hydroxy-3-methylglutaryl-coenzyme A reductase, an integral glycoprotein of the endoplasmic reticulum

(membrane-bound polysomes/cotranslational insertion/signal recognition particle/glycosylation/uncleaved signal sequence)

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ABSTRACT Using a cell line, C100, that overproduces 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase; EC 1.1.1.34) 100-fold, we have studied the synthesis and insertion of this protein into the endoplasmic reticulum. The enzyme is synthesized on membrane-bound polysomes. It is cotranslationally but not post-translationally inserted into dog pancreatic microsomes. This cotranslational insertion is dependent upon signal recognition particle. HMG-CoA reductase is glycosylated with an oligosaccharide(s) of the "high-mannose" type sensitive to endo-β-D-N-acetylglucosaminidase H. Partial determination of the NH_2-terminal amino acid sequence of the *in vitro* translation product and the mature polypeptide indicate they are the same and demonstrate there is no cleavage of an NH_2-terminal signal sequence.

3-Hydroxy-3-methylglutaryl-coenzyme A reductase [HMG-CoA reductase; mevalonate: $NADP^+$ oxidoreductase (CoA-acylating), EC 1.1.1.34] catalyzes the rate-limiting step in the cholesterol biosynthetic pathway. We and others (1–3) have isolated cell lines resistant to the cytotoxic effects of compactin that overproduce this key enzyme. It has been demonstrated that enzyme overproduction results from increased levels of HMG-CoA reductase mRNA (4), which are attributable, in part, to an amplification of the gene for HMG-CoA reductase (4).

We have examined one cell line, derived from baby hamster kidney (BHK) cells, called C100, which is resistant to 225 μM compactin. In the course of demonstrating increased levels of enzyme, we attempted to solubilize HMG-CoA reductase from the microsomal membranes by using the established method of solubilization of HMG-CoA reductase, described by Brown *et al.* (5), which involves subjecting microsomes to several freeze–thaw cycles. Since the enzyme can be solubilized in this way without detergents, it has been considered to be a peripheral membrane protein. Recently, Ness *et al.* (6) found that addition of the protease inhibitor leupeptin prevents solubilization by the freeze–thaw method. Since then, we (2) and others (7, 8) have found that solubilization of HMG-CoA reductase in the presence of leupeptin requires detergent and demonstrated that the apparent molecular of the protomer is 92,000. This suggests that MHG-CoA reductase is actually an integral membrane protein and that forms of the enzyme with apparent molecular weights less than 92,000 are proteolytic products.

As a part of characterizing the increased levels of enzyme in these cell lines, we and others also demonstrated that the *in vitro* translation product and the enzyme isolated from cells comigrate on $NaDodSO_4$ gel electrophoresis (7). This observation supports the claim that the 92-kilodalton (kDa) polypeptide is the undegraded protomer of HMG-CoA reductase. To further compare the translation product and mature enzyme *in vivo*, the peptide maps of the two were compared. The digestion patterns were similar, but there were distinct differences (unpublished observations). These differences suggested that HMG-CoA reductase is modified post-translationally. In this report we examine the biosynthesis of HMG-CoA reductase, considering the events that occur both during and after translation.

MATERIALS AND METHODS

Materials. L-[^{35}S]Methionine (1200 Ci/mmol; 1 Ci = 37 GBq) and D-[3,4-^{3}H(n)]mannose (44.2 Ci/mmol) were from New England Nuclear. L-[4,5-^{3}H]Leucine (160 Ci/mmol) was from Amersham. Placental RNase inhibitor for use in wheat germ *in vitro* translations was from Bio-Tec. Molecular weight standards for gel electrophoresis (Bio-Rad or Pharmacia) include phosphorylase b as the 92 kDa marker. Recently, it has been suggested that the molecular mass of this protein is actually 97 kDa. Compactin was the gift of Akira Endo. Antibody directed against rat liver HMG-CoA reductase was the kind gift of Hans-Stephan Jenke. We thank James Rothman for the gift of endoglycosidase H and Peter Walter for purified signal recognition particle (SRP) and KCl-extracted microsomes. All other chemicals were of the highest quality from commercial sources.

Cell Growth. The selection and maintenance of the C100 cell line has been described (2). Cells were grown as monolayers in minimal essential medium (ME medium; GIBCO) supplemented with nonessential amino acids and 5% delipidated fetal calf serum. Serum was delipidated by solvent extraction (9) or by removal of lipoproteins with 2% (wt/vol) Cab-o-sil (Packard) (10). All cells were grown in the presence of 225 μM compactin as described (2).

***In Vitro* Translation Systems.** Rabbit reticulocyte lysate was prepared as described by Pelham and Jackson (11) or purchased from Amersham. When it was prepared, methionine was omitted from lysate to be used in translations with [^{35}S]methionine, and leucine was omitted from lysate to be used in translations with [^{3}H]leucine. Translation conditions were those recommended by Amersham. Translation reactions were carried out for 1 h at 30°C except where indicated. Wheat germ extract was prepared as described (12) and translations were carried out as described by Theologis and Ray (13) without nuclease treatment of the extract. Inclusion of placental RNase inhibitor significantly increased the translation of HMG-CoA reductase. Translation reactions were for 60 min at 30°C. Total cellular RNA was prepared by the method of Strohman *et al.* (14) and stored at −20°C as an ethanol precipitate. Poly(A)$^+$ RNA was prepared by the method of Aviv and Leder (15).

Microsome Preparation. Dog pancreatic microsomes were prepared from fresh tissue by the method of Shields and Blobel (16). Microsomes to be included in wheat germ translation reactions were passed through a Sepharose CL-2B col-

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Abbreviations: HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; SRP, signal recognition particle; endoglycosidase H, endo-β-D-N-acetylglucosaminidase H; kDa, kilodalton(s).

umn according to Walter *et al.* (17) to reduce inhibition of translation.

Polysome Separation. Membrane-bound and free polysomes were separated by the method of Ramsey and Steele (18). In some experiments, the polysomes were further purified according to those authors. Polysomes were then added directly to *in vitro* translation mixtures (5–10) A_{260} units/ml). In other experiments, fractions containing the crude separated polysomes were pelleted by ultracentrifugation and the RNA was extracted by the method of Strohman (14). Both methods gave the same results.

Labeling of HMG-CoA Reductase *in Vivo*. Cells to be labeled with [^{35}S]methionine were transferred to media lacking methionine and containing 5% dialyzed delipidated serum. Cells in a 150-cm^2 flask were labeled in 10 ml of medium containing 1 mCi of [^{35}S]methionine for 4 hr at 37°C. Cells to be labeled with [^{3}H]mannose were incubated in 10 ml of medium with 0.1% glucose (10% of the glucose in the normal growth medium) containing 5 mCi of [^{3}H]mannose for 4 hr at 37°C. At the end of the labeling period, flasks were washed with medium and cells were dissolved in 4 ml of solubilization buffer C (described in ref. 2). Cells to be labeled with [^{3}H]leucine were incubated in 10 ml of medium containing 5 mCi [^{3}H]leucine as the only leucine for 4 hr at 37°C. Cells were harvested as above, in 1 ml of solubilization buffer C. Extracts were stored frozen at −20°C.

Tunicamycin Treatment. A stock solution of tunicamycin at 1.25 mg/ml in 25 mM NaOH was stored at −20°C. [^{35}S]Methionine labeling and tunicamycin treatment were performed simultaneously. Four microliters of tunicamycin solution of 25 mM NaOH was added to 2 ml of fresh medium (the same medium described above for [^{35}S]methionine labeling) containing [^{35}S]methionine at 100 μCi/ml in a 75-cm^2 flask. Flasks were incubated 2½ hr at 37°C with 5% CO_2. Cells were harvested as described above. This treatment did not affect protein synthesis as judged by incorporation of [^{35}S]methionine into trichloroacetic acid-precipitable form.

Treatment with Endo-β-D-*N*-acetylglucosaminidase H. HMG-CoA reductase labeled *in vivo* with [^{35}S]methionine was immunoprecipitated as described (2). After the final wash, the complexes were eluted from the IgGsorb with 30 μl of the following buffer: 0.1 M Tris·HCl, pH 6.8/2% NaDodSO$_4$/30 mM dithiothreitol. Then 0.2 unit of endoglycosidase H in 0.3 M sodium citrate buffer, pH 5.5/0.1% NaDodSO$_4$ or 20 μl of this buffer alone was added to the HMG-CoA reductase sample and incubated overnight (12–16 hr) at 24°C. After acetone precipitation, the protein was dissolved in 30 μl of urea/NaDodSO$_4$ dissociation buffer as described below and gel electrophoresis was performed.

Immunoprecipitation. Immunoprecipitation of HMG-CoA reductase was performed as described (2). Complexes were eluted from the IgGsorb for gel electrophoresis with 30 μl of dissociation buffer containing 8 M urea, 5% NaDodSO$_4$, 0.25 M Tris·HCl at pH 6.2, and 470 mM 2-mercaptoethanol. Use of buffer containing 8 M urea helps prevent aggregation prior to electrophoresis (7). Samples were heated to 85°C for 3 min before electrophoresis.

Electrophoresis. Acrylamide gel electrophoresis was performed by the procedure of Laemmli (19). For most experiments, gels contained 10% acrylamide and were 11 cm long. Where indicated, 22-cm-long gels, containing 5% acrylamide, were used. All gels were fluorographed by the method of Laskey (20). In some cases, fluorogram bands were scanned with a Joyce–Loebl microdensitometer. Areas under peaks were quantitated by using a Numonics (Lansdale, PA) digital planimeter.

Amino Acid Sequence Analysis. Automated Edman degradation was performed by Alan Smith (Protein Chemistry Laboratory, Biochemistry Department, University of California at Davis).

RESULTS

We have shown that resistance of the cell line C100 to 225 μM compactin is achieved by overproduction of HMG-CoA reductase protein (2) and mRNA (unpublished observations). The enzyme is present in wild-type cells at such low concentrations that some experiments are difficult. Thus, the overproducing cell line is an attractive experimental system. The availability of this cell line and the recent demonstration that HMG-CoA reductase is an integral membrane protein prompted us to investigate its synthesis and insertion into the endoplasmic reticulum.

Synthesis of HMG-CoA Reductase on Membrane-Bound Polysomes. Membrane-bound and free polysomes (or purified RNA from each fraction, data not shown) were added to the *in vitro* reticulocyte lysate translation system. The gel analysis of translation products using the purified polysomes is shown in Fig. 1. Total translation products from free and bound polysomes fractions, respectively, are shown in lanes 1 and 2. Immunoprecipitates of these bound and free polysome translation products are shown in lanes 3 and 4. HMG-CoA reductase is clearly synthesized from RNA in the bound polysome fraction (lane 3) and not from the free polysomes.

Cotranslational Association of HMG-CoA Reductase with Dog Pancreatic Microsomes. HMG-CoA reductase synthesized in an *in vitro* translation system remains in the supernatant fraction after centrifugation and thus behaves as a soluble protein. (Fig. 2; compare lanes 1 and 2). However, when dog pancreatic microsomes are added to the reticulocyte translation mixture, HMG-CoA reductase sediments with the added microsomes and, we assume, inserts into the endoplasmic reticulum (Fig. 2; compare lanes 3 and 4). When the microsomal membranes are heated at 60°C for 10 min prior to addition to the translation system [a treatment known to inactivate the translocation system (21)], HMG-CoA reductase does not insert into the endoplasmic reticulum (Fig. 2; compare lanes 5 and 6). Likewise, when active microsomes are added post-translationally, the enzyme does not insert into the membranes (Fig. 2; compare lanes 7 and 8). These results demonstrate that HMG-CoA reductase inserts into the endoplasmic reticulum cotranslationally but not post-translationally. It should also be noted that with these electrophoresis conditions there is no apparent difference in molecular weight of the HMG-CoA reductase synthesized in the presence and absence of microsomes (Fig. 2; compare lanes 1 and 4).

Association of HMG-CoA Reductase with the Endoplasmic Reticulum Depends on SRP. As shown above, HMG-CoA reductase fails to insert into membranes that have been heated. This indicates that the insertion process may depend on a protein component of the membranes. Walter and Blobel (22) have recently described a protein–RNA complex called

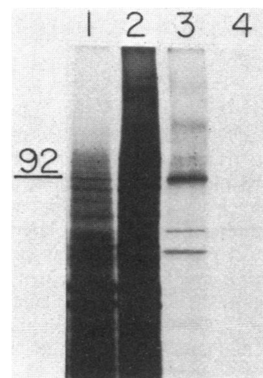

FIG. 1. Immunoprecipitation of HMG-CoA reductase from translation products of membrane-bound and free polysomes. Membrane-bound and free polysomes were isolated and translated in the reticulocyte lysate system (8 A_{260} units, 22-μl translation volume). Polyacrylamide gel electrophoresis was in dissociation buffer. The total translation products (1.5 × 10^6 cpm) are shown in lanes 1 (free) and 2 (bound). Immunoprecipitates from 1 × 10^6 cpm of total translations are presented in lanes 3 (bound) and 4 (free). Only the upper portion of the gel is shown. The position of the 92-kDa marker is indicated.

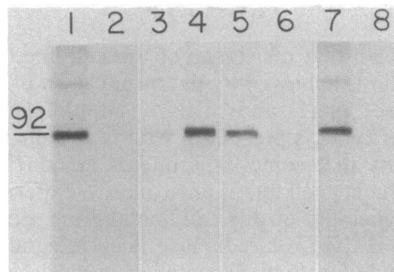

FIG. 2. Analysis of HMG-CoA reductase synthesized in the presence of dog pancreatic microsomes. Total cellular RNA (2 μg) was translated in the reticulocyte lysate system (22 μl). For separation of membrane-bound and soluble products, incubation mixtures were diluted with 150 μl of phosphate-buffered saline containing 100 μM leupeptin and 0.5 μl of packed carrier microsomes. Samples were subjected to centrifugation in a Beckman Airfuge at 28 pounds/inch^2 for 10 min. Supernatant and pellet fractions were immunoprecipitated and analyzed by gel electrophoresis. Lanes 1 (supernatant) and 2 (pellet), no microsomes added; lanes 3 (supernatant) and 4 (pellet), microsomes added during translation; lanes 5 (supernatant) and 6 (pellet), microsomes heated for 10 min at 60°C and added during translation; lanes 7 (supernatant) and 8 (pellet), translation carried out for 45 min, mixtures brought to 225 mM methionine and further incubated 30 min, then 5 A_{260} units of microsomes was added and incubated 45 min longer. Only the upper portion of the gel is shown.

SRP that is required to direct certain nascent proteins to the endoplasmic reticulum. Dog pancreatic microsomes contain sufficient endogenous SRP in a membrane-bound form to allow translocation. When this SRP is stripped off the microsomes by KCl extraction, translocation function is lost. It can be restored by adding back purified SRP. The rabbit reticulocyte lysate contains some SRP in a soluble form (23) and is thus not a suitable system for studying an SRP-dependent process. For this reason we used the wheat germ translation system, which does not contain significant soluble SRP (23). When salt-extracted microsomes were added to the translation system, 90% of the HMG-CoA reductase remained in soluble form (Fig. 3; compare lanes 1 and 2). The addition of SRP inhibited total protein synthesis by 40%, as determined by a decrease in trichloroacetic acid-precipitable radioactive material. Densitometric scanning showed that the synthesis of HMG-CoA reductase was also inhibited by 60%. However, in the presence of SRP, 50% of the total HMG-CoA reductase associated with microsomes. It is clear from this experiment that SRP is required for the insertion of HMG-CoA reductase into the endoplasmic reticulum.

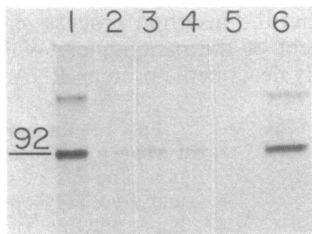

FIG. 3. Effect of SRP on association of HMG-CoA reductase with dog pancreatic microsomes. Poly(A)$^+$ RNA (1 μg) was translated in the wheat germ extract system (25-μl translation volume) in the presence of 10 A_{260} units of native microsomes or 1 equivalent of salt-washed microsomes (18). Soluble (lanes 1, 3, and 5) and membrane-bound (lanes 2, 4, and 6) translation products were isolated, immunoprecipitated, and analyzed by gel electrophoresis as described in the legend of Fig. 2 and *Materials and Methods*. Lanes 1 and 2, translations in the presence of salt-washed microsome; lanes 3 and 4, salt-washed microsomes plus 5 equivalents of SRP (18); and lanes 5 and 6, native microsomes. Only the upper portion of the gel is shown.

Post-translational Modification of HMG-CoA Reductase. As discussed above, differences between peptide maps of the *in vitro* translation product and the mature enzyme suggest post-translational modification. However, the two forms appear to comigrate on our gels. In Fig. 4, lanes 2 and 3, is shown HMG-CoA reductase analyzed by an electrophoresis system more sensitive to small differences in molecular weights. We find that the mature enzyme is about 2000 daltons larger than the *in vitro* translation product. This difference shows that HMG-CoA reductase is modified after translation.

We used the antibiotic tunicamycin, which prevents protein *N*-glycosylation reactions, to test the possibility that HMG-CoA reductase is glycosylated. The data shown in Fig. 4, lanes 1 and 2, clearly indicate that this occurs. HMG-CoA reductase from cells incubated without tunicamycin (lane 2) migrates more slowly than that from cells incubated with the drug (lane 1).

Incubation of cells with [^{3}H]mannose provides additional evidence that HMG-CoA reductase is glycosylated. In Fig. 5, we see that HMG-CoA reductase is labeled by [^{3}H]mannose (Fig. 5*A*, lane 2). Further, the label can be completely removed by incubation of the protein with endoglycosidase H (Fig. 5*A*, lane 3), showing that the ^{3}H label in the protein is mannose and that it exists in the high-mannose oligosaccharide form. When HMG-CoA reductase is labeled *in vivo* with [^{35}S]methionine and then subjected to endoglycosidase H digestion, the treated enzyme (Fig. 5*B*, lanes 1 and 3) is smaller than the untreated enzyme (lanes 2 and 4), since the carbohydrate moiety has been removed. It can also be seen in Fig. 5*B* that during the 12-hr incubation with endoglycosidase H there was significant degradation of the enzyme, yielding a prominent proteolysis product of about 63 kDa. This fragment, which has been demonstrated before (2), represents the catalytically active cytoplasmic domain of the enzyme. As can be seen from the data in Fig. 5*B*, the apparent molecular weight of this fragment is not altered by endoglycosidase H. In addition, this fragment is not labeled by [^{3}H]mannose (Fig. 5*A*). It thus appears not to be glycosylated.

HMG-CoA Reductase Does Not Have a Cleaved NH_2-terminal Signal Squence. HMG-CoA reductase grown in the presence of tunicamycin appears to migrate with HMG-CoA reductase made *in vitro* (Fig. 4; compare lanes 1 and 3). This indicates that glycosylation can fully account for the apparent molecular weight difference between the two forms. Cleavage of a typical NH_2-terminal signal sequence would lower the molecular weight of the *in vivo* form by about 2000, which should be detectable by this electrophoresis system.

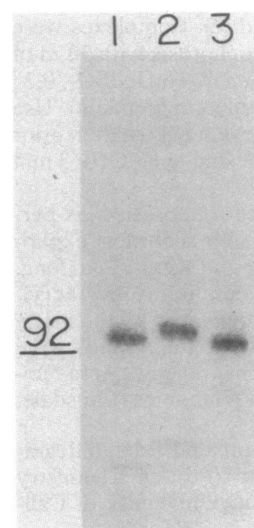

FIG. 4. Effect of tunicamycin on the apparent molecular weight of HMG-CoA reductase. Cells were labeled with [^{35}S]methionine in the presence (lane 1) or absence (lane 2) of tunicamycin and HMG-CoA reductase was immunoprecipitated from the cell extracts. Immunoprecipitate of a reticulocyte translation reaction is shown in lane 3. Samples were analyzed by electrophoresis in 5% acrylamide, on a 22-cm gel. Only the bottom portion of the gel is shown.

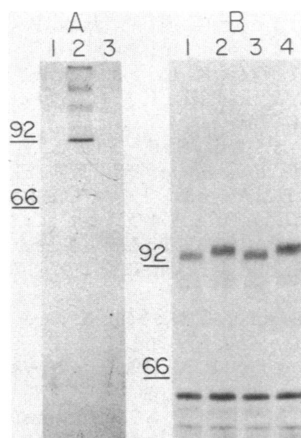

FIG. 5. Effect of endoglycosidase H on HMG-CoA reductase. Cells were labeled with [^{3}H]mannose (*A*) or [^{35}S]methionine (*B*) for 4 hr. The immunoprecipitates were incubated with endoglycosidase H (*A*, lane 3, and *B*, lanes 1 and 3) or buffer (*B*, lanes 2 and 4). (*A*) Lane 1, extracts immunoprecipitated with nonimmune serum, then incubated with buffer.

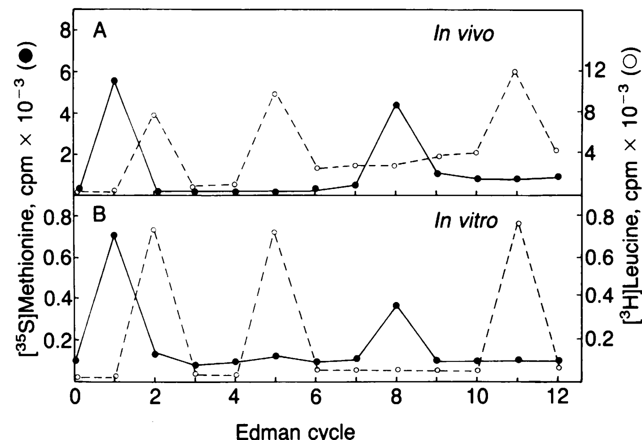

FIG. 6. NH_2-terminal amino acid sequence of radiolabeled HMG-CoA reductase. HMG-CoA reductase was labeled *in vivo* and *in vitro* with [^{35}S]methionine and [^{3}H]leucine. Immunoprecipitations were performed on 3 ml of *in vitro* translation product, double-labeled with 2.5 mCi of [^{35}S]methionine and 4.5 mCi of [^{3}H]leucine, or 0.5 ml of cell extract (labeled as in *Materials and Methods*). Immunoprecipitates labeled *in vivo* with [^{35}S]methionine (6×10^5 cpm) were extracted with 88% (wt/vol) formic acid and subjected to automated Edman degradation after addition of apomyoglobin as internal standard. Immunoprecipitated *in vitro* material and *in vivo* [^{3}H]leucine labeled protein were subjected to gel electrophoresis. The unfixed gel was dried and autoradiographed. The HMG-CoA reductase band was cut out of the gel and allowed to swell in electrophoresis reservoir buffer, and the protein was electroeluted at 200 V for 18 hr. The protein was dialyzed against water overnight and then freeze-dried. After dissolving in 0.5 ml of 88% formic acid, the protein was loaded on a Bio-Rad P6DG column (21 cm high, 95 ml bed volume) equilibrated with formic acid, for desalting. Fractions containing radioactivity (1.2×10^5 cpm of [^{35}S]methionine, 2.7×10^5 cpm of [^{3}H]leucine *in vitro*, 2.2×10^6 cpm of [^{3}H]leucine *in vivo*) were pooled, dried under a stream of nitrogen, and subjected to automated Edman degradation as described for *in vivo* [^{35}S]methionine-labeled material. The radioactivity released at each cycle of the sequencer is presented for the *in vivo* mature enzyme in *A*, and the *in vitro* translation product is presented in *B*. Each cleavage cycle was confirmed by HPLC analysis of the residues released from apomyoglobin.

The absence of any apparent difference led us to suspect that no NH_2-terminal cleavage occurred during synthesis and insertion into the endoplasmic reticulum. To be certain, we compared the partial NH_2-terminal amino acid sequences of the *in vitro* and *in vivo* forms of HMG-CoA reductase. HMG-CoA reductase was radiolabeled with [^{35}S]methionine and [^{3}H]leucine both *in vitro* and *in vivo* and the immunoprecipitated polypeptides were subjected to automated amino acid sequence analysis. As can be clearly seen in Fig. 6, both *in vitro* and *in vivo* enzyme have methionine at positions 1 and 8, and leucine at positions 2, 5, and 11.

DISCUSSION

An overall model for synthesis of secreted proteins is emerging. Many aspects of this model appear to apply to various integral membrane proteins as well (21–25). The first 20 or so amino acids of the nascent protein are made on polysomes in the cytoplasm. This signal sequence is hydrophobic and rapidly binds to SRP. Translation is halted until SRP directs the polysome to the endoplasmic reticulum. The signal sequence is then vectorially discharged into the lumen of the endoplasmic reticulum and translation continues. Once in the lumen of the endoplasmic reticulum, the signal sequence is cleaved by a signal peptidase. During synthesis, the nascent peptide serves to bind the polysome to the membrane. *In vitro* studies show that if microsomal membranes are not available early in translation, the protein rapidly folds into a conformation that does not allow integration into membranes. The protein then remains in a soluble form, even if membanes are added later.

It is not clear how general this model is for the synthesis of integral membrane proteins. The integral membrane proteins of the endoplasmic reticulum that have been studied so far (26, 27), appear to fall in two classes. Cytochrome b_5 and cytochrome b_5 reductase are made on free polysomes and insert into the membrane post-translationally. Cytochrome P-450, its reductase, and epoxide hydrolase are synthesized on membrane-bound polysomes and insert into membranes co-translationally. All these proteins are made *in vitro* in their mature forms, though those made on membrane-bound polysomes all have hydrophobic NH_2 termini that may act as signal sequences. It has been proposed that such a sequence may be a characteristic feature of proteins of the endoplasmic reticulum (26).

HMG-CoA reductase falls into the same class as cytochrome P-450 reductase and epoxide hydrolase. Though it is made on membrane-bound polysomes and cotranslationally inserted into the membrane, no signal sequence is cleaved from the NH_2 terminus. The presence of 3 leucine residues in the first 11 residues suggests that the NH_2-terminal segment of HMG-CoA reductase may be hydrophobic. It is not known whether the NH_2 terminus remains associated with the membrane in the mature protein as is the case for cytochrome P-450 reductase (26) and as has been reported for cytochrome P-450 (28).

We have also shown clearly that HMG-CoA reductase inserts into the membrane via the SRP-mediated pathway. In addition, we have made the important observation that HMG-CoA reductase is glycosylated. Since the enzymes responsible for *N*-glycosylation are located in the lumen of the endoplasmic reticulum (29), this demonstrates that a portion of HMG-CoA reductase penetrates the membrane and is present on the luminal surface. These results also relate to the interesting observation of Volpe and Goldberg (30) that tunicamycin treatment of C-6 glial cells results in reduced HMG-CoA reductase activity. On the basis of the data presented in this paper, we propose a tentative model for the topology of HMG-CoA reductase in the endoplasmic reticulum as depicted in Fig. 7.

When Okada *et al.* (26) studied the synthesis of several endoplasmic reticulum proteins, they found two common features. None of the proteins were glycosylated, and none had a cleaved NH_2-terminal signal sequence. They proposed that one or both of these features might be involved in preventing these proteins from being transported from the endo-

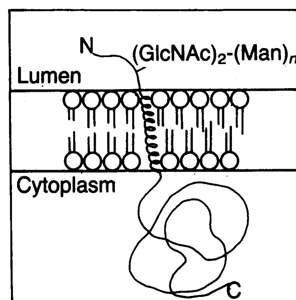

FIG. 7. Tentative model for the topology of HMG-CoA reductase in the endoplasmic reticulum.

plasmic reticulum to other organelles. We have found that HMG-CoA reductase is glycosylated. This demonstrates that glycosylation does not necessitate migration from the endoplasmic reticulum. Our findings are consistent with the proposal that a hydrophobic uncleaved NH_2-terminal sequence is a common feature of proteins that remain in the endoplasmic reticulum. It is interesting to note that proteins of the *Escherichia coli* cytoplasmic membrane (which, like endoplasmic reticulum proteins, remain in the membrane in which they are synthesized) also have uncleaved NH_2-termini, while those that are exported to other compartments are processed.

Note Added in Proof. After this manuscript was submitted for publication a report appeared demonstrating that HMG-CoA reductase is a glycoprotein (31).

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