Characterization of UT2 Cells

THE INDUCTION OF PEROXISOMAL 3-HYDROXY-3-METHYLGUTARLYL-COENZYME A REDUCTASE*

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In the liver 3-hydroxy-3-methylglutarlyl-coenzyme A (HMG-CoA) reductase is present not only in the endoplasmic reticulum but also in the peroxisomes. However, to date no information is available regarding the function of the peroxisomal HMG-CoA reductase in cholesterol/isoprenoid metabolism, and the structure of the peroxisomal HMG-CoA reductase has yet to be determined. We have identified a mammalian cell line that expresses only one HMG-CoA reductase protein and that is localized exclusively to peroxisomes. This cell line was obtained by growing UT2 cells (which lack the endoplasmic reticulum HMG-CoA reductase) in the absence of mevalonate. The cells exhibited a marked increase in a 90-kDa HMG-CoA reductase that was localized exclusively to peroxisomes. The wild type Chinese hamster ovary cells contain two HMG-CoA reductase proteins, the well characterized 97-kDa protein, localized in the endoplasmic reticulum, and a 90-kDa protein localized in peroxisomes. The UT2 cells grown in the absence of mevalonate containing the up-regulated peroxisomal HMG-CoA reductase are designated UT2*. A detailed characterization and analysis of this cell line is presented in this study.

In mammalian cells, 3-hydroxy-3-methylglutarlyl-coenzyme A (HMG-CoA) reductase is the rate-limiting enzyme for the synthesis of mevalonic acid, the precursor of cholesterol and other non-sterol isoprenoids. We and others (1–4) have demonstrated that HMG-CoA reductase is localized in two distinct intracellular compartments, endoplasmic reticulum (ER) and peroxisomes. ER HMG-CoA reductase is a 97-kDa transmembrane glycoprotein. A short non-conserved sequence links the multiple transmembrane domain to the highly conserved catalytic domain, which extends out into the cytosol. Because of its role in cholesterol biosynthesis, the regulation of HMG-CoA reductase has been intensely studied. The levels of the ER enzyme are regulated by transcription (5–7), translation (8, 9), and enzyme degradation (10, 11). Another critical role for this enzyme has emerged in recent years, due to the requirement of farnesyl diphosphate and geranyl-geranyl diphosphate in isoprenylation of proteins (12).

Keller et al. (1) were the first to demonstrate that in the liver HMG-CoA reductase is present not only in the ER but also within the peroxisomes. The function of the peroxisomal reductase in cholesterol/isoprenoid metabolism has yet to be defined. However, it is clear that the ER and peroxisomal HMG-CoA reductases can be regulated differently and, therefore, may play different functional roles (2, 13). The ER reductase has a diurnal cycle distinct from that of the peroxisomal reductase (13). However, the two reductases can also be regulated coordinately. Both reductase activities are induced by cholestyramine (a bile acid resin) (2). No information is available regarding the function of the peroxisomal reductase in cholesterol/isoprenoid metabolism, nor has the structure of the peroxisomal HMG-CoA reductase been determined. Accordingly, to facilitate our studies of the function, regulation, and structure of the peroxisomal HMG-CoA reductase, we have identified a mammalian cell line that expresses only one HMG-CoA reductase protein of 90 kDa and that is localized exclusively to peroxisomes. These cells provide a model system to study the peroxisomal HMG-CoA reductase independent of the ER reductase. A detailed characterization and analysis of this cell line is presented in this study.

EXPERIMENTAL PROCEDURES

Materials—Biochemicals were purchased from Sigma. Electrophoresis supplies, AG1-X8-200–400-mesh formate resin, Zeta Probe GT membrane (used for Northern analysis) and Trans-Blot Transfer Medium (used for Western analysis) were purchased from Bio-Rad. All cell culture media and fetal calf serum were purchased from Life Technologies, Inc. Lipoprotein-deficient media were obtained from PerImmune. 3-Hydroxy-3-methylglutarlyl coenzyme A, (methylglutarlyl-3-14C)- and (RS)-[3H]mevalonic acid was purchased from NEN Life Science Products. 123I-Protein A was obtained from Amersham Corp. Cholestyramine (Questran) was obtained from Bristol Laboratories, and mevinolin (Mevacor) was from Merck.

Animals—Male Sprague-Dawley rats (100–180 g) were maintained on a 12-h light/dark cycle. Water was given ad libitum, and rats were treated for 7 days with a standard laboratory diet containing 5.6% cholestyramine. Rats were fasted overnight and killed by decapitation 2 h into their light cycle.

Cell Culture—UT2 cells were obtained from Dr. J. Goldstein. CHO cells were maintained in 1:1 Dulbecco’s modified Eagle’s media: F12, supplemented with 5% fetal calf serum (FCS), fungizone, and Pen/Strep, in a 37 °C incubator with 5% CO2. UT2 cell cultures were maintained in the same media and supplemented with 0.2 mM mevalonate. We also maintained the UT2 cells in the presence of fetal calf serum (10% FCS) but in the absence of mevalonate. After 3 days in media lacking mevalonate more than 25% UT2 cells remained. The surviving UT2 cells were single cell cloned and designated UT2* and maintained in media lacking mevalonate.

Isolation of Subcellular Organelles from CHO, UT2*, and UT2 Cells—Cell suspensions were pelleted and washed twice with 20 mM KPO4, pH 7.5, 150 mM NaCl, and once with homogenization buffer, 250 mM sucrose, 5 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.1% ETOH. Cells were resuspended in homogenization buffer, gently dispersed with two strokes of a glass/Teflon homogenizer, and transferred to a nitrogen...
cavitation bomb. After a 10-min incubation at 4 °C and 62–63% p.s.i., cells were collected dropwise from the bomb. The suspension was gently homogenized until 80% cell rupture was observed, centrifuged at 750 relative centrifugal field for 5 min, and the pellet resuspended, rehomogenized, and recentrifuged. Supernatants were combined and applied to a linear Nycodenz (20–45% (w/w)) gradient (14). The gradient was then further purified by equilibrium density centrifugation on a linear Nycodenz (20–45% (w/w)) gradient (14). The gradient contained all of the above protease inhibitors except PMSF, DTT, and methionine.

**Cell Fractionation and Preparation of Rat Liver Peroxisomes—Liver homogenates—Cells were first fractionated by differential centrifugation to obtain a peroxisome-enriched fraction, (containing peroxisomes, smaller mitochondria, and microsomes), and a microsomal fraction (14).** The homogenization buffer contained 0.25% n sucrose, 5 mM Tris-HCl, 1 mM EDTA, 0.1% EtOH, 1.28 g/ml aprotinin, 10 μg/ml cycloheximide, 125 ng/ml pestatin A, 250 mg/ml antipain, 125 ng/ml chymotain, 50 μM leupeptin, 100 μM PMSF, 20 μM DTT, 2 mM methionine, 15 μg/ml calpain I, 15 μg/ml calpain II, pH 7.5. The peroxisome-enriched fraction was visualized with a Molecular Dynamics PhosphorImager system. The monoclonal HMG-CoA reductase antibody (clone A-9) was obtained from Drs. Brown and Goldstein’s laboratory (6), and several polyclonal HMG-CoA reductase antibodies were obtained from Dr. P. Edwards (22).

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**Characterization of a Novel HMG-CoA Reductase**

**Double Label Immunofluorescence Microscopy of CHO and UT2**

Cells—Cells were fixed in 3% formaldehyde and 0.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, and infused with London Resin white acrylic resin (London Resin Co., London). After dehydration with ethanol, thin sections were cut on a Reichert Ultra microtome. Immunolabeled sections were poststained in 2% 50–50% tert butanol and uranyl acetate, and stained with a Philips CM12 transmission electron microscope (20, 21). The monoclonal HMG-CoA reductase antibody (clone A-9) was obtained from Drs. Brown and Goldsteins’ laboratory (6), and several polyclonal HMG-CoA reductase antibodies were obtained from Dr. P. Edwards (22).

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cell extract, compared with 80 pmol/min/mg for UT2* cells, in the presence of LPDS, and 200 pmol/min/mg for UT2* cells in the presence of LPDS plus 0.5 μM lovastatin (I). The cells were harvested at 60–70% confluence and homogenized in the presence of Triton X-100 and protease inhibitors, as described under “Experimental Procedures.” 100 μg of cell extract was assayed for HMG-CoA reductase activity. Care was taken to dilute out lovastatin before assaying, to ensure full activity measurements. Each value represents the average of five to six experiments, ± S.D.

As expected, the HMG-CoA reductase activity in CHO cells is down-regulated by the addition of FCS and up-regulated by the addition of LPDS, and further increased by the addition of LPDS and lovastatin (I). Very similar regulation is observed in the UT2* cells.

**Fig. 2. Immunoblotting analysis of HMG-CoA reductase from CHO and UT2* cells.** Cells and cell extracts were prepared as described in Fig. 1 legend. Duplicate samples (200 μg) from each treatment were solubilized in sample buffer, electrophoresed on a 7.5%, 12.5-cm polyacrylamide gel, and processed for immunoblotting using polyclonal anti-reductase IgG. Blots were then incubated with Protein A-horseradish peroxidase and detected with Amersham’s enhanced chemiluminescence reagents.

**Fig. 3. Relative immunoblot density of the 97- and 90-kDa proteins in CHO cells and the 90-kDa protein in UT2* cells.** The immunoblot illustrated in Fig. 2 was scanned on a Molecular Dynamics Densitometer and quantitated by use of ImageQuant.

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**Fig. 1. Activity of HMG-CoA reductase in whole cell extracts from UT2, UT2*, and CHO cells.** CHO and UT2* cells were cultured by standard methods in 5% FCS. UT2 cells were grown in the presence of 5% FCS and 0.2 mM mevalonate. 24 h before harvesting the cells, the monolayers were placed in media containing either 5% FCS, 5% LPDS, or 5% LPDS plus 0.5 μM lovastatin (I). The cells were harvested at 60–70% confluency and homogenized in the presence of Triton X-100 and protease inhibitors, as described under “Experimental Procedures.” 100 μg of cell extract was assayed for HMG-CoA reductase activity. Care was taken to dilute out lovastatin before assaying, to ensure full activity measurements. Each value represents the average of five to six experiments, ± S.D.

Isolation of Organelles from UT2* Cells by Density Gradient Centrifugation—We utilized density gradient centrifugation to determine whether the 90-kDa HMG-CoA reductase protein is localized to the ER or peroxisomes. UT2* cell organelles were isolated from a post-nuclear fraction on a metrizamide linear gradient. Fig. 4 illustrates one of three typical gradients. The separation of endoplasmic reticulum (as determined by cytochrome c reductase), peroxisomes (as determined by catalase), and the distribution of HMG-CoA reductase activity is shown. As can be seen from the catalase distribution, the majority of the intact peroxisomes are found at the dense end (right) of the gradient, well separated from the peak ER fractions. A portion of the catalase activity is solubilized, as a result of rupture of the peroxisomes during the isolation procedure and migrates at the light end of the gradient. The distribution of HMG-CoA reductase activity parallels the distribution of the peroxisomal marker, catalase. The cytosolic fraction is located at the light end of the gradient, and the mitochondrial fraction is also well separated from the peroxisomal fractions (data not shown).

**Isolation of Organelles from CHO Cells by Density Gradient Centrifugation—**In contrast to UT2* cells, CHO cell HMG-CoA

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reductase activity was localized to both the ER and peroxisomes. Fig. 5 illustrates the separation of the organelles in CHO cells. One of three typical gradients is represented. There is a slight contamination of the peroxisomal fractions by the ER, as indicated by the distribution of cytochrome c reductase (marker enzyme for endoplasmic reticulum proteins), catalase (marker enzyme for peroxisomal proteins), and HMG-CoA reductase activity is localized both in the peak ER fractions as well as the peak peroxisomal fractions (panel C).

Fig. 5 shows the results of a typical study in which the density gradient fractions from UT2* and CHO cells were analyzed for both HMG-CoA reductase activity and protein (immunoblot). The results clearly demonstrate that UT2* cells (panel A) express an HMG-CoA reductase that is both localized to the peroxisomes and is smaller in size than that observed in the ER (fractions 5–8) of normal CHO cells (panel B). Immunoblots of the fractions from CHO cells (panel B) demonstrate
that a 97-kDa HMG-CoA reductase is predominantly localized to ER fractions with some contamination in peroxisomal fractions. In contrast, the 90-kDa HMG-CoA reductase is localized exclusively to the peroxisomal fractions (fractions 13–15). To demonstrate localization of the organelles on the gradient, equal volumes of the fractions (instead of equal protein) were loaded on the gel. This resulted in a slight downward shift of the 97-kDa protein in the ER fractions 5–8, in panel B, due to the high levels of protein in these fractions. In addition, there is an excellent correlation with reductase activity and immunoblot density levels in both cell lines. The known 97-kDa ER HMG-CoA reductase protein was not observed in any fraction in the UT2* cells (panel A).

**Fig. 7.** Demonstration of peroxisomal localization of HMG-CoA reductase in UT2* cells by double label immunofluorescence. The cells were simultaneously labeled for HMG-CoA reductase (panels A and B) using a monoclonal antibody (clone A-9) and for peroxisomal proteins (panels C and D) using an affinity purified rabbit polyclonal antibody made against the peroxisomal targeting signal (SKL at the C terminus). The majority of the immunolabeling pattern for UT2* cell HMG-CoA reductase (panel B) is superimposable on the punctate labeling pattern for peroxisomes (panel D) within the same cell (panels B and D, arrowheads).

**Fig. 8.** Demonstration of a peroxisomal localization of HMG-CoA reductase in UT2* cells by immunoelectron microscopy. Panel A, immunolabeled with an IgG fraction of rabbit polyclonal catalase antibody (20 μg/ml). Panel B, immunolabeled with an IgG fraction of a rabbit polyclonal HMG-CoA reductase antibody (25 μg/ml). Panel C, double labeling, the rabbit anti-catalase and a monoclonal antibody against HMG-CoA reductase, clone A-9 (18 μg/ml), were applied simultaneously followed by a solution of 10-nm colloidal gold adducts of affinity purified goat antibodies to rabbit IgG and 5-nm colloidal gold adducts of affinity purified goat antibodies to mouse IgG. P, peroxisomes. Bar = 0.05 μm.

**Fig. 9.** Distribution of HMG-CoA reductase activity and protein in density gradient fractions derived from UT2 cells. A postnuclear fraction of UT2 cells was fractionated on a linear metrizamide gradient. HMG-CoA reductase activity was measured in each fraction, and 0.4-ml aliquots of each fraction were trichloroacetic acid-precipitated. Precipitating proteins were reuspended in sample buffer and electrophoresed on 10%, 18.5 cm polyacrylamide gels. HMG-CoA reductase was visualized as described previously. A sample from rat liver (ER) is also included.

**Localization of HMG-CoA Reductase in Peroxisomes of CHO and UT2* Cells Using Immunofluorescence**—To verify further the subcellular localization of HMG-CoA reductase in UT2* cells, we examined the immunofluorescence pattern obtained with an HMG-CoA reductase antibody. CHO cells and UT2* cells were simultaneously labeled for HMG-CoA reductase and for peroxisomal proteins. A rabbit polyclonal antibody made against the peroxisomal targeting signal (SKL, at the C terminus) was used to label peroxisomal proteins. The SKL antibody has been shown to be specific for peroxisomal proteins (26).
immunofluorescence pattern obtained for HMG-CoA reductase in CHO cells (Fig. 7, panel A) was consistent with ER labeling; however, the pattern obtained for HMG-CoA reductase in UT2* cells was consistent with peroxisomal labeling (Fig. 7, panel B). The majority of the immunofluorescence pattern of HMG-CoA reductase in UT2* cells was directly superimposable over that for the peroxisomal targeting signal antibody (arrowheads in panels B and D).

We also observe some co-localization of HMG-CoA reductase labeling with that of the peroxisomal marker in the CHO cells (arrowheads in Fig. 7, panels A and C). These results indicate that, in CHO cells, HMG-CoA reductase is localized to both the ER and peroxisomes. In contrast, in UT2* cells, HMG-CoA reductase appears to be exclusively localized to peroxisomes.

Localization of HMG-CoA Reductase in Peroxisomes of UT2* Cells Using Immunoelectron Microscopy—To further confirm the localization of HMG-CoA reductase to peroxisomes in UT2* cells, these cells were processed for immunoelectron microscopy. As expected, indirect gold immunolabeling for catalase showed specific immunolabeling of peroxisomes (Fig. 8, panel A). Panel B, demonstrates the localization of HMG-CoA reductase to a similar organelle, utilizing a polyclonal HMG-CoA reductase antibody. The immunolabeling is restricted to the matrix of organelles that morphologically resemble peroxisomes. To unambiguously determine that HMG-CoA reductase is contained in the peroxisomes, we also performed double labeling experiments using both rabbit anti-catalase and a monoclonal antibody against HMG-CoA reductase. Panel C shows that 5-nm gold particles representing antigenic sites for HMG-CoA reductase (arrowheads) are present in catalase-positive organelles (10-nm gold particles), demonstrating the co-localization of catalase and HMG-CoA reductase to the same organelle, and confirming the localization of HMG-CoA reductase to peroxisomes. The small peroxisome in panel C shows immunolabeling for catalase but not for HMG-CoA reductase.

Taken together, all of the above data indicate that UT2* cells contain an HMG-CoA reductase localized only to the peroxisomes. When peroxisomal fractions are isolated from UT2 cells (suppressed conditions) and immunoblotted for HMG-CoA reductase, a 90-kDa protein band and HMG-CoA reductase activity can also be detected in the peak peroxisomal fractions (Fig. 9, fractions 13–15). However, since in the UT2 cells the reductase is not up-regulated, the levels of the reductase protein are very low.

Characterization of the Peroxisomal Reductase in UT2* Cells by Different HMG-CoA Reductase Antibodies—We tested the abilities of a number of HMG-CoA reductase antibodies to immunoprecipitate the 90-kDa protein from UT2* cells, as well as the 97- and 90-kDa proteins from CHO cells (Fig. 10, panel A). The polyclonal, anti-C-terminal, anti-G peptide, and the anti-H peptide antibodies all immunoprecipitated the 97- and 90-kDa proteins from 35S-labeled CHO cell lysates and the 90-kDa protein from 35S-labeled UT2* cell lysates. These proteins were specifically precipitated as they were competed by an excess of the corresponding free peptides (Fig. 10, panels B and C). Clearly, the 90-kDa protein is antigenically similar to the ER HMG-CoA reductase and must contain multiple conserved antigenic sites.

The 90-kDa Band in CHO Cells Is Not a Product of the 97-kDa Band—To determine if a precursor-product relationship existed between the 97- and 90-kDa bands in CHO cells, a pulse-chase experiment was performed in CHO cells (Fig. 11).
The results indicate that there is no precursor-product relationship between the 97- and 90-kDa reductase proteins. The data are expressed as the percentage of HMG-CoA reductase remaining at each time point. The estimated half-life from the slope of the 97-kDa band agreed well with published reports (18), and the estimated half-life of the 90-kDa band in CHO cells appears to be similar.

Localization of the 90-kDa Band in Highly Purified Peroxisomal Fractions from Rat Liver—Successful and reproducible separation of the 97- and 90-kDa reductase proteins requires specific conditions that include the presence of 7M urea, 8% SDS, and 1.1 M 2-mercaptoethanol in the sample buffer, and either 10% acrylamide gels that are 16.5 cm in length or 7.5% acrylamide gels that are 12.5 cm in length. In unpublished studies we have observed that analysis of high levels of peroxisomal proteins in SDS-urea gels fails to resolve the 97- and 90-kDa proteins because of an artifactual shift in the migration of the 90-kDa protein. This finding may explain why the two reductase proteins have not always been identified.

Using these modified conditions, in peroxisomes isolated from rat liver in the presence of aprotinin, cycloheximide, pepstatin, antipain, chymostatin, leupeptin, PMSF, DTT, and methionine in the homogenizing buffer, and in the presence of aprotinin, cycloheximide, pepstatin, antipain, chymostatin, and leupeptin in the gradient solutions, we are able to demonstrate a 90-kDa band in peroxisomes that cross-reacts with a...
FIG. 15. Induction of peroxisomal HMG-CoA reductase in UT2* cells.
The UT2 cell peroxisomal reductase is induced when the cells are grown in the absence of 0.2 mM mevalonate and suppressed when the cells are grown in the presence of 0.2 mM mevalonate.

number of different HMG-CoA reductase antibodies (Fig. 12). The peroxisomal sample contains a 90-kDa band and the ER sample contains a 97-kDa band, similar to that observed in CHO cells. If peroxisomes are isolated without the addition of the above listed protease inhibitors, we are not able to observe the 90-kDa band in purified peroxisomes. In the absence of protease inhibitors, purified peroxisomes contain a ~50-kDa protein band that cross-reacts with the reductase antibodies.

Analysis of HMG-CoA Reductase Activity in CHO and UT2* Cells—Fig. 13 illustrates HMG-CoA and NADPH saturation curves for HMG-CoA reductase from CHO and UT2* cells. As illustrated, the enzyme from CHO cells is fully saturated at 50–100 μM HMG-CoA concentrations, as previously reported (24). The enzyme from UT2* cells is also fully saturated at the same concentrations, 50–100 μM HMG-CoA (panel B). In addition, both reductase activities from CHO and UT2* cells exhibit similar saturation curves for NADPH (panels C and D). NADPH cannot be substituted for NADPH in UT2* cells, and the addition of HMG-CoA reductase inhibitor (lovastatin) to the assay mixture in UT2* cells completely inhibited the activity (data not shown). To demonstrate that we were specifically measuring HMG-CoA reductase activity attributed to a protein sharing antigenic epitopes with the ER reductase in UT2* cells, we covalently coupled a polyclonal HMG-CoA reductase antibody to Sepharose and incubated it with a solubilized extract of UT2* cells that contained reductase activity. After a 3-h incubation, the antibody-Protein A-Sepharose was removed and the supernatant assayed for HMG-CoA reductase activity. The results demonstrated that the polyclonal reductase antibody quantitatively immunoprecipitated the HMG-CoA reductase activity. There was no binding of reductase activity when a control polyclonal IgG antibody was used (data not shown). These results show that HMG-CoA reductase activity is being measured. The pH profile is also similar for CHO and UT2* cell HMG-CoA reductase activities (panels E and F). Thus, all the biochemical determinations of HMG-CoA reductase in UT2* cells are consistent with measurement of reductase activity.

HMG-CoA Reductase mRNA Levels in CHO, UT2, and UT2* Cells—The mRNA from HMG-CoA reductase in mammalian cells consists of at least two predominant messages, 4.2 and 4.7 kb (27). In hamster, the 5'-untranslated region of the transcript ranges from 68 to 670 nucleotides in length (28). It has been shown that multiple initiation sites and four different donor sites are used to excise intron 1 in the 5'-untranslated region (29). Hamster and human HMG-CoA reductase transcripts not only contain a myriad of 5'-untranslated region lengths but also have multiple polyadenylation signals (28). It is thought that together the multiple polyadenylation signals and the 5'-untranslated regions account for the 4.2- and 4.7-kb reductase transcript lengths.

Poly(A) RNA was isolated from CHO, UT2, and UT2* cells. The RNA was separated on a 14-cm gel and hybridized with the full-length cDNA probe (pRed227). Fig. 14 illustrates the results. The reductase probe detected 4.2- and 4.7-kb bands of HMG-CoA reductase mRNAs in UT2* cells and similar size bands in CHO cells (panel A). No HMG-CoA reductase message was detected in UT2 cells. We consistently observe two distinct bands of reductase message in UT2* cells, whereas in CHO cells we are seldom able to resolve the message in two clear distinct bands. In addition, the size of the message appears to be slightly different between the two cell types. The significance of these observations is not clear at this time. The relative mRNA levels (corrected for glyceraldehyde-3-phosphate dehydrogenase) in CHO and UT2* cells are illustrated in panel B. UT2* cells contain 60% of reductase message found in CHO cells.

DISCUSSION

We have shown that CHO cells contain two reductase proteins, the well characterized 97-kDa protein, localized in the ER and a 90-kDa protein localized in peroxisomes. The UT2* cells only express the 90-kDa protein, which is localized exclusively in peroxisomes. The localization of this protein to peroxisomes was demonstrated by four different methods as follows: 1) analytical subcellular fractionation and measurement of enzyme activities; 2) immunoblotting for HMG-CoA reductase in the isolated fractions with a monospecific antibody; 3) immunofluorescence microscopy; and 4) immunoelectron microscopy. All four methods produced consistent results. The conclusion that the 90-kDa protein is predominantly localized in peroxisomes is HMG-CoA reductase based on the following: 1) seven different monospecific HMG-CoA reductase antibodies cross-react with this protein; 2) the polyclonal, anti-C-terminal, anti-G peptide, and the anti-H peptide antibodies all immunoprecipitated the 90-kDa protein from 35S-labeled UT2* cell lysates, and these proteins were specifically precipitated as they were competed by an excess of the corresponding free peptides; 3) the HMG-CoA reductase antibody specifically immunoprecipitated the HMG-CoA reductase activity; 4) the protein and HMG-CoA reductase activity levels are regulated coordinately; and 5) the HMG-CoA reductase activity is completely abolished in vitro by the addition of lovastatin.

Our hypothesis is that all wild type cells contain two forms of HMG-CoA reductase (as illustrated in CHO cells in Fig. 2 and Fig. 6, panel B). The UT2 cells lack the ER HMG-CoA reductase, and the peroxisomal reductase is suppressed due to growth of the cells in the presence of mevalonate, as indicated by the low levels of the 90-kDa protein in UT2 cells, Fig. 9. Therefore, these cells require mevalonate for growth. However, when mevalonate is removed the peroxisomal reductase is up-regulated, as illustrated in Fig. 6, panel A, and the cells can grow without mevalonate. When these cells are placed back in UT2 cell media (containing mevalonate), the peroxisomal reductase activity levels again decrease. Thus, this is a physiological regulation that is reversible. This concept is illustrated in Fig. 15. In addition, it is clear that the up-regulated peroxisomal enzyme in these cells is sufficient in fulfilling the cell’s requirement for HMG-CoA reductase activity.

It is significant that a number of previous publications have reported the presence of two distinct protein bands in whole cell extracts that cross-react with HMG-CoA reductase antibodies. The expected 97-kDa band, as well as a protein migrating around 94–90-kDa, has been reported in UT1 (27), hamster C-100 (30), and HepG2 cells (31). The interpretations of these results have ranged from concluding that the band represents a cross-reacting antigen (30) to the possibility that the two proteins represent two reductases with homologous sequences (27). Our data favor the latter interpretation.
Characterization of a Novel HMG-CoA Reductase

24587

How Can the Dual Subcellular Localization of the Same or Similar Protein be Explained?—Numerous examples are being found of proteins that reside and function in more than one subcellular compartment (32). The multicompartmentalized isoforms may be encoded by multiple genes, each encoding different targeting information, or encoded by the same gene. Several mechanisms have evolved to enable the same gene to encode and differentially express multiple forms of topogenic signals (reviewed in Ref. 32). These mechanisms include alternative forms of transcription initiation, translation initiation, splicing, and post-translational modification (32).

In mammals, only one gene has been found to encode HMG-CoA reductase. However yeast, fungi, and plants all contain more than one HMG-CoA reductase gene. Yeast, fungi, and Arabidopsis thaliana all contain two genes (33–35), three in Hevea (36), at least three in tomato (37), and even larger multigene families in maize and potato (38). A. thaliana HMG-CoA reductase 1 (HMG1) is detected in all tissues, whereas the HMG2 is restricted to young seedlings and roots (39). Arabidopsis thaliana HMG1 is believed to function as a housekeeping form of reductase, and HMG2 may have a specialized role in actively dividing cells (35). Similarly in yeast, the HMG1 and HMG2 genes are differently expressed (40). Additionally, in yeast when HMG1 is deleted, the organism remains viable indicating that HMG2 can replace the function of HMG1 (37). The presence of multiple genes is consistent with the hypothesis that different isoforms of HMG-CoA reductase are involved in separate subcellular pathways for isoprenoid biosynthesis.

The origin of the 90-kDa peroxisomal reductase still remains to be determined. We do not observe a precursor-product relationship between the 97- and 90-kDa bands in CHO cells (Fig. 11). These data suggest that a post-translation modification is not the source of the 90-kDa protein. The mutation identified in the ER reductase gene in UT2 cells indicates that neither a 97- nor a 90-kDa protein can be translated. In addition, any possible alternative splicing mechanism will produce proteins of sizes much less than 90 kDa. These data suggest that the peroxisomal reductase may be a product of a second gene.

The UT2* cell line expressing only the 90-kDa HMG-CoA reductase. The identification and characterization of this cell line will now permit these studies.

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