The regulated degradation of a 3-hydroxy-3-methylglutaryl-coenzyme A reductase reporter construct occurs in the endoplasmic reticulum

Lloyd W. Lecureux and Binks W. Wattenberg*
Cell Biology Unit, The Upjohn Company, Kalamazoo, MI 49001, USA
*Author for correspondence

SUMMARY

The rate-limiting enzyme in cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase, is regulated at a number of levels. One important mechanism is regulation of the half-life of the protein by a controlled proteolytic system. This comes about in response to downstream products of the sterol biosynthetic pathway. Little is known about this system, including where in the cell this regulated degradation occurs. HMG CoA reductase resides in the endoplasmic reticulum. To localize the site of regulated degradation of HMG CoA reductase, we used a construct that fuses the N-terminal membrane-anchoring domain of HMG CoA reductase in-frame with β-galactosidase as a reporter domain (HM-Gal). HM-Gal has previously been shown to reproduce faithfully the degradative properties of native HMG CoA reductase (Chun et al. (1990) J. Biol. Chem. 265, 22004-22010). CHO cells transfected with DNA encoding HM-Gal were exposed to mevalonic acid, which enhances the rate of HMG CoA reductase degradation several fold, and leads to the reduction of the steady state levels of HM-Gal by 80-90%. To accumulate HMG CoA reductase at the site of degradation, cells were simultaneously treated with N-acetyl-leucyl-leucyl-norleucinal (ALLN), which inhibits the protease responsible for reductase degradation. HM-Gal was localized morphologically by immunofluorescence and biochemically by measuring β-galactosidase activity in Percoll gradients of cellular homogenates. Using either technique HM-Gal localization was indistinguishable from that of ER markers in both control cells and in cells treated to accumulate HMG CoA reductase at the site of degradation. We conclude that the regulated degradation of HMG CoA reductase occurs in the ER or an ER-like compartment.

Key words: protein turnover, cholesterol, regulated proteolysis

INTRODUCTION

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase, the rate limiting enzyme in cholesterol biosynthesis, is one of the most elaborately regulated enzymes known (reviewed by Goldstein and Brown, 1990). Regulation of this enzyme occurs at the levels of transcription, translation and post-translational modification, and by alterations in the rate of degradation of the protein. Enhanced degradation of HMG CoA reductase can be induced by both sterol and non-sterol products of the mevalonic acid pathway. In the presence of these substances the turnover of the enzyme may be accelerated by up to 5-fold. This is clearly important in determining the steady state levels of the enzyme. In some situations this may be one of the predominant regulatory mechanisms (Ness et al., 1991).

The structure of HMG CoA reductase places important constraints on the mechanism governing regulated degradation. HMG CoA reductase is a resident protein of the endoplasmic reticulum (ER). The reductase is targeted to and anchored in the membrane of the ER by an N-terminal domain consisting of eight transmembrane segments (Skalnic et al., 1988, Roitelman et al., 1992; Liscum et al., 1985; Olender and Simoni, 1992). This is connected by a linker sequence to the globular, C-terminal catalytic domain situated in the cytosol. Surprisingly, despite the fact that the cytosolic catalytic domain is the ultimate object of regulatory control, when the catalytic domain is expressed without the membrane anchor, thus becoming soluble, the degradative rate of the catalytic domain is no longer regulated (Gil et al., 1985). An elegant series of studies by the laboratories of Simoni, and Brown and Goldstein, demonstrated that the membrane anchor domain is both necessary and sufficient for regulated degradation of the reductase (Gil et al., 1985; Jingani et al., 1987; Simoni et al., 1988; Chun et al., 1990; Chun and Simoni, 1990). One important demonstration of this principle was the construction of a fusion protein consisting of the N-terminal membrane anchor domain fused in-frame to bacterial β-galactosidase (Skalnik et al., 1988). This construct entirely lacks the native reductase catalytic domain. When transfected into Chinese hamster ovary (CHO) cells the behavior of this construct is virtually identical to that of native HMG CoA reductase with regard to regulated degradation. However, because the transfected gene is transcribed from the SV40 promoter, it is not
subject to the transcriptional regulation that characterizes the native gene. This greatly simplifies analysis of the data. In detailed experiments it was shown that measurements of steady state levels of β-galactosidase activity reflected the rate of degradation conferred by the HMG CoA reductase membrane anchor (Skalnik et al., 1988; Chun et al., 1990).

Despite considerable advances in recent years, the mechanism that confers regulated degradation on the reductase remains, in large part, a mystery. It is known that specific structural properties of the membrane anchor region are important for maintaining this regulation (Rottelman et al., 1992; Jingami et al., 1987; Skalnik et al., 1988; Chun and Simoni, 1992). There is also a requirement for energy and an unidentified, short-lived protein (Chun et al., 1990). The nature of the protease, or proteolytic system, is mostly uncharacterized, except for the finding that proteolysis is sensitive to the cysteine protease inhibitor N-acetyl-leucyl-leucyl-norleucinal (ALLN) (Inoue et al., 1991).

A key piece of information for understanding how products of the mevalonic acid pathway turn on degradation of the reductase is the subcellular locale of the degradation process. One possibility is that, when stimulated, a transport mechanism delivers the reductase to a degradative compartment. Alternatively, the reductase could be degraded in situ in the ER. In the latter case degradation is activated either by turning on the degradative enzyme(s) in the ER or by an alteration in the reductase itself, creating a conformation of the reductase that is now susceptible to existing protease activity. One study indicated that regulated proteolysis was sensitive to inhibition of lysosomal function by weak bases, suggesting that proteolysis takes place in a lysosomal setting (Parker et al., 1989). This effect has not been universally observed, however (Tanaka et al., 1986; Inoue et al., 1991). Simoni and colleagues reported that regulated degradation was unaffected by the fungal metabolite Brefeldin A (Chun et al., 1990). This toxin has the property of disrupting membrane traffic between the endoplasmic reticulum and the Golgi apparatus (reviewed by Klausner et al., 1992). In addition, Brefeldin A treatment leads to a mixing of ER and Golgi components (Lippincott-Schwartz et al., 1989). Because of the Brefeldin-A-resistant nature of regulated degradation it has been concluded that regulated degradation takes place in the endoplasmic reticulum. However, it has not been shown that Brefeldin A inhibits autophagy or other delivery of proteins to the lysosome from the ER. In addition, the participation of a novel degradative compartment cannot be ruled out by those experiments. We, therefore, set out to determine the site of regulated degradation. To accomplish this we have utilized an experimental technique that has been used classically to identify sites of degradation. In this analysis, the proteolytic enzymes are inhibited, thus the protein of interest accumulates at the site of proteolysis. By using a combination of morphological and biochemical approaches, we have confirmed that the degradation of HMG CoA reductase occurs in the ER or an ER-like compartment.

**MATERIALS AND METHODS**

**Materials**

Mevalonic acid lactone (MVA), cytochrome c type III, 4-methylumbelliferyl-β-D-galactoside (MUG), p-nitrophenyl-β-N-acetylglucosaminide (PNPNA), acetyl-leucyl-leucyl-norleucinal (ALLN) and monoclonal antibodies against β-galactosidase were purchased from Sigma Chemical Co. Antibodies against endoplasmic reticulum (Louvard et al., 1982) were a generous gift from J. Lippincott-Schwartz (NIH). Percoll (d=1.3 g/ml) was purchased from Pharmacia (Piscataway, NJ). Rhodamine- and fluorescein-labeled goat antimouse IgG were obtained from Kirekegaard & Perry Laboratories, Inc. (Gaithersburg, MD). Lucifer Yellow was from Molecular Probes, Inc. (Eugene, OR). Coverslip mounting buffer, Aqua-Poly/Mount, was purchased from Polysciences, Inc. (Warrington, PA).

**Cells**

CHO HM-Gal cells were generated by transfection with pSV2HMGAL (Skalnik et al., 1988), a generous gift from Robert D. Simoni (Department of Biological Sciences, Stanford University, Stanford, CA), by the calcium phosphate method. High-level expressors were selected as a pool by fluorescence-activated cell sorting based on cleavage of the dye fluorescein-digalactoside (Molecular Probes, Eugene, OR) per the manufacturer’s instructions. Cells were maintained in α-MEM/10% FCS plus 500 µg/ml geneticin. Elevated levels of HM-Gal were obtained by switching the medium to α-MEM/10% lipoprotein-depleted serum (LPDS) plus 0.4 µM mervenolin and 40 µM mevalonic acid.

**Indirect immunofluorescence**

Cells were grown for 24 hours on 25 mm glass coverslips in 12-well culture dishes in medium used to enhance HM-Gal, as described above. The medium was replaced with the same medium plus 20 mM MVA, or 10 µg/ml ALLN, or 20 mM MVA plus 10 µg/ml ALLN, and incubated for 18 hours. Lucifer Yellow (2 mg/ml) was added to some of the cells and incubated for 1 hour. Cells were washed with medium, then medium was replaced with the original medium without Lucifer Yellow for 30 minutes. All cells were washed with PBS and fixed with 4% paraformaldehyde (w/v) for 15 minutes at room temperature. Cells receiving primary antibody were washed three times with permeabilization buffer (0.1% saponin plus 1% BSA in PBS) and incubated in the same for an additional 15 minutes. Monoclonal antibodies against β-galactosidase and rabbit anti-CHO ER were added at the appropriate dilution in permeabilization buffer and incubated for 1 hour at room temperature. Primary antibodies were removed and cells washed three times in permeabilization buffer. Rhodamine-labeled goat anti-mouse, or a combination of fluorescein-labeled goat anti-mouse plus rhodamine-labeled goat anti-rabbit, was added in permeabilization buffer for an additional hour at room temperature. Cells were washed three times in permeabilization buffer before mounting on slides in a drop of Aqua-Poly/Mount. Cells were viewed and photographed in a Carl Zeiss IHR5 fluorescent microscope.

**Percoll gradient fractionation**

Cells were grown for 24 hours in 75 cm² flasks, as described above, then medium was replaced with LPDS, LPDS plus 20 mM MVA, LPDS plus 10 µg/ml ALLN, or LPDS plus 20 mM MVA and 10 µg/ml ALLN, and grown for an additional 18 hours. Medium was removed and cells were washed in PBS on ice before scraping off into 4.0 ml breaking buffer (10 mM Tris-HCl, 0.3 M sucrose and 2 mM EDTA, pH 7.5). The cells were broken by nitrogen cavitation at 450 psi (lb/in²; 1 lb/in² = 6.9 kPa) for 15 minutes at 4°C; complete breakage was verified by Trypan Blue staining. Cells were centrifuged at 1000 g for 5 minutes and the post-nuclear supernatant retained; 3.2 ml post nuclear supernatant, 2.8 ml 90% Percoll (in 10 mM Tris, 0.3 M sucrose and 2 mM EDTA, pH 7.6) and 4 ml breaking buffer were mixed and underlaid with 0.5 ml 2.5 M sucrose in a 1.2 cm × 6.5 mm ultracentrifuge tube. The Percoll gradient was formed at 35,000 g in 1 hour at 4°C. Fractions (0.5 ml) were collected from the bottom of the tube using a peristaltic pump, and assays for subcellular organelles were done directly from the fractions.
Assays for subcellular organelles

**HM-Gal (β-galactosidase)**

The method is one adapted from that described by Miller (1972). In general, 100 µl Z buffer plus 0.5% Triton X-100 and 15 µl 1% MUG plus 5% β-mercaptoethanol are added to either a 96-well plate containing 25 µl Percoll fraction or a culture plate containing washed (PBS) cells, and they are then incubated at 37°C for 60 minutes. The reaction is stopped with 30 µl 0.3 M glycine plus 15 mM EDTA, pH 11.2 and then read in a fluorescent plate reader (EX filter, 395 nm; EM filter, 460 nm).

Lyso-somes (hexosaminidase)

This method was adapted to a 96-well plate format from that of Hall et al. (1978). To each well is added 30 µl citrate buffer (0.1 M citrate, 10 mM NaCl, 0.1% Triton X-100, pH 4.4), 10 µl 5 mM NPNAG, 10 µl Percoll fraction and incubated for 1 hour at 37°C. The reaction is stopped by the addition of 100 µl 0.5 M sodium carbonate, pH 10.3. The plate is read at A405 in a spectrophotometric plate reader.

Endoplasmic reticulum (cytochrome c reductase)

This method was taken from Sottocasa et al. (1967). Here, 400 µl cytochrome c reagent (50 mM sodium phosphate, 300 µM KCN and 740 µg/ml cytochrome c, pH 7.5), 50 µl Percoll fraction, plus 50 µl 1 mM NADPH is added to a 1 ml cuvette at 37°C. The rate of reduction of cytochrome c is measured at A550 at 30 second intervals.

Measurement of the effect of ALLN on vesicular stomatitis virus G-protein carbohydrate maturation

Wild-type CHO cells were plated into 6-well plates (35 mm/well, Corning Glass Co., Corning, NY) at subconfluence. For infection with vesicular stomatitis virus (VSV), medium was replaced with 1 ml/well of methionine-free MEM (Gibco, Gaithersburg, MD) containing 150 µg/ml of [35S]methionine (Amersham, Arlington Heights, IL) and then it was incubated with or without 10 µg/ml ALLN. After 45 minutes the infection medium was replaced with complete medium (10% fetal calf serum) with or without ALLN was added for 4 hours. This medium was then removed and 0.5 ml/well of methionine-free MEM with or without ALLN was added for 4 hours. This medium was then removed and complete medium (10% fetal calf serum) with or without ALLN was added for 4 hours. This medium was then removed and complete medium containing 150 µg/ml of [35S]methionine and 10 µg/ml ALLN, plus 50 µl Percoll fraction and incubated for 1 hour at 37°C. The reaction is stopped with the addition of 100 µl 0.5 M sodium carbonate, pH 10.3. The plate is read at A405 in a spectrophotometric plate reader.

**RESULTS**

For these studies, a recombinant protein consisting of the membrane-spanning domain of HMG CoA reductase fused in-frame to β-galactosidase was used (Skalnik et al., 1988). It has...

![Fig. 1. Mevalonate-accelerated degradation of HM-Gal is inhibited by the protease inhibitor ALLN. Cells were grown in medium with lipid-depleted serum for 48 hours in a 96-well plate. The medium was replaced with varying concentrations of mevalonic acid with (hatched bars) or without (open bars) 10 µg/ml ALLN and incubated for 18 hours, at 37°C. β-Galactosidase was quantified as described in Materials and Methods. The mean of quadruplicate determinations was used for each value.](image1)

![Fig. 2. ALLN does not inhibit protein transit from the ER to the Golgi. Cells infected with vesicular stomatitis virus were subjected to a pulse/chase protocol as described in Materials and Methods. The sensitivity of the VSV G protein carbohydrate to Endo H was then determined at different times of chase as a measure of the transit of G protein between the ER and the Golgi. Cells were treated either with solvent alone (0.1% DMSO, circles) or with 10 µg/ml ALLN and incubated for 30 minutes.](image2)

![HMG CoA reductase degradation in the ER](image3)
been well established that this fusion protein closely follows the behavior of native HMG CoA reductase with regard to regulated degradation (Chun and Simoni, 1990). This construct serves two purposes. β-Galactosidase can be used as a convenient marker, which can be detected by sensitive biochemical and immunological techniques. In addition this construct is driven by the SV40 promoter, and can therefore be subject to transcriptional regulation by products of the reductase pathway. This allows the measurement of the degradation process without interference from transcriptional regulation.

To identify the site where the regulated degradation of HMG CoA reductase occurs, we blocked degradation with a specific protease inhibitor under conditions where degradation would normally be stimulated. In this way the protein is allowed to accumulate at the site of degradation. The peptidic cysteine protease inhibitor N-acetyl-leucyl-leucyl-norleucinal (ALLN) inhibits the regulated degradation of HMG CoA reductase (Inoue et al., 1991). Fig. 1 illustrates that mevalonic acid-stimulated degradation of HMG CoA reductase is inhibited by ALLN, as reported previously (Inoue et al., 1991). Even at levels of mevalonic acid that reduce the steady state levels of the HM-Gal construct by over 80%, ALLN completely maintains steady state levels of the protein at the level of untreated cells. Consequently, in the presence of ALLN at 20 mM mevalonic acid, 80% of the HM-Gal protein is accumulated at the site of degradation, although degradation itself has been inhibited by the protease inhibitor.

**ALLN does not influence protein trafficking**

It is important to ensure that ALLN is preventing degradation of the reductase construct directly by inhibiting proteolysis and not indirectly by preventing transport of the reductase out of the ER to a separate proteolytic compartment. To test this, the effect of ALLN on the transit of the membrane glycoprotein, G protein, of vesicular stomatitis virus, from the ER to the Golgi was examined. The maturation of the carbohydrate on G protein from an immature, ER form, to a more mature form that has been processed in the Golgi can be measured by sensitivity of the carbohydrate to cleavage by endoglycosidase H (Robbins et al., 1977). The ER form is sensitive to cleavage and the Golgi form is resistant. This difference can easily be detected by analysis of the products by gel electrophoresis. Wild-type CHO cells were infected with VSV and one set was treated with ALLN (Fig. 2). The cells were then pulse labeled with [35S]methionine and then chased with cold methionine for the indicated lengths of time before immunoprecipitation and treatment with Endo H. The resulting gels were analyzed on a Phosphor-imager and the Endo-H-sensitive and Endo-H-resistant G protein bands were quantified for each time of chase. Shown is the proportion of the total G protein that acquires the Endo-H-resistant, Golgi form of carbohydrate as a function of time of chase. It is readily apparent from this analysis that ALLN has no effect on the time-course of degradation.

Fig. 4. HM-Gal is co-localized with the ER in cells grown under conditions stimulating accelerated degradation. Cells were sparsely grown on glass coverslips for 24 hours in LPDS medium. Medium was replaced with LPDS, LPDS plus 20 mM MVA, LPDS plus 10 µg/ml ALLN, or LPDS plus 20 mM MVA and 10 µg/ml ALLN, and grown for an additional 18 hours. In a parallel experiment cells were grown in a 96-well plate using the same conditions to verify β-galactosidase enhanced degradation by MVA and prevention of degradation by ALLN (data not shown). Cells were fixed and permeabilized with 0.1% saponin as described in Materials and Methods. Cells were double stained with polyclonal rabbit anti-endoplasmic reticulum and monoclonal anti-β-galactosidase antibodies followed by rhodamine-labeled goat anti-rabbit and fluorescein-labeled goat anti-mouse antiserum (A,B). Another set of cells were incubated for 1 hour with 2 mg/ml Lucifer Yellow and chased for 30 minutes with the same medium, and fixed, permeabilized and stained with monoclonal anti-β-galactosidase, followed by a secondary stain of fluorescein-labeled goat anti-mouse (C,D). Cells were fixed and mounted as described in Materials and Methods. Photos were taken at ×640 magnification using either fluorescein or rhodamine emission filters.
transport of this model membrane protein out of the ER to the Golgi.

Another test of the influence of ALLN on intracellular trafficking is an examination of endocytic delivery to the lysosome. Cells were allowed to ingest Lucifer Yellow. Under normal conditions, Lucifer Yellow is endocytosed and delivered to the lysosome. Fig. 3 illustrates that delivery of Lucifer Yellow to the lysosome is unaffected in ALLN-treated cells. Thus ALLN does not interfere with vesicular traffic, especially to the lysosome, the classical degradative compartment.

Localization of the site of inhibition by immunofluorescence
To determine the site of degradation, cells were treated with or without mevalonic acid, to induce the degradative process, and with or without ALLN, to inhibit induced degradation. The cells were then examined by immunofluorescence in double-labeling experiments with antibodies directed against β-galactosidase (to localize HM-Gal), or a polyclonal antibody against the ER (Fig. 4A,B). Additionally, lysosomes were labeled with Lucifer Yellow in some cells (Fig. 4C,D). A comparison of the localization of the HM-Gal protein with the ER marker in untreated cells (top pair, A,B) illustrates that by and large the two markers co-distributed in a dispersed granular pattern surrounding the nucleus. The ER marker exhibited a tendency to stain in a somewhat more focal pattern. This may indicate that the predominant antigen(s) recognized by this antiserum are restricted to a sub-compartment of the ER. The co-distribution of the ER and HM-Gal staining is sharply in contrast to that of the lysosomal marker, which is distributed in a characteristic punctate pattern (top pair, C,D). Treatment with either mevalonic acid or ALLN alone (middle pairs in A,B and C,D) does not significantly alter these distributions. It should be noted that although mevalonic acid treatment strongly diminishes the HM-Gal signal, the exposures of the photographs of these slides were lengthened to allow visualization of the distribution of the remaining protein. When HM-Gal degradation is stimulated with mevalonic acid and simultaneously blocked with ALLN, HM-Gal remains co-distributed with the ER marker (bottom two pairs, A,B). Again there are minor differences in distribution between these two markers, as there were in untreated cells. There was no overlap between the distribution of the lysosomal marker and the HM-Gal protein (bottom two pairs, C,D). This morphological examination indicates that under conditions of stimulated degradation, HM-Gal, and by inference HMG CoA reductase, remains in the ER.

Density gradient analysis of the site of HM-Gal-stimulated degradation
To complement the morphological analysis of the site of degradation, the density properties of the HM-Gal-containing organelle were examined by Percoll gradient fractionation (Fig. 5). Cells were either left untreated or treated with mevalonic acid in the absence or presence of ALLN. When cells were untreated, β-galactosidase activity exactly co-fractionated with the ER marker NADPH-dependent cytochrome c reductase. This activity was well separated from the lysosomal marker, hexosaminidase. Mevalonic acid virtually eliminated the β-galactosidase activity (B), but did not alter the density of either the ER or lysosomes. ALLN treatment alone

Fig. 5. In Percoll gradients, HM-Gal-containing membranes comigrate with the ER, not the lysosomes, in cells grown under conditions where accelerated degradation occurs. Cells were grown in 75 cm² culture flasks for 24 hours in culturing medium as described in Materials and Methods and replaced with: LPDS (A); LPDS plus 20 mM MVA (B); LPDS plus 10 µg/ml ALLN (C); or LPDS plus 20 mM MVA and 10 µg/ml ALLN (D), and cultured for an additional 18 hours before fractionation as described in Materials and Methods. Fractions of 0.5 ml were collected from the bottom of the tube. β-Galactosidase (filled circles, HM-Gal protein), hexosaminidase (open squares, lysosomal marker) and cytochrome c reductase (open circles, ER marker) were assayed directly from the fractions, as described in Materials and Methods.
did not alter the distribution of any of the markers, but did have a significant impact on the total hexosaminidase activity (C). The reason for this effect is unclear. When ALLN is added to mevalonic acid-treated cells to block stimulated degradation, the β-galactosidase activity of the HM-Gal construct is localized exclusively and exactly to the position of the ER marker activity. The lysosomal activity, while markedly reduced, remained localized to the dense portion of the gradient. These results support the findings of the immunofluorescence experiments which identify the ER as the site of regulated degradation of HMG CoA reductase.

**DISCUSSION**

We have used both morphological and biochemical techniques to localize the site of HMG CoA reductase-regulated degradation. The results from both approaches indicated that the proteolytic machinery resides within the ER, or a compartment with identical localization and density properties. The interpretation of these experiments relies on two assumptions. The first is that the behavior of the HM-Gal construct reflects that of native HMG CoA reductase. HM-Gal and native reductase have nearly identical half-lives both in the basal state and under conditions where degradation is accelerated (Chun et al., 1990). Furthermore, it has been shown that the membrane anchor domain is necessary and sufficient for localization of the reductase to the ER (Skalnik et al., 1988). All available evidence indicates that the catalytic portion of the molecule has little to do with regulation of the localization of the enzyme or its rate of degradation. Therefore, it seems quite unlikely that properties of the HM-Gal construct differ significantly from those of the native reductase. We have also assumed that ALLN exerts its protective effect on the reductase by directly inhibiting the protease responsible for regulated degradation of the reductase. Alternatively, ALLN could have an undetected effect on transport within the cell. In this case its protective effect would result from blocking transport of the reductase from the ER to a degradative compartment. We measured transport of the endocytic marker, Lucifer Yellow, during ALLN treatment and found that its delivery to the lysosome was unimpeded. In addition, we have shown that the movement of a membrane protein, VSV G protein, out of the ER to the Golgi is unaffected by ALLN treatment. Coupled with the demonstration that ALLN is a specific inhibitor of cysteine proteases, our data indicate that this alternative explanation for ALLN action is untenable.

In addition to HMG CoA reductase, a wide array of proteins have been found to be degraded within the ER. Examples include subunits of the T cell and asialoglycoprotein receptors, viral membrane glycoproteins and apolipoprotein B, among others (reviewed by Klausner and Sítia, 1990; Lippincott-Schwartz et al., 1988). Of these, only HMG CoA reductase and apolipoprotein B are known to be subject to regulated degradation. Despite the similar site of degradation, there are significant differences in the properties of degradation of these various proteins. For example, HMG CoA reductase degradation is not sensitive to N-tosyl-L-phenylalanine chloromethyl ketone, whereas T cell receptor-α-subunit degradation is sensitive (Inoue and Simoni, 1992). Conversely, perturbation of calcium homeostasis disrupts reductase degradation but not T cell receptor subunit degradation. Overall, these studies suggest that there is a complex and intricate proteolytic system in the ER and that different proteins are degraded by different aspects of it.

The localization of the proteolytic system to the ER raises an important question about the degradation of the cytoplasmic portions of HMG CoA reductase. When present as a soluble protein within the cytosol, the catalytic domain is exceedingly stable (Gil et al., 1985). Therefore, it is unlikely that the proteolytic system merely clips the reductase near the membrane surface to release the catalytic domain. Indeed we and others have been unable to detect any proteolytic fragments of the reductase under a variety of conditions (Roitelman et al., 1992) and Yue and B. Wattenberg, unpublished results), indicating that the entire reductase protein is degraded in a rapid and efficient manner. This most likely results from a precessive proteolysis. This could be due to a cytoplasmically oriented protease that starts near the ER membrane and moves in a C-terminal direction along the polypeptide chain. Alternatively, the cytoplasmic domain might be impelled into the lumen of the ER for degradation by a luminal proteolytic system. This could be accomplished, for example, by re-initiating translocation of the HMG CoA chain through a neutralization of the stop-transfer signal that must exist for the eighth transmembrane domain. Many other possibilities remain. However, the results we report here indicate that the search for components of the regulatory degradation system for HMG CoA reductase should center on examination of the ER.

**REFERENCES**


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