Studies in different liver-derived cells in culture indicate that apolipoprotein (apo) B-100 production is regulated largely by intracellular degradation and the ubiquitin-proteasome pathway is a major mechanism for the degradation. The proteasomal degradation of apoB-100 was postulated to be an intrinsic property of the protein that occurs even in the presence of optimal amounts of lipids supplied to the cell. We examined apoB-100 and apoB-48 biogenesis in CaCo2, a human colon carcinoma cell line. To our surprise, apoB-100 and apoB-48 were, quantitatively secreted by CaCo2 cell; essentially none of the newly synthesized apoB was degraded before secretion in a 2-h period whether the cells were cultured on filter or on plastic. Furthermore, although ubiquitin immunoreactivity was readily detected in the intracellular apoB isolated from HepG2 cells, little or no ubiquitin was detectable in the intracellular apoB from CaCo2 cells. The amounts of free ubiquitin and total and non-apoB ubiquitin-proteins were comparable in HepG2 and CaCo2 cells, indicating that CaCo2 cells have the necessary machinery for tagging ubiquitin chains onto cellular proteins for proteasomal degradation. Incubation in lipoprotein-deficient serum did not induce apoB degradation, but the addition of a microsomal triglyceride transfer protein inhibitor led to apoB degradation in CaCo2 cells. Finally, similar proportions of apoB-48 are quantitatively recovered in the cellular lysate and secreted into the medium. This apparent intrinsic susceptibility of apoB-100 to proteasome-mediated degradation has turned out to be an intrinsic property of the protein, and the phenomenon is neither universal nor inevitable. The unconditional use of apoB as a paradigm for intracellular protein degradation is not warranted.
ApoB Escapes Intracellular Degradation in CaCo2

EXPERIMENTAL PROCEDURES

Materials—Millipore filters (PHLA03050 and PHA01250) were from Millipore. Nitrocellulose membrane was from Schleicher & Schuell. Silica (fumed), N-ethylmaleimide, rabbit polyclonal antibody against bovine ubiquitin antigen, bovine lipoprotein-deficient serum, trypsin, soybean trypsin inhibitor, and N-acetyl-l-lysyl-norleucine (ALLN) were from Sigma. Gamma-Glide G-Sepharose was from Pharmacia Biotech. Lactealyxin was obtained from Calbiochem. Mouse monoclonal antibody against ubiquitin and goat polyclonal antibodies against human apoB and apoA-I were from Chemicon. Monoclonal antibody against human apoB (1D1) was kindly provided by Dr. R. W. Milne (Ottawa Heart Institute). Monoclonal antibody against protein disulfide isomerase was from Stressgen Biotechnologies Corp. Methionine, methionine, RPMI 1640, and methionine-free RPMI 1640 were from ICN. Triton-X-100 gradient gels were from Norvex. An MTP inhibitor (BMS-197636) (26) was kindly provided by Dr. David Gordon (Bristol-Myers Squibb Pharmaceutical Research Institute).

Cell Culture—HepG2 and CaCo2 cell lines were from American Type Culture Collection and were maintained at 37 °C in an atmosphere with 5% CO2 and in RPMI 1640 medium (Life Technologies, Inc.) containing 20% fetal calf serum (FCS, HyClone), 2 mM penicillin, streptomycin (100 μg/ml) (Life Technologies, Inc.).

Pulse-chase Analysis and Immunoprecipitation—CaCo2 cells were either plated directly on 24-well plates (Falcon) or on 12-mm Millipore filters that were placed in the 24-well plates in RPMI 1640 medium containing 20% FCS, penicillin (100 unit/ml), and streptomycin (100 μg/ml) (Life Technologies, Inc.).

Pulse-chase analysis of apoB and apoA-I was performed as detailed previously (7, 27) on cells that were ~3 weeks post-confluence. In brief, the cells were preincubated for 30 min in minitube-free medium followed by a 60-min labeling with [35S]methionine (100 μCi/ml). Chase was initiated by replacing the labeling medium with RPMI 1640 medium containing 20% FCS and cold methionine (15 giliter). At the times indicated, the culture medium was harvested, and the cell lysates were lysed and immunoprecipitated with polyclonal antibodies against apoB and apoA-I as described previously (16, 27). Pharmacologic manipulations before and during pulse-chase periods were indicated in the figure legends. Immunoprecipitates were released by boiling for 5 min in SDS-PAGE buffer in the presence of 5% 2-mercaptoethanol and analyzed by SDS-PAGE on 6% gel or 4–12% gradient gel (for apoB) or on 4–20% gradient gel (for apoA-I). Gels were dried, and the autoradiographic image was captured with a phosphor storage system (CycloneTM, Packard) and analyzed by OptiQuant Image analysis software. Data were expressed as digital light units/h.

Immunoblot Analysis—Western blot analysis was performed on the cell culture medium or cell lysate as described previously (16, 27). The lipoproteins in the culture medium were concentrated with fumed silica (28), and the concentrated proteins were treated with DTT and with cold phosphate-buffered saline and eluted in SDS-PAGE buffer containing 5% 2-mercaptoethanol by boiling for 5 min. The cell lysates were washed with cold phosphate-buffered saline and lysed in 2% sodium cholate in HEPES-buffered saline (50 mM HEPES, 200 mM NaCl, pH 7.4) containing 1 mM phenylmethylsulfonyl fluoride, 0.1 mM ALLN, 5 mM N-ethylmaleimide, and complete protease inhibitors (Roche Molecular Biochemicals). Insoluble material in the cell lysate was removed by centrifugation at 10,000 × g for 10 min. ApoB was immunoprecipitated as described above. The proteins were separated by SDS-PAGE, transferred overnight onto nitrocellulose membranes, probed with monoclonal anti-ubiquitin or anti-apoB antibodies that were peroxidase-conjugated using an EZ-LinkTM Plus Activated Peroxidase kit (Pierce), and detected by enhanced chemiluminescence (ECL kit, Amersham Pharmacia Biotech). The relative intensity of the immunoblot bands was quantified by AlphaImagerTM 2000 Documentation & Analysis system (Alpha Innotech Corp.).

Sucrose Gradient Ultracentrifugation of Lipoproteins—The lipoproteins present in the medium were separated by sucrose gradient ultracentrifugation according to Borén et al. (29) as described previously (27). A 50 ml homogenate was layered on the following solution, which was made by stretching the bottom of the tube: 2 ml of 47% sucrose, 2 ml of 25% sucrose, a 5-ml sample in 12.5% sucrose, and 3 ml of phosphate-buffered saline. The gradients were centrifuged in a Beckman SW40 rotor at 35,000 rpm on 4 °C for 65 h. Gradients were collected from the top of the tube into 12 fractions. Lipoproteins in each fraction were concentrated with silica and washed with cold phosphate-buffered saline, and apolipoproteins were eluted into SDS-PAGE buffer and separated by 6% SDS-PAGE as detailed above. ApoB was visualized by immunoblot analysis using a monoclonal antibody against human apoB (1D1).

Microsome Preparation and Trypsin Digestion—HepG2 cells and CaCo2 cells were cultured in 75-cm2 flasks in RPMI 1640 medium containing 20% fetal bovine serum. At ~3 weeks post-confluence, microsomes were then prepared and trypsin-digested as described previously (16). Briefly, two flasks of each cell type were harvested by scraping the cells into phosphate-buffered saline. The cells were pelleted by low speed centrifugation and homogenized in 4 ml of 0.25 M sucrose and 10 mM HEPES (pH 7.4) on ice. The homogenate was first centrifuged at 10,000 × g for 15 min at 4 °C, and the resulting supernatant was centrifuged at 100,000 × g for 60 min to pellet the microsomes. The microsomes were resuspended in an appropriate volume of 0.25 M sucrose and 10 mM HEPES. The protein concentration of the microsomes was adjusted to 1 μg/ml. Trypsin was then added to the microsome (50 μl) at a final concentration of 200 μg/ml. The mixture was incubated at room temperature for 25 min. The reaction was terminated by adding soybean trypsin inhibitor at a final concentration of 5 mg/ml. The samples were mixed with SDS-PAGE loading buffer and then denatured by boiling at 100 °C for 5 min. The proteins were separated by SDS-PAGE on 4–12% gradient gels, and Western blot analysis of apoB was performed with peroxidase-conjugated 1D1 monoclonal antibody as described above. The membrane was stripped and reprobed with monoclonal antibody against protein disulfide isomerase. Since the microsomes contained relatively much more apoB in HepG2 and CaCo2 cells, each lane was loaded 14.3 μg of total microsomal proteins from CaCo2 cells and 5.7 μg total microsomal proteins from HepG2 cells.

Construction of Adenoviral Vector Containing Human MTP Large Subunit and Transduction of Cells with Adenoviral Vectors—Construction of adenoviral vector containing human MTP large subunit (AdMTP) was as described previously (27). Another viral vector containing luciferase (AdLuc) was used as a control. At ~3 weeks post-confluence, we transduced the HepG2 and CaCo2 cells with 2 × 109 viral particles/ml. After the first day post-transduction, the medium was changed for a further 2-day culture, at the end of which the culture medium was collected for immunoblot analysis of apoB as described above. The cells were lysed, and the supernatant from the lysate was used for immunoblot analysis of MTP large subunit as described previously (27).

RESULTS AND DISCUSSION

When CaCo2 cells grown on plastic are compared with those grown on membrane filters, there is a difference in the relative levels of expression of apoB-100 versus apoB-48 (30, 31). We examined the accumulation and secretion of apoB-100 and apoB-48 by CaCo2 cells under both culture conditions (Fig. 1A). For cells grown on plastic (Fig. 1A, lanes 11–15), there is abundant secretion of apoB-100 into the medium. The amount of apoB-48, in contrast, is barely detectable. For cells grown on filters, we examined the protein secreted from both the apical and the basolateral regions (Fig. 1A, lanes 1–10). The majority (~80%) of the apoB is secreted from the basolateral region, and the amount of apoB-48 produced went up with the time of plating of the CaCo2 cells, which is related to the differentiation state of the CaCo2 cells (30–32). On days 14–16 after plating, about 50% of the apoB secreted is in the form of apoB-100 (Fig. 1A, lanes 4), much higher than the relative amount of apoB-48 secreted by cells grown on plastic. When we examined the relative amount of the two species of apoB by the cell, the cells grown on filters contained slightly more apoB-48 than apoB-100 (Fig. 1B, lanes 2 and 4), whereas those grown on plastic contained almost all apoB-100 (Fig. 1B, lanes 3 and 2). The apoB3s secreted into the medium are recovered on lipoprotein particles with relative densities that vary from that of high density lipoproteins to that of very low density lipoproteins, with the apoB-48 showing a major preference for the heavier, and apoB-100, the lighter particles (Fig. 1C).

We next performed a pulse-chase experiment to examine what proportion of the newly synthesized apoB was degraded intracellularly before secretion (Fig. 2). To our surprise, we found that both apoB-100 and apoB-48 were recovered quantitatively from inside the cell plus the medium; we detected

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essentially no intracellular degradation of the 35S-labeled apoB during a 2-h pulse-chase experiment in CaCo2 cells. This was true whether the cells were grown on filters (Fig. 2, F and G) or on plastic (Fig. 2E). In this experiment, apoA-I was also quantitatively secreted from these cells (Fig. 2, B and C), although in some other experiments we actually observed a small amount of (up to about 20%) of degradation of apoA-I (see below). Because of the unexpected finding on apoB, we examined the production of apoB and apoA-I in HepG2 cells in a parallel experiment.

We found that, as previously reported by our laboratory (7, 27) and other laboratories (4, 5), during a 2-h chase a significant proportion (38% in this experiment (Fig. 2D), consistently between 35–65% in other experiments, data not shown) of the newly synthesized apoB-100 was degraded intracellularly before secretion, but apoA-I was quantitatively secreted by the HepG2 cells (Fig. 2A). The pulse-chase analysis on apoB production in CaCo2 cells is quite reproducible; in multiple experiments we consistently found essentially no intracellular degradation of the newly synthesized apoB-100 or apoB-48.

We and others have shown that, in HepG2 cells, a substantial portion of intracellular apoB-100 is tagged for proteasomal degradation by ubiquitination (7, 8, 11, 16). We next examined whether apoB in CaCo2 cells is ubiquitinated. Intracellular apoB was isolated by immunoprecipitation, and the presence of immunoreactive ubiquitin in the purified apoB was examined by Western blotting. As shown in Fig. 3 and as reported previously (7, 16), apoB-100 isolated from HepG2 cells was heavily ubiquitinated (Fig. 3, lane 11). Furthermore, the degree of ubiquitination was greatly enhanced when the proteasomal degradation of apoB was inhibited by lactacystin (Fig. 3, lane 12). In contrast, we detected hardly any immunoreactive ubiquitin in the apoB isolated from CaCo2 cells, irrespective of whether the cells were cultured on plastic or on filter (Fig. 3, lanes 7–10). The left-hand panel of Fig. 3 shows that substantial amounts of apoB-100 (and substantial, although smaller, amounts of apoB-48 for the filter-grown sample) are present in the sample, and the almost complete absence of immunoreactive ubiquitin was not caused by an absence of apoB on the membrane. This experiment was repeated three times, and consistently, very little or no ubiquitin was detectable in the intracellular apoB isolated from CaCo2 cells, whereas abundant amounts of immunoreactive ubiquitin were present in the apoB isolated from HepG2 cells.

Since to our knowledge CaCo2 cells have not been used in previous experiments on proteasomal protein degradation, we examined this cell line for the presence of ubiquitinated proteins. Total lysates were isolated from CaCo2 and HepG2 cells, and immunoreactive ubiquitinated proteins in these lysates were analyzed by Western blotting. In Fig. 4A, the left-hand panel (lanes 1–6) presents data on the immunoreactive ubiquitin in 15 μg of total cellular lysate proteins, and the right-hand panel (lanes 7–12), the immunoreactive ubiquitin in apoB isolated from 315 μg of total lysate proteins, i.e. ~20-fold more starting material. It is evident that abundant amounts of ubiquitinated proteins were detected in intracellular proteins isolated from both types of cells. It was also readily detected in the intracellular apoB isolated from HepG2 cells (lanes 11 and 12) but not the apoB isolated from CaCo2 cells, whether the cells were grown on plastic (lanes 7 and 8) or filters (lanes 9 and 10). Furthermore, the ubiquitin immunoreactive proteins increased in the presence of lactacystin (Fig. 4A, lanes 2, 4, 6, and 12). We then determined the relative amounts of free ubiquitin in CaCo2 and HepG2 cells by Western blotting (Fig. 4B, bottom panel).
We found that CaCo2 and HepG2 cells contained approximately similar amounts of free ubiquitin, a substrate used by the cell to tag apoB and other proteins for proteasomal degradation. We next depleted the cell lysates of apoB by immunoprecipitation and checked the apoB-free proteins for the presence of immunoreactive ubiquitin. It is clear from Fig. 4B (top panel) that substantial amounts of ubiquitinated proteins are represented in these apoB-free proteins in both CaCo2 and HepG2 cells. Therefore, there is no apparent defect in the ability of CaCo2 cells to modify the intracellular proteins by ubiquitination. At least some of the ubiquitinated proteins were destined for proteasomal degradation, because the amount of ubiquitinated proteins was greatly enhanced in the presence of the proteasomal inhibitors, lactacystin or ALLN (Fig. 4, A and B).

As noted previously, the supply of optimal amounts of oleate reduces, but does not abolish, apoB degradation in HepG2 cells (20), which indicates the importance of lipids in preventing apoB degradation. Since under basal conditions, apoB production in CaCo2 cells seems to have escaped degradation, it may be that lipids are not limiting in these cells like they are in HepG2 cells. We therefore examined the effect of limiting amounts of lipid on apoB biogenesis in CaCo2 cells. CaCo2 cells were grown either in 20% FBS or in bovine lipoprotein-deficient serum for 2 days before we examined the intracellular fate of apoB-100 in CaCo2 cells. CaCo2 cells were grown either in 20% FBS or in bovine lipoprotein-deficient serum (Fig. 5). Although the amount of apoB produced in the lipoprotein-deficient serum was reduced by about 25% compared with nonlipoprotein-deficient serum (compare groups 3 and 1), the mild limitation of lipid availability associated with the use of lipoprotein-deficient serum did not stimulate intracellular degradation of apoB-100 in CaCo2 cells (compare groups 4 and 3).

We next turned our attention to the role of MTP, another well studied factor that may be important in stabilizing apoB in the endoplasmic reticulum (33, 34). MTP transfers lipids to the endoplasmic reticulum and the nascent apoB peptide chain and facilitates its correct folding, thereby preventing its intracellular degradation (34, 35). To compare the role of MTP in apoB-100 production in HepG2 and in CaCo2 cells, we acutely increased the intracellular MTP content by adenovirus-mediated transfer of the large subunit cDNA for MTP to the two types of cells. As shown in Fig. 6, this maneuver stimulated the accumulation of the MTP large subunit protein in both cell types, as had been demonstrated by us in HepG2 cells (27). Furthermore, as reported previously (27), there was a marked stimulation of apoB-100 secretion into the culture medium in Ad-MTP-treated HepG2 cells compared with control vector (AdLuc)-treated cells (Fig. 6, left-hand panel). In contrast,
there was only a minimal difference in the amount of apoB-100 secreted into the culture medium of CaCo2 cells following treatment by these vectors (Fig. 6, right-hand panel). We conclude from this observation that under basal conditions there is optimal expression of MTP in CaCo2 cells protecting the newly synthesized apoB, whereas HepG2 cells produce suboptimal amounts of MTP, which can be boosted by adenovirus-mediated gene transfer. However, this is not the whole story, because we recently showed that even marked overexpression of MTP in HepG2 cells fails to completely inhibit the polyubiquitination and proteasomal degradation of apoB (27). This contrasts the essential lack of degradation in the CaCo2 cells under basal conditions. There must be additional factors that account for the difference in the stability of apoB between HepG2 and CaCo2 cells.

To further explore the role of MTP and to find out whether we can induce apoB degradation in CaCo2 cells by an even more drastic limitation of apoB lipidation, we treated CaCo2 cells with an MTP inhibitor (BMS-197636) (Fig. 7A). The addition of the inhibitor during the preincubation and labeling periods led to substantial inhibition of incorporation of [35S]methionine into apoB-100 (compare groups 2 and 1), consistent with the interpretation that deficiency of MTP activity causes co-translational degradation of apoB (9). Moreover, whereas there was little degradation of apoB under basal conditions (compare groups 3 and 1), inhibition of MTP during the labeling and chase periods caused substantial (69%, compare groups 3 and 5) intracellular apoB-100 degradation in CaCo2 cells. Substantial intracellular degradation was also observed when the inhibitor was added only during the chase period (compare groups 5 and 1 (55% degradation) and 5 and 3 (52% degradation)).

We performed a parallel experiment examining the effect of MTP inhibition on apoA-I dynamics in CaCo2 cells (Fig. 7B). In this experiment we found that under basal conditions, a small amount (18%) of apoA-I was degraded (compare groups 1 and 5), consistent with the interpretation that deficiency of MTP activity causes co-translational degradation of apoA-I (9).
Procedures. Data are expressed as mean ± S.E. (n = 3). DLU, digital light units.

We next examined whether there is a fundamental difference between HepG2 and CaCo2 cells with respect to the orientation of apoB-100 in the endoplasmic reticulum. In HepG2 cells, the nascent apoB polypeptide is partially exposed to the cytosol and is accessible to exogenously added proteases (16, 36). Furthermore, ubiquitination seems to affect only the cytosolically exposed apoB (16). Therefore, one possibility for the difference between CaCo2 and HepG2 cells in the susceptibility of apoB to proteasomal degradation may be that the nascent apoB polypeptide displays differential accessibility to the proteasomal machinery located in the cytosolic compartment in these two cell types. We examined this hypothesis by comparing the effects of limited proteolysis on microsomes isolated from HepG2 and CaCo2 cells (Fig. 8). Under the conditions of the experiment, the intraluminally located protein, protein disulfide isomerase, is completely protected from trypsin digestion. Therefore, the nonsusceptibility of apoB-100 polypeptide with respect to the endoplasmic reticulum and cytosolic compartments.

In the series of experiments above, we have demonstrated that under the conditions used in these experiments apoB-100 and apoB-48 appear to escape intracellular degradation in CaCo2 cells. ApoB-100 especially is a highly complex protein, and it has been postulated that a significant proportion of apoB-100 molecules always undergoes misfolding and is subsequently removed by the cell largely via the ubiquitin-proteasome pathway (3, 7, 8, 10, 11, 16). MTP plays a role in facilitating the core lipidation of apoB and decreasing intracellular degradation. Supplying HepG2 cells ample amounts of lipids or up-regulating MTP activity by adenosine-mediated gene transfer reduces the number of molecules that are misfolded, but experiments in HepG2 cells indicate that there remains a significant proportion of molecules that undergo proteasomal degradation when the cells are supplemented with oleate (12, 20) and even when they experience a marked stimulation in MTP activity (27). The intracellular degradation of apoB has been observed not only in HepG2 cells but also in other cell types such as McArdle 7777 rat hepatoma cells (14, 37) and primary cultures of rat (1, 6, 38) and rabbit hepatocytes (39). We note, however, that these are all liver-derived cells. The previously purported universal occurrence of this phenomenon has led to the conclusion that the susceptibility to intracellular degradation of apoB is an intrinsic property of the protein. This is a plausible hypothesis, given the huge size and complexity of apoB. Therefore, our observation of the virtual absence of intracellular degradation of apoB-100 and apoB-48 in CaCo2 cells is totally unexpected and revolutionizes our thinking on apoB biogenesis. From a mechanistic standpoint, we found that during the transit of the apoB polypeptide chain in the endoplasmic reticulum, the relative accessibility of the apoB polypeptide chain to the cytosolic compartment is not unique to HepG2 cells but is also observed in CaCo2 cells (Fig. 8). It has been postulated that the unusual orientation of apoB during its transit into the endoplasmic reticulum exposes the nascent protein to polynoubiquitination and proteasomal degradation (12, 16). Our observations indicate that the accessibility of apoB to the cytosolic compartment is not sufficient in itself to promote proteasomal degradation. Therefore other signals unique to apoB/HepG2 or apoB/CaCo2 organellar interactions must account for

**FIG. 7. Effect of MTP inhibitor on apoB biogenesis in CaCo2 cells.** Cells were plated onto 24-well plates. About 3 weeks post-confluence, they were preincubated with methionine-free medium for 30 min, labeled with [35S]methionine for 1 h, and chased with the medium containing cold methionine (15 g/liter) for 0 or 2 h. An MTP inhibitor (BMS-197636) was present during the preincubation, pulse, or chase periods in some of the cells as indicated. Cellular and secreted apoB and apoAI were immunoprecipitated from cell lysate supernatant and cultured medium, respectively. ApoB-100 (panel A) and apoAI (panel B) were quantitated by SDS-PAGE as described under “Experimental Procedures.” Data are expressed as mean ± S.E. (n = 3). DLU, digital light units.

**FIG. 8. Susceptibility of CaCo2 and HepG2 microsomal apoB to trypsin digestion.** Cells were plated onto 75-cm² flasks. About 3 weeks post-confluence, flasks of CaCo2 and HepG2 cells were incubated with 10 nm lactacystin for 2 h, and microsomes were prepared and treated with trypsin as described under “Experimental Procedures.” Microsomal proteins were fractionated by SDS-PAGE and immunoblotted with monoclonal antibody (Ab) against apoB (left-hand panel). The membrane was stripped and reprobed with monoclonal antibody against protein disulfide isomerase (PDI) (right-hand panel).

We note, however, that these are all liver-derived cells. The previously purported universal occurrence of this phenomenon has led to the conclusion that the susceptibility to intracellular degradation of apoB is an intrinsic property of the protein. This is a plausible hypothesis, given the huge size and complexity of apoB. Therefore, our observation of the virtual absence of intracellular degradation of apoB-100 and apoB-48 in CaCo2 cells is totally unexpected and revolutionizes our thinking on apoB biogenesis. From a mechanistic standpoint, we found that during the transit of the apoB polypeptide chain in the endoplasmic reticulum, the relative accessibility of the apoB polypeptide chain to the cytosolic compartment is not unique to HepG2 cells but is also observed in CaCo2 cells (Fig. 8). It has been postulated that the unusual orientation of apoB during its transit into the endoplasmic reticulum exposes the nascent protein to polynoubiquitination and proteasomal degradation (12, 16). Our observations indicate that the accessibility of apoB to the cytosolic compartment is not sufficient in itself to promote proteasomal degradation. Therefore other signals unique to apoB/HepG2 or apoB/CaCo2 organellar interactions must account for
the difference in the susceptibility of apoB to intracellular degradation in these cell types.

ApoB production has been examined in CaCo2 cells in the past (40–43), but the potential role of intracellular degradation in the regulation of apoB biogenesis in these cells was not addressed specifically in any of these publications. Because of the different experimental objectives, e.g. often only apoB secreted into the basolateral media was collected, it is difficult to determine from the published data how much, if any, of the newly synthesized apoB was degraded.

In conclusion, we found that despite the fact that CaCo2 cells have the capacity to tag some of their intracellular proteins for degradation by the proteasome pathway, the apoB-100 and apoB-48 produced by these cells manage to largely escape this degradation by the proteasome pathway, the apoB-100 and apoB-48 produced by these cells manage to largely escape this fate. The intracellular degradation of apoB is neither universal nor inevitable. This observation is important, because it indicates that susceptibility to degradation by this pathway is not intrinsic to apoB and forces us to look for other determinants for the proteasomal degradation of apoB in hepatocytes. The unconditional use of apoB as a paradigm for intracellular protein degradation is not warranted.

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