The Proteasome Participates in Degradation of Mutant \(\alpha_1\)-Antitrypsin Z in the Endoplasmic Reticulum of Hepatoma-derived Hepatocytes*

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Because retention of mutant \(\alpha_1\)-antitrypsin (\(\alpha_1\)-AT) Z in the endoplasmic reticulum (ER) is associated with liver disease in \(\alpha_1\)-AT-deficient individuals, the mechanism by which this aggregated glycoprotein is degraded has received considerable attention. In previous studies using stable transfected human fibroblast cell lines and a cell-free microsomal translocation system, we found evidence for involvement of the proteasome in degradation of \(\alpha_1\)-ATZ (Qu, D., Teckman, J. H., Omura, S., and Perlmutter, D. H. (1996) J. Biol. Chem. 271, 22791–22795). In more recent studies, Cabral et al. (Cabral, C. M., Choudhury, P., Liu, Y., and Sifers, R. N. (2000) J. Biol. Chem. 275, 25015–25022) found that degradation of \(\alpha_1\)-ATZ in a stable transfected murine hepatoma cell line was inhibited by tyrosine phosphatase inhibitors, but not by the proteasomal inhibitor lactacystin and concomitantly and inducible expression of \(\alpha_1\)-ATZ. In each of these cell lines degradation of \(\alpha_1\)-ATZ was inhibited by lactacystin, MG132, epoxomicin, and clasto-lactacycin \(\beta\)-lactone. Using the inducible expression system to regulate the relative level of \(\alpha_1\)-ATZ expression, we found that lactacystin had a similar inhibitory effect on degradation of \(\alpha_1\)-ATZ at high and low levels of \(\alpha_1\)-AT expression. Although there is substantial evidence that other mechanisms contribute to ER degradation of \(\alpha_1\)-ATZ, the data reported here indicate that the proteasome plays an important role in many cell types including hepatocytes.

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‡The abbreviations used are: \(\alpha_1\)-AT, \(\alpha_1\)-antitrypsin; \(\alpha_1\)-ATZ, \(\alpha_1\)-antitrypsin Z; ER, endoplasmic reticulum; PAGE, polyacrylamide gel electrophoresis.
s. Because hepatocytes are the predominant site of synthesis of α1-AT and the cells predominantly affected by the pathobiological process of α1-AT deficiency-associated liver disease, this is a very important issue. In this study we examined hepatocytes in further detail by generating rat and murine hepatoma cell lines that express α1-ATZ. We also examined the role of the relative level of α1-ATZ biosynthesis by generating hepatoma cell lines with regulated expression of α1-ATZ.

**EXPERIMENTAL PROCEDURES**

**Materials**—MG132, epoxomicin, and clasto-lactacystin β-lactone were purchased from Calbiochem (La Jolla, CA), and lactacystin was provided by Dr. E. J. Corey (Harvard University, Boston, MA). Goat anti-human α1-AT was purchased from Cappel. Hepa1–6 cells were purchased from ATCC, and Dr. K. Fournier (University of Washington, Seattle, WA) provided H11 cells. HeLa Tet-Off cells were purchased from CLONTECH (Palo Alto, CA).

**Cell Lines**—Fibroblast cell lines that were transduced with amphotropic recombinant retroviral particles bearing α1-ATZ cDNA and have stable constitutive expression of α1-ATZ (CJZ12B) as described previously (10). The same approach was also used to establish stable constitutive expression of α1-ATZ in the mouse hepatoma cell line Hepa1–6 (Hepa1–6NZ2B) and the rat hepatoma cell line H11 (H11NZ2Z1) (15). Our previous studies have shown that the goat anti-human 1-AT antibody does not recognize endogenous murine 1-AT in Hepa1–6 cells (15). The H11 cell line has been shown to be highly differentiated for hepatocytic function, but, because it is extinguished for expression of HNF-1α and HNF-4, it does not express endogenous rat α1-AT (16). We also established HeLa and Hepa1–6 cell lines with inducible expression of α1-ATZ. Full-length α1-ATZ cDNA was subcloned into the pTRE plasmid provided by CLONTECH, and HeLa Tet-Off cells were transfected with the resulting pTRE α1-ATZ plasmid (HTO/Z). Hepa1–6 cells were transfected with the Tet-On plasmid provided by CLONTECH (Hepa1–6TON4Z2). Candidate colonies were screened by luminometry for inducible expression of a luciferase reporter plasmid after transient transfection. The colony with the optimal profile of low background and highest inducibility was selected for transfection with the pTRE α1-ATZ plasmid. Pulse labeling experiments showed dose-dependent, time-dependent, and reversible induction of α1-ATZ synthesis in the HTO/Z cell line and in the Hepa1–6TON4Z1 cell line.2

**Metabolic Labeling, Immunoprecipitation, and Analytical Gel Electrophoresis**—Cell lines were subjected to pulse-chase radiolabeling as described previously (11, 12). For the pulse period, the cells were incubated at 37 °C for 2 h in 250 μCi/ml Tran35S-label in Dulbecco’s modified Eagle’s medium lacking methionine. The cells were then rinsed vigorously and incubated in Dulbecco’s modified Eagle’s medium with excess unlabeled methionine for time intervals up to 6 h as the chase period. At the end of each chase period, the extracellular medium was harvested and the cells were lysed in phosphate-buffered saline, 1% Triton X-100, 0.5% deoxycholic acid, 10 mM EDTA, and 2 mM phenylmethylsulfonyl fluoride. The radiolabeled cell lysates were subjected to clarification and immunoprecipitation, and immunoprecipitates were analyzed by SDS-PAGE/fluorography exactly as described previously (12). Aliquots of the radiolabeled cell lysates were also subjected to trichloroacetic acid precipitation and scintillation counting of the trichloroacetic acid precipitates to ensure that there was equivalent incorporation between cell lines under comparison. Results were quanti-

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RESULTS

Effect of Proteasome Inhibitors on Degradation of α₁-ATZ in Human Fibroblast Cell Lines—In previous studies we showed that lactacystin inhibits degradation of α₁-ATZ in human fibroblast cell lines genetically engineered for expression of α₁-ATZ (11). Here we examined the effect of several additional proteasome inhibitors to determine whether the effect of lactacystin was generalizable and whether other proteasome inhibitors were more or less effective. The CJZ12B human fibroblast cell line was incubated for 1 h at 37 °C with each inhibitor and then subjected to pulse-chase radiolabeling in the presence of each inhibitor. The highest dose of each inhibitor that did not affect cell viability or incorporation into trichloroacetic acid-precipitable radioactivity was selected for further study. Fig. 1 shows the results for α₁-ATZ present in intracellular lysates after a pulse of 2 h and a chase of 3 h. There was a marked increase in the 52-kDa α₁-ATZ polypeptide in cells treated with lactacystin, MG132, epoxomicin, and clasto-lactacystin β-lactone. At the optimal dose for each inhibitor, there was no significant difference in the effectiveness of lactacystin, MG132, or epoxomicin. Clasto-lactacystin β-lactone was slightly less effective in this cell line.

A pulse-chase experiment in the CJZ12B cell line with multiple time points is shown in Fig. 2. The results show that, in the absence of inhibitor (control, top panel), α₁-ATZ was synthesized as a 52-kDa polypeptide, which disappeared from intracellular over 1–2 h and was almost completely degraded by 4–6 h of the chase period with negligible amounts secreted into the extracellular fluid. A similar amount of α₁-ATZ was present in the intracellular contents at time 0 after lactacystin treatment, but only began to disappear from intracellular contents by 4–6 h. There was no evidence for secretion of α₁-ATZ into the extracellular fluid after lactacystin treatment. The halftime for disappearance of α₁-ATZ was 82 min \( (r^2 = 0.82) \) in the absence and 219 min \( (r^2 = 0.80) \) in the presence of lactacystin. These data indicate that degradation of α₁-ATZ in genetically engineered human fibroblasts was decreased, but not completely abrogated, by several different proteasome inhibitors. There is no evidence that one of these inhibitors, lactacystin, mediated an increase in secretion of α₁-ATZ, even when lower doses (as low as 10 \( \mu \)M) and longer preincubation periods (as long as 2 h) were used (data not shown).

Effect of Proteasome Inhibitors on Degradation of α₁-ATZ in Hepatoma Cell Lines—We used amphotropic recombinant retroviral particles to establish hepatoma cell lines with stable expression of α₁-ATZ. First, we applied this strategy for the murine hepatoma cell line Hepa1–6. This cell line was used by Cabral et al. (14). Antibody to human α₁-AT does not recognize...
endogenous murine $\alpha_1$-AT expressed in this cell line (data not shown). Once human $\alpha_1$-ATZ expression was established in Hepa1–6 (Hepa1–6N2Z9B), we examined the effect of proteasomal inhibitors. The results of a pulse-chase experiment with one time point and multiple inhibitors are shown in Fig. 3. There was a marked increase in the 52-kDa $\alpha_1$-ATZ polypeptide after incubation with lactacystin, MG132, epoxomicin, and clasto-lactacytin $\beta$-lactone. The results of a pulse-chase experiment in Hepa1–6N2Z9B cells with multiple time points and MG132 are shown in Fig. 4. In cells that had been preincubated in the absence of MG132 (control), there was a 52-kDa polypeptide at time 0 intracellular. It disappeared progressively over 1–3 h of the chase period and was only faintly seen at 4 and 6 h of the chase period. Negligible amounts of $\alpha_1$-ATZ were secreted into the extracellular fluid. In the presence of MG132, a similar amount of $\alpha_1$-ATZ was present at time 0 intracellular with very little disappearance until 4 h of the chase period. There was no increase in secretion of $\alpha_1$-ATZ in the presence of MG132. The half-time for disappearance of $\alpha_1$-ATZ was 70 min ($r^2 = 0.91$) in the absence and 224 min ($r^2 = 0.92$) in the presence of MG132. These data indicate that degradation of $\alpha_1$-ATZ in Hepa1–6 cells is reduced by proteasome inhibitors.

Next we established expression of $\alpha_1$-ATZ in the rat hepatoma cell line H11. This cell line is highly differentiated for hepatocytic function, but completely lacks endogenous $\alpha_1$-AT expression because expression of transcription factors HNF-1$\alpha$ and HNF-4 is extinguished (15). At the end of a pulse-chase experiment with a 2-h pulse and a 3-h chase in H11N2Z1 cells, there was a marked increase in $\alpha_1$-ATZ remaining intracellular after treatment with lactacystin, MG132, epoxomicin, and clasto-lactacytin $\beta$-lactone (Fig. 5). When MG132 was examined in a pulse-chase experiment with multiple time points in Fig. 6, it was again shown to mediate a decrease in degradation of $\alpha_1$-ATZ. In control, the 52-kDa $\alpha_1$-ATZ began to disappear at 2 h of the chase period. Cells treated with MG132, $\alpha_1$-ATZ began to disappear at 3 h and the rate of disappearance was less at each subsequent time point. MG132 did not mediate an increase in secretion of $\alpha_1$-ATZ into the extracellular fluid. The half-time for disappearance of $\alpha_1$-ATZ was 100 min ($r^2 = 0.95$) in the absence and 153 min ($r^2 = 0.92$) in the presence of MG132.

Effect of Proteasome Inhibitors on Degradation of $\alpha_1$-ATZ in Cells with High Levels of $\alpha_1$-ATZ Biosynthesis as Compared with Those with Low Levels of $\alpha_1$-ATZ Synthesis—To address this issue we established cell lines with inducible (regulated) expression of $\alpha_1$-ATZ. First, we used the tetracycline-regulated expression system (Tet-Off) to establish regulated expression of $\alpha_1$-ATZ in HeLa cells. Using this system, the tetracycline analogue doxycycline suppresses expression of the target gene. To determine whether expression of $\alpha_1$-ATZ in this system is dependent on the concentration of doxycycline and whether we could establish conditions of low and high $\alpha_1$-ATZ expression for proteasome inhibitor experiments, we examined the effect of different concentrations of doxycycline in our first cell line HTO/Z (Fig. 7A). The HTO/Z cell line was incubated at 37°C for 48 h with doxycycline in several different concentrations and then subjected to pulse-labeling for 30 min. The results of immunoprecipitation/SDS-PAGE/fluorography on cell lysates show that there was abundant 52-kDa $\alpha_1$-ATZ polypeptide in the absence of doxycycline, progressively decreasing as the concentration of doxycycline increased. The 52-kDa $\alpha_1$-ATZ polypeptide was undetectable in cells treated with doxycycline at 1.0 ng/ml. Expression of $\alpha_1$-ATZ in this system is also time-dependent and reversible.$^3$ Pulse-chase experiments show that there is intracellular retention of $\alpha_1$-AT in this cell line in a manner that recapitulates the defect in $\alpha_1$-AT deficiency.$^3$

![Fig. 5. Effect of proteasome inhibitors on degradation of $\alpha_1$-ATZ in rat hepatoma cells.](image)

![Fig. 6. Effect of MG132 on the fate of $\alpha_1$-ATZ in rat hepatoma cells.](image)

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concentrations of doxycycline from 0.001 to 0.1 ng/ml mediated of proteasome inhibitor epoxomicin (Fig. 8). At high levels of chase radiolabeling after treatment in the absence or presence doxycycline for 48 h) levels of experiment.

Next, we examined several additional concentrations of doxycycline to establish a concentration that reduced expression, as directed by a lower concentration of doxycycline for 48 h) levels of 1-ATZ formed more insoluble aggregates when the half-time for disappearance of 1-ATZ expression, the presence of epoxomicin (Fig. 8b). In the absence of doxycycline (control), 1-ATZ was again synthesized as a 52-kDa polypeptide, which progressively disappeared over 2–3 h of the chase period to undetectable levels at 4 h and with a small amount of 56-kDa mature 1-ATZ secreted. At this lower level of 1-ATZ expression, the presence of epoxomicin (Fig. 8b, lower panel) was associated with a significant decrease in disappearance of 1-ATZ from the intracellular contents, but no change in the amount of 1-ATZ found in the extracellular fluid. The half-time for disappearance of 1-ATZ was 127 min ($r^2 = 0.93$) in the absence and 279 min ($r^2 = 0.99$) in the presence of epoxomicin. These results indicate that epoxomicin inhibited degradation of 1-ATZ to a similar extent in cells with high levels as compared with the same cells with 100-fold lower levels of 1-ATZ expression. Inhibition of degradation by epoxomicin was not associated with an increase in secretion of 1-ATZ. The results also indicate that there was no major difference in the fate of 1-ATZ, degradation or secretion, in the HTO/Z cell line engineered for high levels as compared with 100-fold lower levels of 1-ATZ expression.

FIG. 7. Synthesis of 1-ATZ in a HeLa cell line engineered for inducible expression. A HeLa cell line engineered for inducible expression of 1-ATZ (Tet-Off), HTO/Z, was incubated at 37°C for 48 h with complete medium alone or supplemented with doxycycline (Dox, DOX) in the concentrations indicated at the bottom of each fluorogram. The cells were then pulse-labeled for 30 min. Cell lysates were subjected to immunoprecipitation for 1-ATZ and the immunoprecipitates analyzed by SDS-PAGE/fluorography.

The results show that, in the absence of lactacystin (control), 1-ATZ was synthesized as a 52-kDa polypeptide at time 0 intracellular, retained for 1 h, and then it underwent progressive disappearance between 2 and 4 h of the chase period. In the presence of lactacystin, a similar amount of the 52 kDa 1-ATZ polypeptide was present at time 0 intracellular, reaching 100-fold lower levels of 1-ATZ expression. Inhibition of degradation by lactacystin mediated an inhibition of 1-ATZ degradation in hepatoma cells with relatively high levels of 1-ATZ expression. The half-time for disappearance of 1-ATZ was 98 min ($r^2 = 0.95$) in the absence and 215 min ($r^2 = 0.94$) in the presence of lactacystin. Lactacystin also mediated an inhibition of 1-ATZ degradation in Hepa1–6 TON4Z1 cells with 30-fold lower levels of 1-ATZ expression, as directed by a lower concentration of doxycycline (data not shown).

Effect of Proteasome Inhibitors on the Solubility of 1-ATZ—Next we examined the detergent-soluble and insoluble fractions from HTO/Z cells treated with MG132 to determine whether 1-ATZ formed more insoluble aggregates when the proteasome was inhibited. HTO/Z cells were subjected to a pulse-chase experiment in the absence or presence of MG132 in several different concentrations, and then the cell lysates were analyzed after separation into soluble and insoluble fractions (Fig. 10). The results show that MG132 mediated a concentration-dependent increase in 1-ATZ in soluble fraction and a dramatic increase in insoluble 1-ATZ. The accumulation of 1-ATZ in the insoluble fraction mediated by MG132 was already apparent at time 0 of the chase period (after a 1-h incuba-

A small amount of the mature 56-kDa 1-ATZ polypeptide was secreted into the extracellular fluid. In the presence of epoxomicin (Fig. 8a, lower panel), a similar amount of 1-ATZ was present at time 0 intracellular, but in this case there was very little disappearance during the chase period. The half-time for disappearance of 1-ATZ was 108 min ($r^2 = 0.95$) in the absence and 193 min ($r^2 = 0.93$) in the presence of epoxomicin. The results also indicate that epoxomicin did not mediate an increase in secretion of 1-ATZ under these conditions. At 100-fold lower levels of 1-ATZ (Fig. 8b), in the absence of epoxomicin (control), 1-ATZ was again synthesized as a 52-kDa polypeptide, which progressively disappeared over 2–3 h of the chase period to undetectable levels at 4 h and with a small amount of 56-kDa mature 1-ATZ secreted. At this lower level of 1-ATZ expression, the presence of epoxomicin (Fig. 8b, lower panel) was associated with a significant decrease in disappearance of 1-ATZ from the intracellular contents, but no change in the amount of 1-ATZ found in the extracellular fluid. The half-time for disappearance of 1-ATZ was 127 min ($r^2 = 0.93$) in the absence and 279 min ($r^2 = 0.99$) in the presence of epoxomicin. These results indicate that epoxomicin inhibited degradation of 1-ATZ to a similar extent in cells with high levels as compared with the same cells with 100-fold lower levels of 1-ATZ expression. Inhibition of degradation by epoxomicin was not associated with an increase in secretion of 1-ATZ. The results also indicate that there was no major difference in the fate of 1-ATZ, degradation or secretion, in the HTO/Z cell line engineered for high levels as compared with 100-fold lower levels of 1-ATZ expression.

We also examined the effect of proteasome inhibitors on degradation of 1-ATZ in a hepatoma cell line with inducible (regulated) expression of 1-ATZ. Here we used the Hepa1–6 cell line and engineered it for Tet-On expression of 1-ATZ. The Hepa1–6 TON4Z1 cell line only expresses 1-ATZ in the presence of doxycycline.3 We examined the effect of proteasome inhibitor lactacystin on 1-ATZ in this cell line under conditions of maximal expression (1 μg/ml doxycycline for 48 h) in Fig. 9. The results show that, in the absence of lactacystin (control), 1-ATZ was synthesized as a 52-kDa polypeptide at time 0 intracellular, retained for 1 h, and then it underwent progressive disappearance between 2 and 4 h of the chase period. In the presence of lactacystin, a similar amount of the 52 kDa 1-ATZ polypeptide was present at time 0 intracellular, but there was very little disappearance of the polypeptide over the duration of the 4-h chase period, indicating that lactacystin mediates an inhibition of 1-ATZ degradation in hepatoma cells with relatively high levels of 1-ATZ expression. The half-time for disappearance of 1-ATZ was 98 min ($r^2 = 0.95$) in the absence and 215 min ($r^2 = 0.94$) in the presence of lactacystin. Lactacystin also mediated an inhibition of 1-ATZ degradation in Hepa1–6 TON4Z1 cells with 30-fold lower levels of 1-ATZ expression, as directed by a lower concentration of doxycycline (data not shown).

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tion and 1-h pulse in the presence of MG132). Interestingly, two distinct 48- and 38-kDa cleavage products of α1-ATZ also appeared in the insoluble pellet in the presence of MG132. Densitometric analysis of the 52-, 48-, and 38-kDa α1-ATZ polypeptides in the insoluble fraction taken together showed that MG132 mediated an 7.4-fold increase in insoluble α1-ATZ. These results indicate that there was a marked increase in insoluble aggregates of α1-ATZ when the proteasome was inhibited and that insoluble aggregates of α1-ATZ underwent two distinct proteolytic cleavage reactions when the proteasome was inhibited.

**DISCUSSION**

A detailed elucidation of the mechanisms by which mutant aggregated α1-AT is degraded in the ER is essential for understanding how the quality control apparatus of the ER works in general and for understanding the specific issue of how a subgroup of α1-AT-deficient individuals become susceptible to liver injury and carcinogenesis. Previous studies have shown that there is a lag in the disposal of this mutant protein in genetically engineered human fibroblasts from “susceptible” deficient patients (10). Moreover, this lag in the ER disposal/quality control mechanism appears to be specific, i.e. it affected the disposal of two polymerogenic mutants of α1-AT, but not a model unassembled membrane protein (19).

Initial studies of the ER degradation of α1-ATZ indicated that the ubiquitin-dependent proteasomal system was involved. Degradation of α1-ATZ in genetically engineered human fibroblast cell lines and in a cell-free microsomal translocation system was inhibited by lactacystin (11). Degradation of α1-ATZ in the cell-free system was shown to be dependent on ATP and, more importantly, a polyubiquitinated calnexin-α1-ATZ complex was shown to be a degradative intermediate (11). Subsequent studies by Novoradovskaya et al. (13) have shown that degradation of α1-ATZ is also inhibited by lactacystin in transfected Chinese hamster ovary cells and in primary cultures of human mononuclear phagocytes. Using an experimen-

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**FIG. 8. Effect of epoxomicin on the fate of α1-ATZ under conditions of low and high level expression in the HTO/Z cell line.** The HTO/Z cell line was incubated at 37 °C for 48 h in the absence (high expression; (A)) or presence of doxycycline at 0.1 ng/ml (low expression; (B)). In each case separate wells were incubated at 37 °C for 1 h in the absence or presence of epoxomicin (pulse: 30 min) and the individual samples analyzed exactly as described in the legend to Fig. 1. The volume of samples from the low-expressing cells used for immunoprecipitation was 8 times greater than the volume for corresponding samples from high-expressing cells. The film for panel B (low expression) was subjected to fluorography for 7 days as compared with 1 day for panel A (high expression). The insets at the right show quantitative data from densitometric scanning of these fluorograms. These data are reported as percentage of α1-ATZ remaining by comparison to time 0, which is arbitrarily set at 100%. The solid line with diamonds is for control, and the dashed line with squares is for lactacystin. IC, cell lysates; EC, extracellular fluid.
The migration of the novel degradation from soluble and insoluble fractions. Immunoprecipitates were divided into aliquot from the starting material was used for immunoprecipitation in the absence of lactacystin or presence of 10 μM lactacystin and then subjected to a pulse-chase protocol in the absence or presence of lactacystin (pulse: 30 min). IC, cell lysates; EC, extracellular fluid.

![Diagram of IC and EC with Mr values](image)

**Fig. 9.** Effect of lactacystin on the fate of α1-ATZ under conditions of high level expression in a Hepa1–6 cell line engineered for inducible expression of α1-ATZ. A Hepa1–6 cell line engineered for inducible expression of α1-ATZ (Tet-On) Hepa1–6TON4Z1 was incubated at 37 °C for 48 h with complete medium supplemented with doxycycline 1 μg/ml. Separate wells were incubated at 37 °C for 1 h in the absence (control) or presence of 10 μM lactacystin and then subjected to a pulse-chase protocol in the absence or presence of lactacystin (pulse: 30 min). IC, cell lysates; EC, extracellular fluid.

**Fig. 10.** Solubility of α1-ATZ in the presence of MG132. The HTOZ cell line was incubated at 37 °C for 1 h in the absence or presence of MG132 in several different concentrations as indicated at the bottom. The cells were then subjected to pulse-chase radiolabeling in the absence or presence of MG132 in the same concentrations (pulse: 60 min) and the cell lysates separated into soluble and insoluble fractions exactly as described under “Experimental Procedures.” An equivalent aliquot from the starting material was used for immunoprecipitation from soluble and insoluble fractions. Immunoprecipitates were subjected to analytical gel electrophoresis exactly as described above. The migration of the novel ~48- and 38-kDa insoluble proteolytic fragments is indicated by the arrows at the right margin.

tal approach in which the degradative machinery in the reticulocyte lysate of the cell-free system is fractionated and reconstituted with purified components, we have recently found evidence for at least three different pathways in the degradation of α1-ATZ, including ubiquitin-dependent and -independent proteasomal mechanisms and at least one nonproteasomal mechanism (12). Subsequent studies have suggested that autophagy may constitute one of the nonproteasomal mechanisms (15) and have substantiated the concept that there are multiple pathways involved in ER degradation of α1-ATZ.

In the most recent study of this issue, Cabral et al. (14) found that lactacystin did not inhibit degradation of α1-ATZ in a stable transfected murine hepatoma Hepa1–6 cell line. Degradation of α1-ATZ in this cell line was decreased by tyrosine phosphatase inhibitors. Lactacystin did inhibit degradation of α1-ATZ Null Hong Kong, a truncated mutant, in a separate stable transfected Hepa1–6 cell line. Taken together, these results indicated that the proteasome did not play a role in degradation of α1-ATZ in hepatoma cell line even though the proteasome was active in these cells, that an entirely separate mechanism for degradation existed, and that this distinct mechanism was specific for α1-ATZ. Taking into consideration the previous results indicating involvement of the proteasome in degradation of α1-ATZ in genetically engineered fibroblasts (11, 13), in primary cultures of human macrophages (13), and in the cell-free microsomal system (11), these authors concluded that the proteasomal mechanism was cell type-specific, either for nonhepatocytic cell types and/or cell types with lower levels of endogenous α1-AT expression than hepatocytes (14).

Because the hepatocyte is an extremely important, if not the most important, site of synthesis of α1-ATZ with respect to the development of liver disease, in this study we sought to examine in further detail the involvement of the proteasome in ER degradation of α1-ATZ in cells of hepatocytic lineage. The results show that lactacystin, MG132, epoxomicin, and clasto-lactacystin β-lactone all inhibit degradation of α1-ATZ in several different types of genetically engineered hepatoma cell lines, including the murine hepatoma Hepa1–6 used by Cabral et al. and a rat hepatoma cell line H11, which has the advantages of being highly differentiated for hepatocytic function, but lacking endogenous expression of α1-AT. Degradation of α1-ATZ in hepatoma cell lines with constitutive and inducible expression of α1-ATZ was blocked to an equivalent extent by proteasomal inhibitors. Finally, studies in HeLa and Hepa1–6 cell lines with inducible expression of α1-ATZ showed that the proteasome was involved in degradation of α1-ATZ at both high and 30–100-fold lower levels of expression. These results suggest that the lack of involvement of the proteasome in degradation of α1-ATZ in the Hepa1–6 cell line generated by Cabral et al. (14) is cell line-specific, perhaps reflecting a type of adaptation. This hypothesis by no means diminishes the importance of the observations of Cabral et al. or the importance of tyrosine phosphatases in the quality control mechanism. There is now ample evidence for multiple mechanisms/pathways in the ER quality control apparatus and for cellular “adaptation.” In fact, gene expression profile analysis has shown marked changes in expression of many genes in yeast cells that accumulate misfolded proteins (20–23). Moreover, if a cellular adaptation mechanism is truly applicable, then the results of Cabral et al. raise the interesting possibility that the adaptation is specific for α1-ATZ, a polymorphic mutant, and not for α1-AT Null Hong Kong, a mutant that is truncated and not likely to be polymorphic.

Three other results of this study deserve comment. First, there is a marked increase in the formation of insoluble aggregates of α1-ATZ and two distinct degradation products appear exclusively in the insoluble fraction when the proteasome is inhibited. These degradation products could theoretically be generated in, and/or accumulate in, the ER or the cytoplasm. Several lines of evidence make it more likely that they are generated in and localize to the ER. α1-ATZ has not been detected outside the ER lumen in intact cells (15) or in the supernatant of cell-free mammalian translocation reactions (11) and proteasome inhibitors do not induce aggresomes in cells that express mutant α1-ATZ (15). Similar degradation products are generated processively in the lumen of microsomal vesicles that have translocated wild type α1-ATZ in a cell-free microsomal translocation reaction (11), suggesting the existence of an endoluminal proteolytic system that recognizes wild type or mutant α1-ATZ when it is retained in the ER for a prolonged period of time. Second, there was no evidence for a significant increase in secretion of α1-ATZ in fibroblasts, hepatoma cells, or HeLa cells in the presence of lactacystin, MG132, epoxomicin, or clasto-lactacystin β-lactone at doses optimal for inhibition of degradation of α1-ATZ. Using the lower doses of lactacystin or longer periods of preincubation with lactacystin described by Novoradovskaya et al. (13), we
observed a lesser degree of inhibition of degradation, but no increase in secretion of α₁-ATZ in fibroblasts, hepatoma cells, and HeLa cells at low and high levels of expression (data not shown). Third, secretion of α₁-ATZ did not increase when the level of its synthesis was decreased. This was shown by modulating the level of synthesis 30–100-fold in tetracycline-regulated cell lines. This result is noteworthy because the rate of polymerization of α₁-ATZ decreases at lower concentrations in purified systems (6). The results in this report therefore suggest that other factors working in concert with polymerization play a role in the fate of α₁-ATZ when it accumulates in the ER.

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