Evidence of Proteasome-mediated Cytochrome P-450 Degradation*

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The cytochrome P-450 family of enzymes performs an incredibly diverse range of detoxification and oxidation reactions within the cell and constitutes between 5 and 10% of protein in hepatic endoplasmic reticulum. In this report it is demonstrated that constitutively expressed membranous P-450s are targeted for destruction by the proteasome, in a process which is ubiquitin-independent and is demonstrated in vitro to require prior labilization of the enzyme. This process was specific for P-450s CYP1A2, CYP2E1, CYP3A, and CYP4A and was not demonstrated to be involved in the turnover of CYP1A1, CYP2B1/2, or NADPH reductase. In reconstitution experiments using purified proteasomes and microsomal fractions, labilized P-450 conformations are protected from 20 S proteasome degradation by substrate addition, with proteolysis occurring while P-450s are still attached to the endoplasmic reticulum.

The cytochrome P-450 multigene family encodes a broad variety of membranous proteins that generally serve to render exogenous or endogenous compounds hydrophobic. They are inducible, share similar topology (1), and constitute between 5 and 10% of endoplasmic reticulum (ER) protein in hepatocytes. The P-450s possess rapid to medium half-lives in the constitutive state (for a review, see Correia (2)), in contrast to NADPH cytochrome c reductase (NADPH reductase) and cytochrome b$_2$, both of which exhibit half-lives on the order of several days (2). These observations suggest a series of specific intracellular events may target the P-450s for rapid proteolysis by an as yet unidentified pathway. Recently, ubiquitination has been associated with membranous proteins, apparently acting as a means of tagging or translocating these proteins for destruction either in the lysosomal vacuole (3) or 26 S proteasome (4, 5). The discrete 20 S proteasome has been implicated in these interactions (4), but unlike the 26 S proteasome it does not possess ATPases capable of unfolding complex globular proteins. Intracellular distribution of the 26 and 20 S proteasomes suggests one third of the 20 S associates with the ER, while the 26 S is found predominantly in the cytosol (6, 7). In this report it is suggested that a major biological role of the 20 S proteasome is to remove cytochrome P-450s from the ER, in a mechanism which is shown in vitro to require prior labilization of the enzyme.

EXPERIMENTAL PROCEDURES

Specialized Reagents—Lactacystin was the generous gift of S. Omura (The Kitasato Institute) and E. Corey (Harvard University). ALLN was purchased from Sigma. All other chemical and consumables were of the highest grade commercially available.

Cell Culture, Microsome Preparation, and Immunoblotting—Isolated hepatocytes were obtained from untreated male Harlan Sprague Dawley rats by the collagenase perfusion method (95% viable) and seeded on a collagen matrix at a density of 1.5 × 10^6 cells/well (6 × wells/plate). Cells exhibited 95% attachment prior to the commencement of experiments. Plated cells were washed thrice with 2 ml of Waymouth’s MB 752/1 medium at pH 7.4, containing 0.2 g/liter NaHCO_3, supplemented with 100 units/ml penicillin, 100 mg/ml streptomycin, 5% fetal bovine serum, and 5 ng/ml dexamethasone. The final wash was conducted with the addition of lactacystin (25 μM per culture well, dissolved in Tris-HCl, pH 7.4, and prefiltered through a 0.22-μm Millipore membrane), ALLN (200 μM per well, dissolved and prefiltered in dimethyl sulfoxide and added such that the final concentration of Me2SO was no more than 0.4% v/v). Controls received culture medium only. Primary cultures were incubated for 24 or 30 h in 5% CO2 at 37 °C. Following this period, culture medium was aspirated off, and cells were washed with 2 ml of ice-cold Tris-HCl, pH 7.4, containing 0.25 M sucrose and 20 μg of leupeptin (buffer 1). After washing, cells were scraped and resuspended in 0.3 ml of the Tris buffer. To obtain sufficient microsomes for analysis, cells from three wells were pooled and used as starting material. Cells were homogenized in Sorvall 2-ml ultracentrifuge tubes using a Potter-Elvejem Teflon pestle in approximately 1 ml of Tris buffer. Homogenates were centrifuged at 9000 × g for 20 min, and the supernatant was removed and centrifuged at 105,000 × g for 1 h. All centrifugation steps were carried out at 4 °C in a Sorvall model TFF-80.2 rotor. The resultant pellet from the 105,000 × g step (microsomal) was resuspended in 200 μl of Tris-HCl, pH 7.4, containing 0.25 M sucrose, 20% N-acetyl-L-cysteine, stored at –70 °C for further use. Approximately 150 μg of microsomal protein were obtained using this method. Antibodies to CYP1A2, CYP4A, and CYP3A were purchased from Amersham Life Science. Polyclonal antisera to CYP2E1 was the gift of B. J. Song (National Institute of Alcohol Abuse and Alcoholism) and CYP1A1/2 and CYP2B1/2, J. Hardwick (Northeastern Ohio Medical College). Bands corresponding to CYP1A1, CYP1A2, CYP2B1, and CYP2B2 have previously been authenticated by the supplier of these antibodies. A polyclonal antibody to NADPH reductase was obtained from Oxford Biomedical Research (Oxford, MI). Anti-ubiquitin IgG was purchased from Sigma. In all cases 5–20 μg of microsomal protein were used to determine P-450 levels by chemiluminescent immunoblotting (Amerham).

Extract Isolation for Dose-response Curves and Ubiquitin Conjugate Isolation—The isolation buffer used for dose-response curves was essentially buffer 1 in the absence of leupeptin. All procedures were conducted at 4 °C. Cells were preincubated with lactacystin for 4 h, isolated by scraping, and lysed by homogenization, and the 9000 × g supernatant was used for analysis of chymotryptic activity. Because it is a reversible proteasome inhibitor, ALLN was added to the extracts 10 min prior to the start of the experiment. Preliminary experiments using purified proteasome revealed ALLN to inhibit >95% of chymotryptic activity following this preincubation step. Activity was determined by the cleavage of fluorogenic free 7-amino-4-methylcoumarin/min from the peptide, succinyl-Leu-Leu-Val-Tyr 7-amino-4-methylcoumarin (SLLVT-AMC). Incubations were carried out at 37 °C in a shaking
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RESULTS

Proteasome inhibitors lactacystin and ALLN were incubated with primary hepatocyte cultures at concentrations of 25 μM and 200 μM respectively and levels of several constitutive P-450s determined by immunoblotting. Fig. 1 shows representative blots of CYP2E1 (Fig. 1A) and CYP4A (Fig. 1B) in the microsomal fraction. Marked loss of CYP2E1 and CYP4A was observed after 24 h of primary culture, with lactacystin and ALLN conferring a substantial protective effect on the loss of the two isoforms shown. No staining was observed in the high molecular mass region of Fig. 1, A and B, following incubation with proteasome inhibitors, suggesting an absence of P-450 ubiquitin conjugation. Proteins identified as P-450s following incubation with proteasome inhibitors appeared to migrate identically on SDS-PAGE as those isolated at t₀ and t₂₄ h, and were therefore not considered to arise via the cleavage action of ubiquitin isopeptidases or other artefactual mechanisms. When the ER fraction was probed with ubiquitin IgG (Fig. 1C), no significant changes were observed in higher molecular mass ubiquitination (>80 kDa) either in the absence or presence of ALLN. It is conceivable that some of the staining observed with the ubiquitin IgG represents free ubiquitin chains, instead of ubiquitin-protein conjugates, and therefore would not be expected to change in the presence of proteasome inhibitors such as ALLN. In the same experiments, levels of CYP1A2 and CYP3A were also determined following 24 h of culture with and without proteasome inhibitors. Again, proteasome inhibitors conferred a significant protective effect on the degradation of these isoforms without high molecular mass staining,² suggesting...
ing the involvement of a proteolytic pathway which is proteasome but not ubiquitin dependent. Using CYP2E1 as an example, several methods were used to determine whether P-450 ubiquitin conjugates were present in these cells, but below the levels of detection used in this study (Fig. 2). First, using a methodology similar to Haas and Bright (12), a comparison of differing buffer systems was employed to establish whether loss of ubiquitin conjugates occurred during preparation. The use of a buffer with a mixture of chelating agents and protease inhibitors had little effect on ubiquitin conjugate loss when compared with that of ice-cold standard buffer containing leupeptin (Fig. 2A). In the time taken for isolation of microsomal fractions (150 min), some of the very high molecular mass (>200 kDa) ubiquitin conjugates were “trimmed” to a slightly lower molecular mass using the standard buffer. Considering a previous report by Wilkinson et al. (13), it is possible that some of this activity represents free polyubiquitin chain disassembly by isopeptidase T or variants thereof. Overall, there was a 20% decrease in the amount of ubiquitin conjugates between 80 and 200 kDa, the region where we have conventionally found most P-450-ubiquitin conjugates to occur. Buffer B appeared to protect against the trimming of higher molecular mass conjugates during this time. A second approach was to take cells extracts and assess the level of proteasome inhibition using chemiluminescence. Time points, molecular masses, and ALLN concentrations are as indicated.

**Fig. 2. Effect of various preparation conditions on the detection of ubiquitin conjugates in primary liver cultures.** A, after 24 h of incubation with 200 μM ALLN, different preparation buffers were used to estimate stability of high molecular mass ubiquitin conjugates during the isolation and subfractionation procedures. Buffer 1 contained ice-cold 0.1 M Tris-HCl, pH 7.4, 0.25 M sucrose, and 20 μg of leupeptin. Buffer 2 contained several additional chelating agents and protease inhibitors as described under “Experimental Procedures.” In each case total cellular extract was used and quenched in SDS-PAGE buffer at the time points shown. Ubiquitin conjugates were measured by densitometry between 80 and 200 kDa. Note the slight “trimming” of ubiquitin conjugates in the 200-kDa range using buffer 1. In B, the same extracts were probed with CYP2E1 IgG and compared with 0-h controls using buffer 2. No differences were observed in the starting concentrations of ubiquitin conjugates or CYP2E1 using either buffer. The position of CYP2E1 is denoted. The identity of the other bands is unknown; however, they do not appear in microsomal isolates and are therefore considered to be cytosolic contaminants. In C, microsomal preparations were run on 6% acrylamide gels, transferred, and subjected to CYP2E1 staining and overexposure using chemiluminescence. Time points, molecular masses, and ALLN concentrations are summarized in Table I. CYP2E1 and CYP3A are turned over quite rapidly in cultured hepatocytes, with CYP1A2 and CYP4A displaying comparatively smaller losses over a 24-h period. Generally, ALLN was found to have a slightly more potent inhibitory effect on P-450 turnover in hepatocytes than did lactacystin, possibly because it affects other proteases in addition to the proteasome. In the case of CYP2E1 and CYP3A the loss from culture was not fully blocked by lactacystin and ALLN, suggesting other proteases may contribute in part to the proteol-
ysis of these forms. With CYP1A2 and CYP4A, lactacystin and ALLN also significantly inhibited protein turnover, with these forms intrinsically more stable than either CYP2E1 or CYP3A in culture. Overall, the amount of constitutive P-450 recovered after 24 h of culture was 2–4-fold higher in the presence of proteasome inhibitors.

Although proteasome-mediated degradation was clearly involved in the destruction of several P-450s, it was necessary to establish negative controls in hepatocyte cultures to interpret these findings in the context of in vivo turnover data, and confirm that proteasome degradation is a specific event. CYP1A1, CYP1A2, CYP2B1, CYP2B2, and NADPH reductase were evaluated in primary cultures following extended incubation (30 h) with ALLN (Fig. 4). As anticipated from Table II, CYP1A2 was rapidly degraded in an ALLN-sensitive manner (Fig. 4A). In contrast, CYP1A1 (Fig. 4A), CYP2B1/2 (Fig. 4B), and NADPH reductase (Fig. 4C) were relatively stable in culture, and levels were unaffected by ALLN. Approximately 40–60% of the initial protein levels were lost over 30 h, suggesting a proteasome-independent pathway degrades these isoforms.

The findings from the first part of this study were compared against existing data on the half-lives of several hemoproteins resident in the ER (Table II). Of those investigated CYP2B1/2 and NADPH reductase have been reported to be the most stable in the ER. CYP2E1, CYP3A, and CYP1A2 possess shorter half-lives in the order of 12 h or less, with CYP1A1 falling in between with a value of approximately 15–16 h. No data were available for CYP4A. In this study, the presence of proteasomal degradation was coincident with P-450s exhibiting reported half-lives of 12 h or less and was not observed in ER proteins exhibiting longer half-lives.

To confirm that the proteasome can directly degrade P-450s in the ER, a reconstituted system was used in which purified

### Table I

| P-450  | 100 | 44 ± 10 | 83 ± 12 | 87 ± 22 |
|--------|-----|---------|---------|---------|
| CYP1A2 |     |         |         |         |
| CYP2E1 | 100 ± 10 | 23 ± 11 | 76 ± 7 | 88 ± 16 |
| CYP3A  | 100 ± 14 | 30 ± 9 | 76 ± 10 | 84 ± 15 |
| CYP4A  | 100 ± 5 | 51 ± 15 | 93 ± 9 | 94 ± 13 |

Changes in P-450 levels from the four major gene subfamilies in cultured hepatocytes following incubation of cells with lactacystin and ALLN

In each case, $t_0$ refers to P-450 determination at the time at which cells were plated and had successfully attached to the collagen substra-

Fig. 3. Dose-response curves of lactacystin and ALLN in primary liver cell cultures. In A, cells were preincubated for 4 h with lactacystin at various concentrations, cell extracts were isolated, and the chymotryptic cleavage of SLLVT-AMC was determined by fluorimetry as described under "Experimental Procedures." In B, extracts were isolated, and ALLN was added at the concentrations denoted. $K_i$ and maximal inhibition values were determined from these plots.

Fig. 4. Effects of ALLN on levels of constitutive and inducible P-450s and NADPH reductase in cultured hepatic microsomes. In these experiments, CYP1A1/2 (A), as well as CYP2B1/2 (B) and NADPH reductase (C), were assessed after 30 h of primary culture. Protein concentration, electrophoresis, and immunoblotting were as described in Fig. 1, A and B, using antibodies that recognize individual isoforms of the rat CYP1A and CYP2B subfamilies. Each lane represents microsomal protein prepared from six culture wells. Histograms at the right represent the mean ± S.D. of four separate determinations. Results are expressed as a percentage of initial levels (100%) and represent the mean ± S.D. of four separate determinations.

| P-450  | $t_0$ | $t_{24}$ |
|--------|------|---------|
|        | −Proteasome inhibitors | +Lactacystin | +ALLN |
| CYP1A2 | 100 ± 18 | 44 ± 10 | 83 ± 12 | 87 ± 22 |
| CYP2E1 | 100 ± 10 | 23 ± 11 | 76 ± 7 | 88 ± 16 |
| CYP3A  | 100 ± 14 | 30 ± 9 | 76 ± 10 | 84 ± 15 |
| CYP4A  | 100 ± 5 | 51 ± 15 | 93 ± 9 | 94 ± 13 |
proteasomes were co-incubated with microsomal fractions derived from rat livers (Figs. 5 and 6). Using purified 26 and 20 S proteasomes, together with suicide inhibitors known to accelerate P-450 proteolysis in vivo, it was possible to provide evidence on the mechanism of P-450 destruction by the cytosolic proteasome. CYP2E1 and CYP3A were used as representative P-450s because specific labilizing agents were available, and they are both turned over by the proteasome in cultured cells. Both CYP2E1 and CYP3A were resistant to proteolysis using purified preparations of rat liver proteasome (Fig. 5), suggesting P-450s must undergo conformational changes in vivo to explain the results presented in Table I. The 26 S proteasome was found to have little effect on deactivated forms of P-4503; however, the 20 S proteasome exhibited substantial proteolytic activity toward membranous P-450s independently of ATP.

### Table II

| Enzyme          | Proteolytic pathway sensitive to proteasome inhibitors in this study | $t_{1/2}$ previously calculated in primary cultures or in vivo | References                  |
|-----------------|---------------------------------------------------------------------|---------------------------------------------------------------|------------------------------|
| CYP1A1          | No                                                                   | 15                                                           | Sadano and Omura (24)        |
|                 |                                                                     | 16                                                           | Shiraki and Guengerich (23)  |
| CYP1A2          | Yes                                                                  | 10                                                           | Shiraki and Guengerich (23)  |
| CYP2B1          | No                                                                   | 19                                                           | Shiraki and Guengerich (23)  |
|                 |                                                                     | 25                                                           | Sadano and Omura (24)        |
| CYP2B2          | No                                                                   | 19                                                           | Shiraki and Guengerich (23)  |
|                 |                                                                     | 25                                                           | Sadano and Omura (24)        |
| CYP2E1          | Yes                                                                  | 6                                                            | Barmada et al. (20)          |
|                 |                                                                     | 7                                                            | Song et al. (21)             |
|                 |                                                                     | 7                                                            | Shiraki and Guengerich (23)  |
| CYP3A           | Yes                                                                  | 9, 14                                                         | Watkins et al. (22)          |
|                 |                                                                     | 12                                                           | Shiraki and Guengerich (23)  |
| CYP4A           | Yes                                                                  | 29                                                           | Shiraki and Guengerich (23)  |
| NADPH reductase | No                                                                   | 35                                                           | Sadano and Omura (24)        |

### Fig. 5

Cytochrome P-450s were isolated as membranous fractions (microsomes) from male Harlan Sprague Dawley rats and exposed to the cytosolic 20 S proteasome using a variety of conditions. With CYP2E1 (A) and CYP3A (B) the suicide substrates CCl₄ and DDEP were used to selectively inactivate these isozymes via NADPH-dependent mechanisms. In the case of CYP1A2 and CYP4A, no specific agents have been reported to modify or inactivate these particular forms in vivo. Microsomes were preincubated in the presence of NADPH for 45 min at 37 °C prior to the addition of 20 S proteasome. Incubation mixtures contained 1 mg of microsomes, 5 mM MgCl₂, 15 units of yeast hexokinase, 5 mM glucose, 2 mM NADPH, and either 10 mM CCl₄, 0.5 mM DDEP, or 0.1 mM troleandomycin. Controls were incubated in the absence of substrate/substrate inactivators. In each case, 2.5 units of cytosolic 20 S proteasome was added to the incubates to start the reaction. After 1 h at 37 °C, reactions were terminated by the addition of SDS-PAGE sample buffer, boiled, and subjected to chemiluminescent immunobloting using isozyme specific antibodies. Results are expressed as a percentage of controls (100%), where controls are defined as microsomal samples without 20 S proteasome. Each column represents the mean ± S.D. of four separate determinations, as quantitated by laser densitometry (Molecular Dynamics, Sunnyvale, CA). ADU denotes arbitrary densitometric units. For simplicity, * denotes a statistically significant difference ($p < 0.05$) between this treatment and all others shown on each histogram.

3 This is an unsurprising observation given the general requirements of the 26 S proteasome for ATP and ubiquitination.
From the data presented it is clear that CYP2E1 and CYP3A are marked for 20 S proteasome destruction by the NADPH-dependent activation of reactive metabolites. In the case of CYP3A, it was found that prolonged storage of microsomes (4 months) or repeated cycles of freeze/thawing labilized this isoform to such an extent that it could be degraded by the 20 S proteasome. The partial labilization these procedures cause could be attenuated by the presence of the CYP3A substrate troleandomycin (TAO, Fig. 5B), suggesting there is an allosteric modification in the ligand bound state that inhibits 20 S proteolysis of CYP3A.

Using carbon tetrachloride (CCl₄) it was possible to track the pattern of CYP2E1 fragmentation by the 20 S proteasome (Fig. 6A). After treatment with this suicide substrate, CYP2E1 is degraded by the 20 S proteasome, with the immediate appearance of a fragment of CYP2E1 at around 32 kDa. This process was blocked in the presence of lactacystin (Fig. 6B). Notably, there is little evidence of any intermediate breakdown products on the blot, suggesting the 20 S proteasome rapidly cleaves a large unfolded segment of CYP2E1 oligopeptide. Resiolation of the microsomal fraction (Fig. 6C) suggests the fragment of CYP2E1 left after initial cleavage is still attached to the ER, awaiting further destruction by the 20 S proteasome. Similar findings were obtained with DDEP-inactivated CYP3A (Fig. 6, B and D); however, no discrete degradation products were reproducibly observed in this study.

**DISCUSSION**

Recent findings suggest a number of ER proteins are targeted for destruction, either by ubiquitin conjugation (4, 5, 9, 10, 14) or possibly by the proteasome itself (4, 15). Evidence for the latter is less rigorous, because ubiquitinated proteins can be identified by a variety of techniques, whereas the direct action of the proteasome can only be inferred in whole cells by the recent advent of specific proteasome inhibitors such as lactacystin (16). The cytochrome P-450 family of enzymes is particularly intriguing from the perspective of protein turnover as there is considerable heterogeneity in the turnover rates of different isoforms, despite apparently similar topologies in the ER membrane (1).

Primary hepatocyte cultures from untreated rats exhibit a characteristic loss of cytochrome P-450s within the first 24 h of plating due to the inability of hepatocytes to maintain steady state levels of P-450 mRNA (17). The expressed proteins are subsequently degraded at varying levels via a pathway that is unknown. This property of primary cultures is somewhat for-
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tuous as it allows the proteolytic pathway of several P-450s to be investigated within one study, an issue which is difficult to address in artificial expression systems. The role of the cytosolic proteasome in P-450 turnover was investigated using lactacystin and ALLN. Reports to date indicate that lactacystin (active metabolite, clasto-lactacystin β-lactone) (18) is a specific inhibitor of the 20 S proteasome (19) and therefore a useful tool to investigate the role of this protease in various metabolic processes (4, 5). ALLN (also known as LLnL) is also a potent inhibitor, although its effects are not entirely confined to the proteasome (19). Because the 20 S proteasome is the catalytic core of the larger 26 S proteasome, it would be expected that these inhibitors will prevent the removal of both ubiquitinated and nonubiquitinated proteins. After 24 h of culture, proteolysis of P-450s from the four major gene families was evaluated and was found to be blocked by lactacystin and ALLN (Fig. 1 and Table I). CYP2E1 and CYP3A were most rapidly removed, with CYP1A2 and CYP4A exhibiting greater stability in cultured cells. Despite these differences in turnover rates, it appears that a feature common to all is their destruction by a mechanism involving the proteasome. Surprisingly, there was no indication of P-450 ubiquitination in these cells (Figs. 1 and 2), contrasting with previous studies in which ubiquitination of P-450s has been shown to occur following exogenous chemical damage (9, 10). Previously, our laboratory and others (9, 10, 14) have observed P-450-ubiquitin conjugates in microsomal preparations as high molecular mass species of immunoreactive bands detectable by Western blotting. How then, are the present observations of ubiquitin-independent P-450 degradation to be reconciled with previous reports? Notably, prior studies on P-450 ubiquitination used highly induced levels of CYP2E1 (10, 14) and CYP3A (9), such that the concentrations were 5–10-fold higher than the control levels employed in this study. Because these isoforms constitute such a large percentage of ER protein in the induced state (2–5%) it is conceivable that these enzymes are not degraded by the proteasome (19). The entrance to the 20 S proteasome is 13 Å in diameter (27) and cannot degrade a native P-450 that is roughly 50 Å across. Therefore the protein must be partially unfolded under its own auspices or molecular chaperones associated with the ER (28). A third possibility is a chaotropic effect exerted by components of the 19 S subunit of the 26 S proteasome; however, this normally requires ubiquitination or antizyme attachment (29). Data presented in Figs. 5 and 6 suggest membranous CYP2E1 and CYP3A are capable of unfolding in the ER to an extent sufficient to allow physical exposure to the 20 S proteasome and subsequent proteolysis. Labeled CYP3A is partially protected from degradation by the addition of the substrate troleandomycin. Although little data is available on the three-dimensional structure of mammalian P-450s, phosphorylation of CYP2E1 and CYP3A1 is blocked upon substrate binding (30, 31) with a concordant shift in enzyme spin-state (32). Based on the data in Fig. 5E, the net result is that ligand-bound P-450s are resistant to proteasome degradation and thereby likely to accumulate when substrates are present (14, 21, 22, 33). With CYP2E1 this scenario is easy to envisage, as CYP2E1 ligands are low molecular mass chemicals and are unlikely to be prevented access to the heme pocket even if changes have occurred in overall protein folding or topology. Whether efficient P-450 catalysis occurs when the enzyme is “induced” in this manner is unclear, and in this context it has been suggested that the coupling of electron transfer to oxygen insertion into the heme-bound substrate is impaired in ethanol-stabilized CYP2E1 (34). Such observations may account for the increase in free radicals and lipid peroxides observed after prolonged ethanol exposure (35). During ethanol withdrawal the ligand is rapidly removed from all tissues, and the protein is subsequently degraded with a half-life of 6 h or less (8). In this respect the 20 S proteasome may be providing a very valuable service by removing a protein that is not only damaged but also hazardous.

The P-450s may be unusually susceptible to destruction by the 20 S proteasome in vivo for several reasons. First, their catalytic mechanism involves oxygen transfer, a pathway by which the potential exists for auto-oxidation (a recurring theme in proteolysis) (36). The P-450 active site is hydrophobic and sequestered from the action of cytosolic antioxidants; thus, contrary to the surface of the protein, the heme and adjacent amino acids are chronically exposed to superoxide radicals and hydrogen peroxide that develop spontaneously from side reactions of normal catalysis (34, 37). Studies using hemoglobin (another hemoprotein) suggest artificial oxidation of this en-
zyme by hydrogen peroxide targets it for destruction by the 20 S proteasome (38). Within this context, it has been shown that individual P-450s do not exhibit the same propensity to “uncouple” their oxygen transfer in reconstituted systems. CYP2B1/2 has been demonstrated to be less susceptible to this side reaction than CYP2E1 (39), thus providing a possible basis for differential proteolytic susceptibility. Because the half-lives of P-450s are in the order of hours rather than minutes, it is anticipated that these changes are of a gradual nature and may therefore be difficult to reproduce in vivo. Clearly, the challenge of identifying the molecular mechanism/s of P-450 ligation in vivo is an important one.

In addition to biochemical factors, the microsomal P-450s are localized in an organelle that is intimately associated with many 20 S proteasomes (6, 7). Current literature suggests the 20 S proteasome association with the ER is related to antigen processing. The 20 S proteasome may function as a pore by which peptides are transferred to transporters associated with antigen processing or as a final pruning system for the selection of appropriate antigenic peptides (7, 40). Given the high constitutive expression and inducibility of many P-450s by environmental chemicals, it is conceivable that shuffling proteasomes around the ER to process damaged P-450 isoforms significantly influences major histocompatibility complex class 1 antigen presentation in the liver. Ethanol withdrawal is an excellent example of a process in which large quantities of proteasome are allocated to the destruction of a highly induced P-450 (CYP2E1). Considering the breadth of membranous proteins starting to emerge as proteasome and/or ubiquitin targets, it will be particularly interesting to study the dynamics of proteasome distribution and antigen processing in the ER.

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