

Subpopulations of proteasomes in rat liver nuclei, microsomes and cytosol

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Mammalian proteasomes are composed of 14–17 different types of subunits, some of which, including major-histocompatibility-complex-encoded subunits LMP2 and LMP7, are non-essential and present in variable amounts. We have investigated the distribution of total proteasomes and some individual subunits in rat liver by quantitative immunoblot analysis of purified subcellular fractions (nuclei, mitochondria, microsomes and cytosol). Proteasomes were mainly found in the cytosol but were also present in the purified nuclear and microsomal fractions. In the nuclei, proteasomes were soluble or loosely attached to the chromatin, since they could be easily extracted by treatment with nucleases or high concentrations of salt. In the microsomes, proteasomes were on the outside of the membranes. Further subfractionation of the microsomes showed that the proteasomes

in this fraction were associated with the smooth endoplasmic reticulum and with the *cis*-Golgi but were practically absent from the rough endoplasmic reticulum. Using monospecific antibodies for some proteasomal subunits (C8, C9, LMP2 and Z), the composition of proteasomes in nuclei, microsomes and cytosol was investigated. Although there appear not to be differences in proteasome composition in the α subunits (C8 and C9) in the different locations, the relative amounts of some β subunits varied. Subunit Z was enriched in nuclear proteasomes but low in microsome-associated proteasomes, whereas LMP2, which was relatively low in nuclei, showed a small enrichment in the microsomes. These differences in subunit composition of proteasomes probably reflect differences in the function of proteasomes in distinct cell compartments.

INTRODUCTION

Proteasomes (multicatalytic proteinase complexes) are high-molecular-mass multisubunit complexes that form the major non-lysosomal degradative machinery of eukaryotic cells [1,2]. From the results of cell fractionation, immunohistochemical, immunofluorescence and immunogold electron microscopic studies (see [3,4] for references), it is clear that proteasomes can be found in both the nucleus and the cytoplasm of animal cells. Also, changes in their nuclear and cytoplasmic distribution have been reported during development, following cell transformation and at different stages of the cell cycle [5–9], and a mechanism for their translocation across the nuclear membrane must exist. In addition, it seems possible that there may be differences in proteasomes localized in the nucleus and the cytoplasm. Preliminary studies with proteasomes isolated from nuclei and cytoplasm showed no obvious differences [10], but variations in proteasome structures have since become apparent.

The proteasome particle has four stacked rings, each comprising seven subunits, and the rings at both ends are formed by α subunits and the two central rings by β subunits. Yeast proteasomes are made up of two of each of 14 different subunits [11]. However, in animal cells, in addition to the 14 subunits that are homologues of the yeast proteasome subunits, genes for three other non-essential β proteasome subunits have been found. Two of these subunits, LMP2 and LMP7, are γ -interferon inducible and are encoded within the major histocompatibility complex (MHC) class II region. LMP2 and LMP7 are closely related to subunits δ and MB1 respectively, and there is now evidence from the work of several different groups (e.g. [12–14]) to show that the MHC-encoded subunits can replace δ and MB1 in some proteasomes and also modulate the proteolytic activities

(reviewed in [15]). There is some evidence to suggest that LMP2 and LMP7 may enhance the efficiency of presentation of viral (and other) antigens by the MHC class I pathway [16–18]. Another variable subunit, MECL1, is also induced by γ -interferon [19] and is closely related to subunit Z [20]. The presence of LMP2, LMP7 and MECL1 subunits in many cells and tissues must give rise to subpopulations of proteasomes. However, it is not clear whether the proteasomes found in different subcellular localizations are identical or whether their characteristics vary to accomplish the different functions. We have demonstrated previously, using immunogold procedures, that proteasomes are present in the nucleus and in the cytoplasm, and that some proteasomes appear to be closely associated with the endoplasmic reticulum [21]. In this study we have investigated further aspects of the localization of proteasomes in the nucleus and associated with the endoplasmic reticulum, and also demonstrated, by immunoblot analysis of well-characterized subcellular fractions isolated from rat liver, some differences in the subunit composition of proteasomes in different locations.

MATERIALS AND METHODS

Materials

The materials and their sources were as described previously [9,21,22] with the following additions: trypsin (bovine pancreas), soybean trypsin inhibitor, 3,3'-diaminobenzidine, 4',6'-diamidino-2-phenylindol dihydrochloride, Butvar B-98, poly-L-lysine hydrobromide (70 000–150 000 Da), DNase I and RNase A (Sigma, St. Louis, MO, U.S.A.); dithiothreitol (Boehringer, Mannheim, Germany); enhanced chemiluminescence Western blotting kit (Amersham, Amersham, Bucks, U.K.); Paraplast (Manoject Scientific, Kildare, Ireland); Vectastain kit (Vector

Abbreviations used: MHC, major histocompatibility complex; DNP, dinitrophenol.

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Labs., Burlingame, CA, U.S.A.); Bacto Fa-mounting fluid (Difco, Detroit, MI, U.S.A.); hydrogen peroxide (Merck, Darmstadt, Germany); xylene (Panreac, Montcada i Reixac, Spain); sodium pentobarbital (Abbot Labs., Madrid, Spain). All other reagents were of the best analytical quality available.

Proteasomes and antibodies

Proteasomes were prepared from rat liver, and anti-proteasome antibodies (anti-DNP-MCP) were prepared in rabbits, using dinitrophenol (DNP)-modified proteasomes as described previously [23]. Purified IgGs were obtained using Protein A-agarose. The specificity of the antibodies in rat liver was checked as described previously [21,23]. Control IgG preparations for immunolabelling experiments were obtained by removing anti-proteasome IgG with purified rat liver proteasomes bound to cyanogen bromide-activated Sepharose 4B [9] or by preparing IgG fractions from preimmune serum. Proteasome-subunit-specific monoclonal antibodies (MCP72 for C8, MCP257 for C9 and MCP165 for Z) were those raised against human proteasomes [20]. Antibody to MB1 was produced in rabbits immunized with the peptide DNVADLHEKYSGSTC, coupled to a purified protein derivative of tuberculin as described previously [24]. Anti-LMP2 antibodies were raised in a mouse and a rabbit by immunization with rat LMP2 protein that was produced in *Escherichia coli* using the Qiaexpress system (QIAGEN, Chatsworth, CA, U.S.A.), and anti-LMP7 antibodies were raised against the recombinant protein in a rabbit. Rat LMP2 and LMP7 cDNAs were derived from a DA rat lymphoblast library (E. Deverson, I. Milisav, J. Coadwell, J. C. Howard and G. W. Butcher, unpublished work). Anti-(carbamoyl phosphate synthetase) monoclonal antibodies were generously provided by Dr. J. Cervera (Instituto de Investigaciones Citológicas, Valencia, Spain). Polyclonal antibodies against recombinant subunits were affinity purified against the purified protein.

Isolation of rat liver fractions

Fed or 20-h-fasted male Wistar rats (Interfauna Ibérica, Sant Feliu de Codines, Spain) weighing 200–250 g were used throughout. Purified nuclear fractions were obtained as described previously [25]. Microsomal and cytosolic fractions were the sediment and supernatant, respectively, from the last of three successive centrifugations at 4 °C (600 g, 10 min; 17000 g, 10 min; and 150000 g, 60 min). Microsomes were washed once by resuspending the sediment in 10 vol. of 0.25 M sucrose. Mitochondria were prepared as described previously [26].

All further washings (up to five) of nuclear and microsomal pellets were carried out by resuspending them in 10 vol. of 0.25 M sucrose. For the trypsin treatment of microsomes, 100 µg of microsomes were incubated for 1 h at 37 °C with increasing amounts of trypsin as indicated in the corresponding Figure, followed by the addition of twice as much soybean trypsin inhibitor. After the incubation, the samples were boiled for 3 min in SDS/PAGE sample buffer containing 2 mM PMSF.

A nuclear-matrix fraction was obtained from the purified nuclei following two different procedures [27,28]. To separate plasma membrane, rough and smooth endoplasmic reticulum and Golgi complex, the method described by Fleischer and Kervina [29] was followed. In addition, rough and smooth endoplasmic reticulum were prepared by the method described by Graham [30] and Golgi complexes and their *cis*- and *trans*-Golgi fractions were purified by the method of Bretz et al. [31]. The purity of the fractions was judged by standard enzymic measurements and/or electron microscopy as reported previously [22,32].

Immunoblotting

Rat liver fractions and proteasomes were subjected to SDS/PAGE (15% acrylamide) in slab gels [33] followed by electrophoretic blotting to nitrocellulose membranes using either a blotting tank or a semi-dry transfer cell (Bio-Rad, Richmond, CA, U.S.A.). The blots were stained for 2 min with Ponceau S solution [0.2% (w/v) in 3% (w/v) trichloroacetic acid], destained with PBS and reacted for 16 h at 4 °C with the various anti-proteasome antibodies and then for 1 h at 20 °C with goat anti-rabbit or anti-mouse IgG-alkaline phosphatase conjugates. The enzyme was suitably developed with 5-bromo-4-chloro-3-indolyl phosphate and Nitro Blue Tetrazolium by standard procedures. Densitometric analysis of the Western blots was carried out in a 2202 Ultrascan laser densitometer from Pharmacia LKB (Uppsala, Sweden) with a Hewlett Packard (Palo Alto, CA, U.S.A.) 3396 Series II integrator. The linearity of the method was established using proteasomes purified from rat liver as a standard in the blotting assay.

Electron microscopy, immunocytological and immunohistological procedures

Rat liver portions (approximately 1 mm³) and cellular fractions were fixed and embedded, either in Vestopal W (for conventional electron microscopy) or in Lowicryl K4M (for immunogold procedures), by standard procedures [21,32]. The immunogold procedures and the calculation of labelling density (number of gold particles/µm² of cell compartment area) were carried out as described previously [21].

For the immunoperoxidase detection of proteasomes, rats were anaesthetized with sodium pentobarbital (50 mg/kg of body weight) and perfused transcardially, via a peristaltic pump set to deliver 12 ml/min. Brief perfusion of cold saline to rinse the vascular tree was followed by 0.5 ml/g of body weight of cold 30% sucrose in PBS. The liver was fixed by immersion in 4% paraformaldehyde/0.1 M phosphate buffer, pH 7.4, for 4 h at 4 °C, washed overnight in PBS and embedded in paraffin (Paraplast) by standard procedures. Sections (7 µm thick) were mounted on microscope slides coated with poly-L-lysine, deparaffined, and bathed for 1 h at room temperature in 1% H₂O₂ in 50 mM Tris/HCl (pH 7.6)/0.9% NaCl to inhibit endogenous peroxidase activity. After an overnight incubation in the various antibodies, the sections were stained using the Vectastain kit, based on the avidin-biotin-peroxidase complex method and following the manufacturer's instructions. The resulting label was detected by using 3,3'-diaminobenzidine as a chromogen.

For immunofluorescence, isolated nuclei from rat liver were dried on Butvar B-98-coated slides and fixed for 5 min with absolute methanol at -20 °C. Incubation with anti-DNP-MCP was for 12 h and, after washing with PBS containing 500 mM NaCl and 0.5% Tween 20, the slides were incubated with goat anti-rabbit IgG-fluorescein isothiocyanate-conjugated F(ab')₂ fragment for 1 h at room temperature. Slides were washed with PBS containing 500 mM NaCl and 0.5% Tween 20 and mounted in Bacto Fa-mounting fluid, pH 7.2. In all the preparations DNA was stained with 1 µg/ml 4',6-diamidino-2-phenylindol dihydrochloride in PBS.

RESULTS

Immunoblot localization of proteasomes in rat liver fractions with polyclonal anti-proteasome antibodies raised against the whole 20 S proteasome

When four different rat liver fractions (nuclei, mitochondria, microsomes and cytosol) were tested by Western-blot analysis

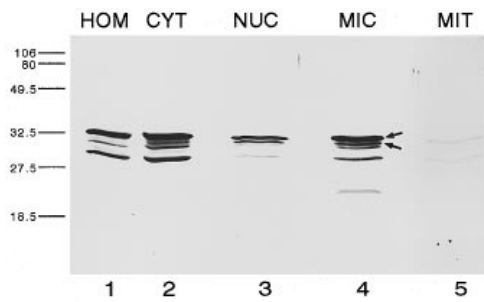


Figure 1 Immunolocalization of proteasomes in rat liver fractions

Proteins from the various fractions (25 μ g for homogenate and cytosol and 100 μ g for nuclei, microsomes and mitochondria) were separated by SDS/PAGE and immunoblotted with polyclonal anti-DNP-MCP antibodies. The two bands used for the densitometric analysis (the same for all fractions) are marked by arrows in lane 4. HOM, homogenate; CYT, cytosol; NUC, nuclei; MIC, microsomes; and MIT, mitochondria. The positions of molecular-mass markers and their size in kDa are indicated on the left.

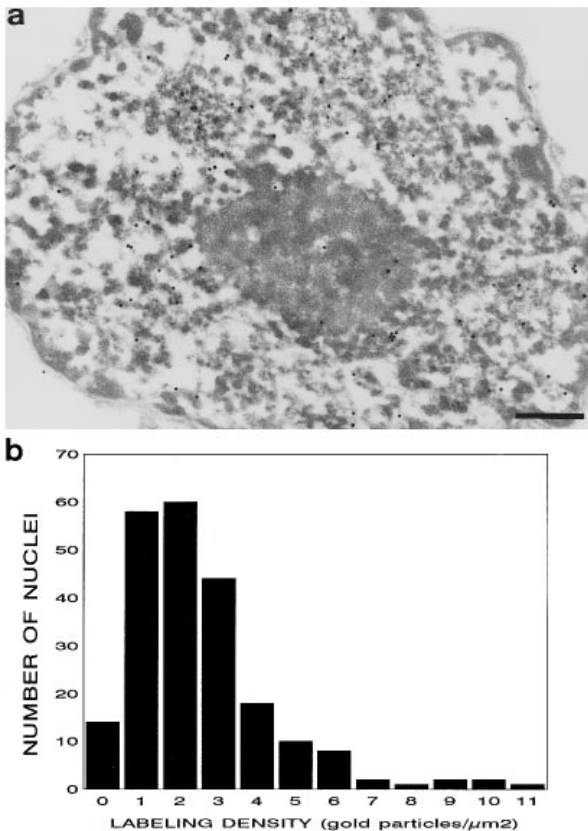


Figure 2 Localization of proteasomes in isolated nuclei

(a) Electron microscopic immunogold localization of proteasomes using polyclonal anti-DNP-MCP antibodies. The distribution of proteasomes shown in this representative nucleus was similar to that found in the nuclei from intact rat liver tissue. The labelling densities in the experiments with these preparations were 2.7 (anti-DNP-MCP labelling) and 0.7 (control labelling) gold particles/ μ m². The bar is 0.5 μ m long. (b) Frequency histogram of immunogold labelling densities, using anti-DNP-MCP antibodies, in individual nuclei from several isolation experiments.

with a polyclonal anti-(20 S proteasome) antibody (anti-DNP-MCP), proteasomes were found clearly associated with cytosol, nuclei and microsomes (Figure 1). By being careful to make them

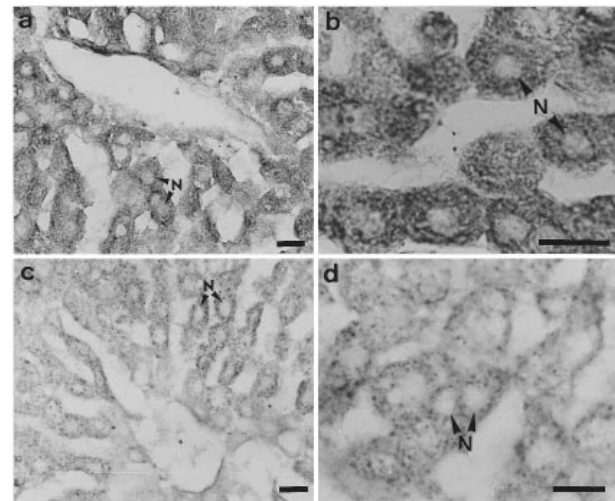


Figure 3 Localization by immunoperoxidase of proteasomes in rat liver

Immunoperoxidase with rat liver using anti-DNP-MCP antibodies (a,b) or, as a control of the procedure, an antibody against the mitochondrial enzyme carbamoyl phosphate synthetase (c,d). No immunoreaction was observed in incubations carried out with control IgG preparations (see the Materials and methods section). Some nuclei (N) are indicated by arrows. The bars represent 50 μ m.

quantitative, densitometric analyses of the Western blots from four independent cell-fractionation experiments showed that, for the same amount of bulk protein in each fraction, the level of proteasomes in the cytosol is 10-fold higher than in the nuclei and 6–7-fold higher than in the microsomal fraction. The results were essentially similar with fed and starved rats, except that in the latter a small quantity of proteasomes was sometimes found associated with the mitochondrial fraction. This immunoreactivity may be due to the proteasomes that, under starvation conditions, are incorporated for degradation into the lysosomes [22]. Similar qualitative and quantitative results were obtained using a second polyclonal anti-(20 S proteasome) serum. In some experiments, in addition to the proteasomal bands, a lower-molecular-mass band was sometimes evident in the microsomal fraction. Since this band only appeared in some preparations its nature was not further investigated.

We investigated whether the low quantity of proteasomes found in the nuclei could be due to their release during the homogenization, subfractionation and/or extensive washing procedures. This is unlikely, since we calculated, by immunogold quantification of the proteasome labelling in the nuclei from the various purification steps, that the losses represent, at most, 20–25% of the proteasomes originally present in the nuclei from rat liver. The immunogold localization of the proteasomes in the isolated nuclei was similar to that already reported in the rat hepatocytes [21]: i.e. inside and mostly at the periphery of heterochromatin and of nucleolar areas (Figure 2a). The distribution of individual nuclei according to their number of gold particles/ μ m² corresponds to a normal distribution (Figure 2b). This indicates that different nuclei contain a similar quantity of proteasomes.

Immunofluorescence studies of the isolated nuclei (results not shown) and immunoperoxidase staining of intact liver (Figure 3) with anti-DNP-MCP confirm the presence of proteasomes in the liver nuclei, although in much lower amounts than in the cytosol. Further washings (up to five) of the purified nuclear fraction did not decrease the quantity of proteasomes associated with these

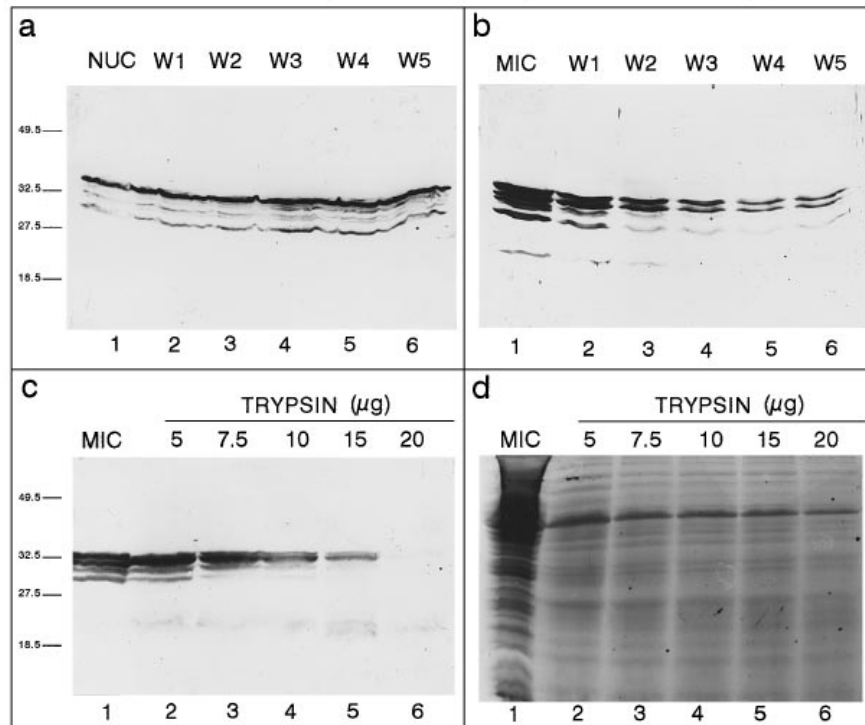


Figure 4 Association of proteasomes with the nuclei (NUC) and with the microsomes (MIC)

(a,b) Effect of five different sequential washes (W1–W5) on the association of proteasomes with the purified nuclei and microsomes respectively. Proteins (100 μ g) from the various fractions were separated by SDS/PAGE and immunoblotted with anti-DNP-MCP antibodies. (c,d) Effect of various trypsin treatments on the association of proteasomes with microsomes. Microsomes (100 μ g) were incubated for 1 h at 37 °C with the indicated amounts of trypsin followed by the addition of twice as much soybean trypsin inhibitor. After incubation, the samples were boiled for 3 min in SDS/PAGE sample buffer containing 2 mM PMSF, subjected to SDS/PAGE and either immunoblotted with anti-DNP-MCP antibodies (c) or stained with Coomassie Brilliant Blue R-250 (d). The positions of molecular-mass markers and their size in kDa are indicated on the left.

fractions, as shown by immunogold procedures (results not shown) and immunoblot analysis (Figure 4a). In contrast, the proteasomes associated with the microsomal fraction were progressively washed out (Figure 4b). However, on the basis of the washing volume used, the observed decrease in the quantity of proteasomes associated with the microsomes with progressive washing is much lower than expected if this association were merely due to proteasomal contamination of these fractions. Also, the microsome-associated proteasomes were progressively eliminated by a trypsin treatment (Figure 4c), which does not greatly affect the microsomal pattern of bands in SDS/PAGE (Figure 4d). We conclude, therefore, that the proteasomes associated with the microsomal fraction are on the outside of these preparations, probably loosely attached to the microsomal membrane.

Further insights into the nuclear and endoplasmic reticulum localization of the proteasomes

To gain more insights into the nuclear and endoplasmic reticulum localization of the proteasomes we further subfractionated these two preparations. Figure 5 shows the electron microscopic appearance of some of the fractions used in our experiments to illustrate their purity.

The nucleus of eukaryotic cells contains a nuclear matrix, which is operationally defined as the non-chromatin nuclear proteins that remain after treatment with nucleases, chaotropic agents and high concentrations of salt. Figures 6(a) and 6(b) show immunoblots of various nuclear subfractions obtained by

the successive treatment of the isolated nuclei by two different procedures with nucleases, elevated salt concentrations and non-ionic detergents. In both cases, most of the nuclear-associated proteasomes are removed by the above-mentioned treatments (which extract the chromatin and the soluble and associated non-histone proteins and histones). The microsomal fraction mainly contains rough and smooth endoplasmic reticulum, components of the Golgi complex and plasma membrane. No proteasomes were found associated with purified fractions enriched in plasma membrane (results not shown) or rough endoplasmic reticulum (Figures 6c and 6d). In contrast, we found, using monoclonal and polyclonal antibodies, that most of the proteasome labelling was associated with the microsomal fraction enriched in smooth endoplasmic reticulum (Figures 6c and 6d) and in Golgi components. When the Golgi complex was further fractionated, it was found that the labelling was mainly associated with the fraction enriched in the *cis* part of the Golgi complex (Figures 6e and 6f). Immunogold analysis of the isolated fractions confirmed that the labelling associated with microsomes is found in the fractions devoid of rough endoplasmic reticulum (results not shown).

Analysis of variable subunit composition of proteasomes in rat liver nuclei, microsomes and cytosol

Using specific antibodies for two α subunits (C8 and C9), and comparing the results obtained with the polyclonal antibodies raised against the whole 20 S proteasome (anti-DNP-MCP), we were unable to find differences in the distribution of the labelling

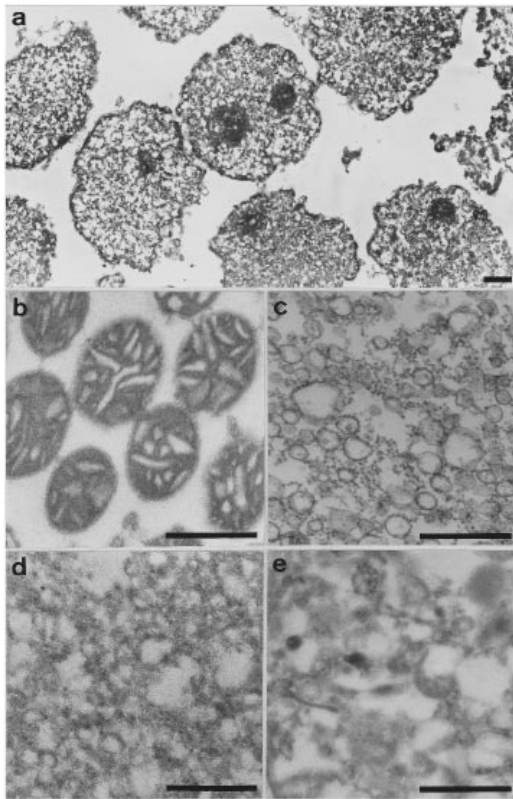


Figure 5 Electron microscopic morphological appearance of various rat liver subcellular fractions used in these studies

Nuclei (a); mitochondria (b); rough endoplasmic reticulum (c); smooth endoplasmic reticulum (d); Golgi complex (e). Bars: 1 μm (a) and 0.5 μm (b–e).

between the nuclear, microsomal and cytosolic preparations (see, e.g., Figure 1, Figures 7a and 7b and Table 1). However, the distribution of the β subunits LMP2 and, especially, Z (compare Figures 7c and 7d and Table 1) was different: LMP2 was slightly enriched in the microsomes and at relatively lower amounts than the α subunits in the nucleus whereas Z was almost absent from microsomes and enriched 3–4-fold in the nuclei when compared with the α subunits. Although we also found that two β subunits (LMP7 and MB1) were apparently either present at very low amounts or absent from the microsomes and nuclei (results not shown), the expected intensity of the bands in the nuclei and microsomes, on the basis of the results with the homogenate and cytosol, was outside the linearity of the blotting assay, as estimated using purified rat liver proteasomes as a standard. Attempts to increase the sensitivity of the assay (either using higher amounts of protein of the fractions or the enhanced chemiluminescence assay) were unsuccessful. Therefore, we can only conclude that in rat liver the proteasomes localized in different cell compartments appear to differ in their content in, at least, two β subunits (LMP2 and especially Z).

DISCUSSION

The results reported here provide further evidence for the presence of proteasomes in the nucleus, in the cytoplasm and associated with the endoplasmic reticulum (microsomes). Sub-fractionation of the nuclei showed that most of the proteasomes

are easily detached from the nucleus by treatment with high salt or nucleases, showing that in rat liver most nuclear proteasomes are not associated with the nuclear matrix. In contrast, it has been recently reported [34] that proteasomes are associated with the nuclear scaffold of a simian virus 40-immortalized, Ras-transformed rat hepatocyte cell line. The proteins of the nuclear scaffold change with the differentiation stage of the cell and are believed to play a fundamental role in growth-related functions of the cell nucleus, including regulation of gene expression. The apparent difference between our results may therefore be explained by the fact that the transformed cells are actively dividing.

The localization of proteasomes to the cytoplasmic surface of the microsomes (which form outside-out from the endoplasmic reticulum and associated organelles) and not to the lumen of these structures is relevant to the poorly understood process of protein degradation in the endoplasmic reticulum [35]. Several recent reports have implicated proteasomes in the degradation of endoplasmic reticulum proteins [36,37], although proteasomes are clearly not involved in all the protein degradation associated with this compartment [38]. It appears from the localization of proteasomes outside the lumen of the endoplasmic reticulum that, if proteasomes participate in this degradation, either the proteins should be transported outside the endoplasmic reticulum, or degradation should start with the cytosol-exposed part of these proteins. Interestingly one of the ubiquitin-conjugating enzymes is located in the membrane of the endoplasmic reticulum with its catalytic domain facing the cytosol [39].

The association of proteasomes with the smooth endoplasmic reticulum and *cis*-Golgi fractions reported here is also consistent with their possible role in the degradation of cytoplasmic proteins for antigen presentation. Since in hepatocytes the Golgi complex is scarce (less than 1% of the cell volume; see, e.g., [21]) the association of proteasomes may be due to co-purification of the smooth endoplasmic reticulum physically associated with the *cis*-Golgi or transitional endoplasmic reticulum (a series of smooth membrane limited cisternae and vesicles from the endoplasmic reticulum, some of which may also form part of the *cis*-Golgi network). In fact, another component of the antigen-presentation machinery (TAP1/TAP2 heterodimer, a transporter molecule associated with antigen processing that is responsible for the ATP-dependent transmembrane translocation of selected peptides from the cytosol to the site of MHC class I assembly) [40] have been also found, by immunogold electron microscopy of cryosections, to be located at the endoplasmic reticulum and *cis*-Golgi [41].

As might be expected from current knowledge about the structure of animal cell proteasomes [1,2], our results suggest that there are not significant differences in the composition of α -type subunits in proteasomes from different locations. However, the two β subunits for which we had suitable antibodies showed differences in their relative distribution. Observations that the β -subunit composition of proteasomes affects their activities (reviewed in [15]) must have implications for their functions. LMP2 is only present in low levels in liver proteasomes [42], but our finding of a slight enrichment of this MHC-encoded subunit in microsome-associated proteasomes (Figure 7) suggests that LMP2-containing proteasomes that are involved in antigen processing [17,18] can be localized at the surface of the smooth endoplasmic reticulum, where TAP transporters can translocate peptides across the membrane for association with MHC class I molecules [43]. However, LMP2 is also found in nuclear and cytoplasmic proteasomes in thymus [44] as well as in liver.

The relative enrichment of the variable β subunit Z compared with proteasome α subunits in the nuclei is very striking. The

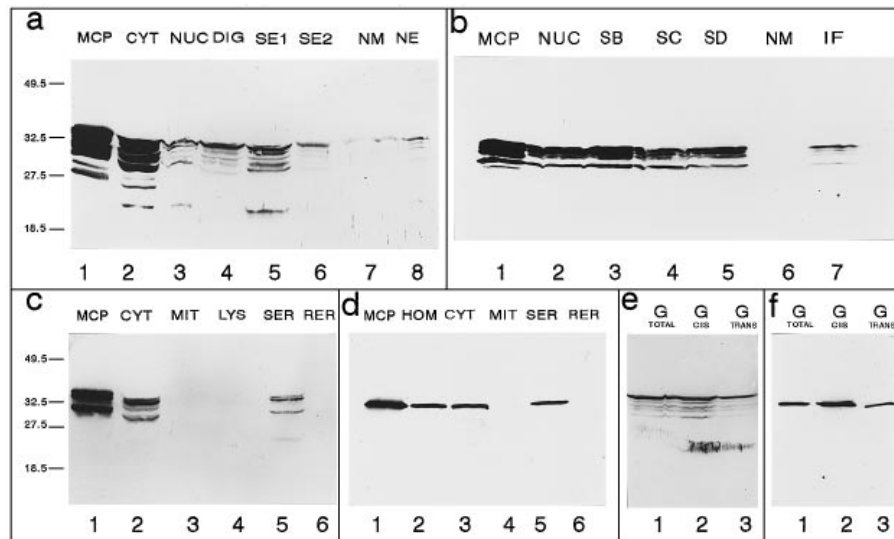


Figure 6 Immunolocalization of proteasomes in several subfractions prepared from rat liver nuclei and microsomes

Proteins (50 μ g) from the various fractions were separated by SDS/PAGE and immunoblotted with polyclonal anti-DNP-MCP antibodies (**a,b,c,e**) or with a monoclonal antibody, MCP257, for an α -type proteasomal subunit (**d,f**). (**a**) Lane 1, proteasomes (1 μ g; MCP); lane 2, cytosol (CYT); lane 3, nuclear fraction (NUC); lanes 4–6, proteins released from nuclei by nucleases (DIG) or high salt (SE1, SE2) treatments; lane 7, nuclear matrix (NM); lane 8, nuclear envelope (NE). (**b**) Lane 1, proteasomes (1 μ g; MCP); lane 2, nuclear fraction (NUC); lanes 3–5, proteins released from the nuclei by Triton X-100 (SB), high salt (SC) or nuclease (SD) treatments; lane 6, nuclear matrix (NM); lane 7, intermediate filaments (IF). (**c**) Lane 1, proteasomes (1 μ g; MCP); lane 2, cytosol (CYT); lane 3, mitochondria (MIT); lane 4, lysosomes (LYS); lane 5, smooth endoplasmic reticulum (SER); lane 6, rough endoplasmic reticulum (RER). (**d**) As (**c**) except with antibody MCP257 and including homogenate (HOM) instead of lysosomes in lane 2. (**e,f**) Fractions enriched for Golgi (G_{TOTAL}); *cis*-Golgi (G_{CIS}); or *trans*-Golgi (G_{TRANS}). The positions of molecular-mass markers and their size in kDa are indicated on the left.

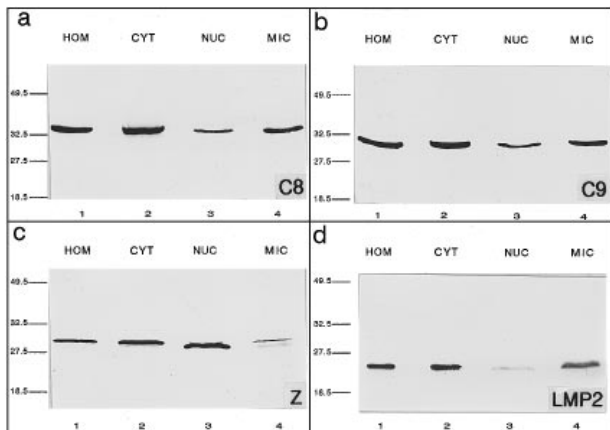


Figure 7 Immunolocalization of α and β proteasomal subunits in rat liver fractions

Proteins from the various fractions (25 μ g for homogenate and cytosol and 100 μ g for nuclei and microsomes) were separated by SDS/PAGE and immunoblotted with two different monoclonal antibodies for α subunits [MCP72 for C8 (**a**) and MCP257 for C9 (**b**)] or with antibodies for β subunits [mouse polyclonal for LMP2 (**c**) and MCP165 for Z (**d**)]. Abbreviations: HOM, homogenate; CYT, cytosol; NUC, nuclei; and MIC, microsomes. The positions of molecular-mass markers and their size in kDa are indicated on the left.

amount of Z in nuclei is 13-fold that found in the microsomes, whereas the amount of LMP2 in the nuclei is only about one-sixth of that found associated with the microsomes. These results demonstrate that different subpopulations of proteasomes do indeed have different subcellular locations. Although it would

Table 1 Subcellular distribution of the proteasomes and some of its various subunits

Immunoblots, derived from the number of independent cell fractionation experiments indicated in parentheses after the antibody employed, were prepared as described in the Materials and methods section. They were suitably developed and densitometrically analysed. The data were calculated for the same amount of bulk protein in each fraction and are presented as a percentage \pm S.D. of the total absorbance (sum of absorbances of the cytosol, nuclear and microsomal fractions) in one fraction. *, **, *** Differences from anti-DNP-MCP significant at $P < 0.05$, 0.025 and 0.0005 respectively.

Antibody	Subunit	Cytosol	Nuclei	Microsomes
Anti-DNP-MCP (4)	Several	83.2 \pm 3.4	5.4 \pm 1.6	11.3 \pm 2.0
MCP72 (8)	C8	83.1 \pm 3.0	5.1 \pm 2.1	11.8 \pm 1.4
MCP257 (4)	C9	83.9 \pm 2.8	5.3 \pm 1.6	10.7 \pm 1.2
Anti-LMP2 (5)	LMP2	83.8 \pm 3.5	2.2 \pm 1.8**	14.0 \pm 1.9*
MCP165 (6)	Z	80.4 \pm 5.3	18.2 \pm 4.0***	1.4 \pm 0.9***

clearly be of interest to investigate the localization of the other variable β subunits, we do not at present have suitable antibodies. More detailed studies would be required to determine possible variations in subunit composition of proteasomes in different cells, in the various locations and in different situations.

We thank Dr. Fernando Martínez-García and Cristian Font from University of Valencia for their help with immunohistochemical studies and A. Montaner for technical assistance. The work was supported in part by Dirección General de Investigación Científica y Técnica del Ministerio de Educación y Ciencia (PB94-1281), Fondo de Investigación Sanitaria de la Seguridad Social (93/0408) and by grants from the Ministerio de Educación y Ciencia (SAB94-0030) and the Biotechnology and Biological Sciences Research Council. A. P. is a fellow from Ministerio de Educación y Ciencia, and A. J. R. is a Lister Institute–Jenner Research Fellow.

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