REVIEW ARTICLE

Proteasomes: multicatalytic proteinase complexes

A. Jennifer RIVETT

Department of Biochemistry, University of Leicester, Leicester LE1 7RH, U.K.

INTRODUCTION

The multicatalytic proteinase complex (proteasome) is a high-molecular-mass (approximately 700 kDa) intracellular proteinase which has been isolated under a variety of different names from a wide variety of eukaryotic cells and tissues (reviewed, Rivett, 1989a; Orlowski, 1990). The proteinase complex is composed of at least 24 subunits which include many different polypeptides arranged in a cylindrical structure. Other multi-subunit complexes with cylindrical structures include 'prosome', that are widely distributed 19 S ribonucleoprotein particles which were thought to be involved in the control of translation (Schmid et al., 1984; Martins de Sa et al., 1986), erythrocyte cylindrin (Harris, 1988), and a number of other partially characterized 16–22 S cylindrical particles. 'Prosome' and certain related particles have been shown to have proteolytic activity and to share antigenic cross-reactivity with the multicatalytic proteinase (Falkenburg et al., 1988) and are therefore believed to be the same. The particles are most often referred to either as multicatalytic proteinase complexes (Dahlmann et al., 1988; Orlowski and Wilk, 1988) or, more recently, as proteasomes (Arrigo et al., 1988) and their properties are broadly similar irrespective of the source (Martins de Sa et al., 1986; Tanaka et al., 1988b; Rivett, 1989a; Orlowski, 1990). A related proteinase with a simpler subunit composition has been isolated from the archaebacterium *Thermoplasma acidophilum* (Dahlmann et al., 1989).

The abundance (up to 1% of the soluble protein in cell extracts; e.g. Tanaka et al., 1986; Hendil, 1988) and ubiquitous distribution of proteasomes in eukaryotic cells, as well as observations that some proteasome subunit genes in yeast are essential for cell growth and viability, suggest that they have important functions within the cell. There is evidence to suggest that they play a major role in nonlysosomal pathways of intracellular protein turnover and they have recently been implicated in antigen processing. Proteasomes are not closely related to any other known proteases and it is likely that they represent a novel family of proteolytic enzymes.

POLYPEPTIDE AND RNA COMPONENTS OF PROTEASOMES

Analysis of the subunit composition of highly purified proteasomes by two-dimensional polyacrylamide gel electrophoresis reveals a characteristic pattern of polypeptide components of varying molecular masses (usually in the range of 22–35 kDa) and pI values in the range of pH 4 to 10 (Figure 1). Some changes in subunit pattern have been observed during development in *Drosophila melanogaster* (Haass and Kloetzel, 1989) and chicken (Ahn et al., 1991). The different polypeptides are not always present in equal amounts and the number appears to vary depending upon the species. For example, yeast proteasomes have 14 different types of subunits (Heinemeyer et al., 1991), plant proteasomes 12 to 15 (Schliephacke et al., 1991), and other eukaryotic complexes have been reported to contain up to 25 distinct polypeptides (e.g. Martins de Sa et al., 1986; Rivett and Sweeney, 1991). The proteasome isolated from thermoacidophilic archaebacteria, on the other hand, is a much simpler molecule, being composed of only two different types of

subunit (α , 27 kDa; β , 25 kDa; Dahlmann et al., 1989) and has therefore been particularly useful for structural studies. Although a few of the spots observed on two-dimensional polyacrylamide gels of eukaryotic proteasomes may be related to each other by **proteolysis** (Lee et al., 1990; Rivett and Sweeney, 1991; Weitman and Etlinger, 1992), **phosphorylation** (Haass and Kloetzel, 1989) or **glycosylation** (Tomek et al., 1988; Schliephacke et al., 1991), many are antigenically distinct (Rivett and Sweeney, 1991; Kaltoft et al., 1992). However, it is not yet possible to put a precise value to the number of proteasomal subunits which are the products of different, but related (see below), genes. Even the archaebacterial proteasome can give rise to a more complex pattern on two-dimensional PAGE gels than the two spots expected (Zwickl et al., 1992). Proteolysis of some subunits of eukaryotic proteasomes may occur during purification, storage or activation of the enzyme (Lee et al., 1990; Rivett and Sweeney, 1991; Weitman and Etlinger, 1992) and some specific processing events may also occur *in vivo*. For example, a 41 kDa cross-reacting protein has been detected by using a monoclonal antibody raised against the 32 kDa subunit of human erythrocyte proteasomes (Weitman and Etlinger, 1992), a 38 kDa protein cross-reacts with a monoclonal antibody for a 27 kDa subunit of "prosome" (Kreutzer-Schmid and Schmid, 1990), and the archaebacterial β subunit is known to be post-translationally processed (Zwickl et al., 1992).

There are discrepancies in the literature with regard to the presence and amount of RNA associated with proteasomes. Recent studies have confirmed the presence of one or more small RNA species (approximately 80 nucleotides) in highly purified multicatalytic proteinase and prosome preparations, but the RNA is not present in stoichiometric amounts (Skilton et al., 1990; Coux et al., 1992). Since the RNA is resistant to nuclease attack in the intact particles, the latter observation suggests the possibility of a subpopulation of particles containing RNA. The

Figure 1 Two-dimensional PAGE of proteasomes

Schematic diagram showing the characteristic two-dimensional PAGE pattern of proteasomes purified from rat liver. Closed spots represent subunits which are usually present. Open spots are variable in different preparations and/or present in lower amounts than the dark polypeptides. Small differences are observed in the ratios of the different subunits in different proteinase preparations and differences are apparent between the particles isolated from different eukaryotic sources. All show a complex subunit composition with some major and some minor components. The precise stoichiometry of eukaryotic proteasomes is not yet clear.

Table 1 Size and shape of proteasomes

Particle Shape/subunit arrangement	Diameter (nm)	Height (nm)	Symmetry	References
Rat muscle MCP	Hollow cylinder Stack of four hexagonal rings	11	16	6 Kopp et al., 1986
Rat liver MCP	Reel-shaped Prolate ellipsoid	16	11	Not true 6-fold Baumeister et al., 1988 Tanaka et al., 1988a
	Cylindrical pseudo-helical arrangement	11	17	8 Arrigo et al., 1988 6–7 Djaballah et al., 1993
Duck erythroblast prosomes	Hollow cylinder	12	17	Coux et al., 1992
Related particles	Cylindrical	10–13	16–18	Reviewed in Harris, 1988
Archaeobacterial proteasome	Cylindrical barrel	11	15	7 Dahlmann et al., 1989; Hegerl et al., 1991 7 Pühler et al., 1992

Figure 2 Model of the proteasome

The model shows the cylindrical structure which appears side-on as a stack of four rings. There are six or seven subunits around each ring. The arrangement of subunits within the mammalian complex is pseudo-helical (Djaballah et al., 1993). Based on analogy with the archaeobacterial enzyme (Grziwa et al., 1991), A-type subunits (red) may be located in the outer rings while B-type subunits probably form the inner rings.

number of RNA species appears to depend on the source of the particles and a major proteasomal RNA from HeLa cells and from duck erythroblasts has recently been identified as tRNA^{Lys3} (Nothwang et al., 1992b). The functional significance of these observations remains to be established.

CYLINDRICAL STRUCTURE

The subunits of proteasomes are arranged in a stack of four rings to form a hollow cylindrical structure. Recent detailed structural investigations of the archaeobacterial proteasome by image analy-

sis of negatively stained particles (Dahlmann et al., 1989; Hegerl et al., 1991) and of small three-dimensional crystals (Pühler et al., 1992) show clear 7-fold symmetry similar to that of molecular chaperones of the GroEL family (Zwickl et al., 1990). Each ring contains seven subunits and the stoichiometry of the molecule is $\alpha_{14}\beta_{14}$. An immunoelectronmicroscopic investigation has demonstrated that the α subunits form the outer rings while the β subunits form the inner ones (Grziwa et al., 1991).

There are variations in the literature with respect to the size and shape of eukaryotic proteasomes (Table 1) but these now appear to be resolved. Electron microscopy of negatively stained rat muscle and liver proteasome preparations suggest a cylindrical structure (Baumeister et al., 1988; Djaballah et al., 1993). The particle viewed end-on appears to be ring-shaped and side-on a rectangular shape with four bands, suggesting a stack of four rings. The molecule has two-fold rotational symmetry. The rings do not show the true 6-fold symmetry nor the direct stacking of the model previously proposed for the rat muscle enzyme (Kopp et al., 1986). The arrangement of subunits in the rat liver enzyme appears to be pseudo-helical (Djaballah et al., 1993; Figure 2). This model resembles that for the archaeobacterial enzyme (Pühler et al., 1992), but differs somewhat from the prolate ellipsoid structure suggested earlier for the rat liver proteinase based on X-ray scattering data (Tanaka et al., 1988a). However, it has already been pointed out that the structure of the archaeobacterial enzyme is consistent with the X-ray scattering data of Tanaka et al., (1988a) and that the differences between models lies largely in the interpretation of data (Pühler et al., 1992). The dimensions of the archaeobacterial proteasome and various eukaryotic proteasomes are all similar, bearing in mind differences in measurements obtained using different negative stains (diameter 11–12nm, height 16–20nm; Table 1).

PRIMARY STRUCTURES OF PROTEASOMAL PROTEINS

During the last few years the amino acid sequences of several subunits of human, rat, yeast, and *Drosophila* proteasomes have been deduced from the sequence of cloned cDNA (see Table 2). The sequences of these proteasome subunits show significant identities to each other but not to any other known proteins. Similarity scores obtained by pairwise alignment of different human, rat or yeast proteasomal subunits of the same species (often 20–40 % identity) imply that many of the genes belong to the same gene family. Moreover, the significant sequence similarity of the α subunit of the archaeobacterial particle (Zwickl et al., 1991) to various subunits of eukaryotic proteasomes suggests that these proteasomal proteins are encoded by a gene family of

Table 2 Sequence information for proteasomal subunits

Source	Subunit	Reference
Archaeobacteria (<i>Thermoplasma acidophilum</i>)	α	Zwickl et al., 1991
Yeast (<i>Saccharomyces cerevisiae</i>)	β	Zwickl et al., 1992
	YC1, YC7 α	Fujiwara et al., 1990
	Y7, Y8, Y13	Emori et al., 1991
	PRE1	Heinemeyer et al., 1991
	PRS3	Lee et al., 1992
	scl1 ⁺ (YC7 α)	Balzi et al., 1990
	PUP1	Haffter and Fox, 1991
	PUP2	Georgatsou et al., 1992
	PROS35	Haass et al., 1989
	PROS28.1	Haass et al., 1990a
<i>Drosophila melanogaster</i>	PROS29	Haass et al., 1990b
<i>Xenopus laevis</i>	XC3	Fujii et al., 1991
Rat	β	van Riel and Martens, 1992
	C2	Fujiwara et al., 1989
	C3	Tanaka et al., 1990a
	C5	Tamura et al., 1990
	C8	Tanaka et al., 1990b
	C9	Kumatori et al., 1990b; Sorimachi et al., 1990
	N-terminal seq. 1–7	Lilley et al., 1990
	C1	Aki et al., 1992
	LMP2	Martinez and Monaco, 1991
	MC13	Frentzel et al., 1992a
Human	C2, C3, C5, C8, C9	Tamura et al., 1991
	ν , ι , ζ , δ	De Martino et al., 1991
	RING10	Glynne et al., 1991
	RING12	Kelly et al., 1991
	N-terminal seq. α , β , γ , δ , ϵ	Lee et al., 1990

ancient origin. Several gene duplication events may have contributed to the complex subunit composition of eukaryotic proteasomes, probably via selection for a variety of functional requirements, although there are clearly structural constraints on the extent of subunit divergence within the cylindrical molecule.

The possibility of at least two **groups of closely-related proteasome subunits**, which seemed likely from comparison of directly determined N-terminal amino acid sequence data (Lilley et al., 1990; Lee et al., 1990) with deduced sequences of N-terminally blocked subunits (e.g. Fujiwara et al., 1989; Tanaka et al., 1990a; Tamura et al., 1991) of the rat and human proteinases, has been confirmed by the recent sequence analysis of the second (β) subunit of the archaeobacterial enzyme (Zwickl et al., 1992). Although the two amino acid sequences of the *Thermoplasma acidophilum* proteasome can be aligned to show 24% identity, there are significant differences, particularly in the N-terminal regions. Close examination of sequences of proteasome subunits from other species shows that they can be divided into two groups, A and B (Figure 3), based upon whether they are more closely related to the archaeobacterial α or β subunit, respectively. However, not all eukaryotic proteasome subunits fit neatly into group A or B. For example, the amino acid sequence deduced for C5, although related to the A and B groups, is not characteristic of either (Tamura et al., 1990; Lee et al., 1992). Many of the cloned subunits fall into the A group. The *Thermoplasma acidophilum* α subunit is most similar to the eukaryotic C3, which is very highly conserved between rat (Tanaka et al., 1990a), human (98% identity to rat C3; Tamura et al., 1991) and *Xenopus* (95% identity to rat C3; Fujii et al., 1991). Other A-type subunits are also very highly conserved between different animal species (Tamura et al., 1991).

The archaeobacterial β subunit has an unblocked N-terminus (Zwickl et al., 1992) as do seven of the related rat liver

proteasomal polypeptides (Lilley et al., 1990), while A subunits RC2, RC3, and RC8 have been shown to be N-acetylated (Tokunaga et al., 1990). Sequences of N-terminal regions of different proteasomal A-type subunits are particularly highly conserved, whereas the C-terminal regions are often not. A-type subunits each contain three highly conserved regions (termed PROS-Box I, II, and III by Haass et al., 1990b; Zwickl et al., 1991). Sequences of B subunits share some highly conserved regions but have variable lengths at the N-terminus (Figure 3b). Members of the proteasome gene family are found on several different chromosomes (Lee et al., 1992; Frentzel et al., 1992b).

In general there are no sequence motifs in proteasome subunits which are characteristic of other proteases. Sequences which are related to the conserved regions around catalytic residues in subtilisin-like serine proteases which were identified in the primary structure deduced from the cDNA sequence of RING10 (Glynne et al., 1991) are not conserved in other B-type proteasomal subunits, and in RC1, which is the rat homologue of RING10, the putative catalytic histidine residue is replaced by an asparagine residue (Aki et al., 1992). The fact that proteasome subunits show no obvious relationship to other known proteases suggests that the proteasome represents a **novel family of proteolytic enzymes** (multicatalytic endopeptidase complex; EC 3.4.99.46; and see Rawlings and Barrett, 1993, for a listing of proteinase families).

KINETIC PROPERTIES OF PROTEASOMES

The term multicatalytic was applied to this proteinase complex because it has an unusual enzymological property when compared to other proteinases; it can catalyse peptide bond cleavage on the carboxyl side of basic, hydrophobic and acidic amino acid residues (Wilk and Orlowski, 1983). These three types of pro-

Figure 3 Sequence relationships in proteasome subunits

Proteasomal subunits can usually be assigned to one of two groups based on their similarity to the *Thermoplasma acidophilum* α (A-type) or β (B-type) subunit. Sequence references are given in Table 1. (a) A-type subunits are closely related to the *Thermoplasma acidophilum* α subunit. Sequences have been deduced from the nucleotide sequence of cloned cDNA. The N-terminal sequences are shown with highly conserved residues highlighted. There are two other regions of highly conserved residues within the full-length sequences which are characteristic of A-type subunits (PROS-BOX1 and III, Haass et al., 1990a). At least some A-type subunits are N-acetylated (Tokunaga et al., 1990). (b) B-type subunits have sequences which show some similarities to the *Thermoplasma acidophilum* β subunit. Several B-type subunits are not N-terminally blocked and the sequences shown are aligned to maximize identities with directly determined N-terminal amino acid sequences (Lee et al., 1990; Lilley et al., 1990). Highly conserved residues have been highlighted. Archaeobacterial α and β subunits are related to each other and can be aligned to give 24% identical residues (Zwickl et al., 1992).

teolytic activity have usually been referred to as "trypsin-like", "chymotrypsin-like" and "peptidylglutamyl-peptide hydrolase" activities, respectively. Information concerning the kinetic characteristics of the proteinase has been derived from many studies carried out with the enzyme from a variety of different sources (reviewed in Rivett, 1989a; Orlowski, 1990).

The **multiple proteolytic functions** are believed to be catalysed at independent sites within the complex because the different proteolytic activities respond differently to various activators and inhibitors. From detailed studies with the rat liver and bovine pituitary proteinases (Wilk and Orlowski, 1983; Rivett, 1989b; Orlowski et al., 1991; Yu et al., 1991; Djaballah and

Rivett, 1992; Pereira et al., 1992; Djaballah et al., 1992), it now seems likely that there may be at least five distinct catalytic components within the mammalian proteasome, while the much simpler archaeobacterial enzyme possesses primarily chymotrypsin-like activity (Dahlmann et al., 1989). The early names for the multiple catalytic activities were clearly an oversimplification. For example, peptidylglutamyl-peptide hydrolase activity of the mammalian proteasome, which is usually assayed with Z-Leu-Leu-Glu- β -naphthylamide, is catalysed by at least two distinct components, LLE1 and LLE2 (Djaballah and Rivett, 1992) and there may be as many as three distinct components catalysing chymotrypsin-like activity (Cardozo et al., 1992;

Djaballah et al., 1992). Rather little is known in detail about the specificities of the different catalytic centres of eukaryotic proteasomes and a better system of naming them must therefore await further characterization of catalytic components.

It is difficult to predict sites of cleavage in peptide substrates, but many different bonds can be cleaved (Wilk and Orlowski, 1980; Rivett, 1985a; McDermott et al., 1991). The rat liver and human erythrocyte proteasomes show the same major cleavage sites in insulin B chain (Rivett, 1985a; Dick et al., 1991). Some differences in kinetic properties of the proteinases isolated from bovine pituitary (Orlowski and Michaud, 1989; Orlowski et al., 1991; Pereira et al., 1992) and rat liver (Rivett, 1989b; Arribas and Castaño, 1991; Djaballah and Rivett, 1992; Djaballah et al., 1992) have been reported, but in view of the complexity of the particles and differences in purification protocols it is not easy to assess the significance of such differences.

The effects of protease inhibitors on proteasome activities depends on the substrate used. Dramatic differences in reactivity with different inhibitors have been observed. For example, the peptidyl arginine aldehyde leupeptin inhibits only the trypsin-like activity (Wilk and Orlowski, 1983; Rivett, 1989b). Although results of early studies with protease inhibitors led to some confusion about whether the proteinase should be classified as a serine or cysteine endopeptidase (reviewed by Rivett, 1989a), recent results obtained with the serine protease inhibitors, 3,4-dichloroisocoumarin (Orlowski and Michaud, 1989; Mason, 1990; Djaballah et al., 1992) and 4-(2-aminoethyl)-benzenesulphonyl fluoride (Djaballah et al., 1992), which, at least for some proteasomes, are more potent inhibitors than those used previously (e.g. di-isopropyl fluorophosphate and phenylmethanesulphonyl fluoride), support the view that the enzyme is possibly an **unusual type of serine endopeptidase** although several of the activities are sensitive to inhibition by thiol-reactive reagents. The apparent rate constants for inactivation by inhibitors of serine proteases are mostly very low when compared to those for other proteinases (Djaballah et al., 1992). The precise determination of catalytic mechanism will only become possible with the identification of catalytic residues. Surprising differences in reactivity have been observed between the different catalytic centres of the mammalian proteinase complex. For example, 3,4-dichloroisocoumarin reacts rapidly with some, but does not inhibit all, catalytic components of the rat liver or bovine pituitary enzyme (Djaballah et al., 1992; Pereira et al., 1992) and 4-(2-aminoethyl)-benzenesulphonyl fluoride can selectively inhibit trypsin-like activity (Djaballah et al., 1992). Therefore, although it seems likely that the proteolytic sites are mechanistically related, they obviously differ in important aspects which determine specificity.

PROTEIN DEGRADATION BY PROTEASOMES

The proteinase can degrade a variety of protein and peptide substrates, and activity at all sites is optimal at neutral to weakly alkaline pH values. The multiple proteolytic activities of the proteasome should be advantageous for rapid degradation of the variety of intracellular proteins. Protein substrates, which include certain oxidized proteins (Rivett, 1985b), crystallins (Ray and Harris, 1985), myofibrillar proteins (Mykles and Haire, 1991) and casein, can be degraded to small acid soluble peptides without accumulation of intermediates detectable by SDS/PAGE (Rivett, 1985b). Although it seems likely that the various peptidase activities contribute to protein degradation, an additional distinct catalytic centre has been implicated in the degradation of casein (Pereira et al., 1992). However, this may

simply be a catalytic centre for which a convenient synthetic peptide substrate has not yet been identified.

REGULATION OF PROTEINASE ACTIVITIES

In vitro experiments have shown that sodium dodecyl sulphate, at low concentrations, as well as a variety of other treatments (including polylysine, low concentrations of guanidine hydrochloride, dialysis against distilled water and heat treatment) can cause a dramatic stimulation of one or more activities of proteasomes isolated from different sources (see e.g. McGuire et al., 1989; Tanaka et al., 1990b; Arribas and Castaño, 1990; Mykles and Haire, 1991; Djaballah et al., 1992). Such observations have sometimes led to use of the term "**latent form**" for the non-activated complex, especially for human erythrocyte proteasomes (Lee et al., 1990; Weitman and Etlinger, 1992) but their significance to the regulation of activity *in vivo* is not clear. Sedimentation velocity studies have shown significant changes in conformation associated with activation and inhibition of proteinase activities (Djaballah et al., 1993).

Such stimulatory or inhibitory effects may be mediated *in vivo* by specific **endogenous activator and inhibitor proteins**. Several different endogenous inhibitors have been identified: a 200 kDa (subunit molecular mass 50kDa) inhibitor from human erythrocytes (Li et al., 1991), a 60 kDa (subunit molecular mass 31 kDa) protein from bovine erythrocytes (Chu-Ping et al., 1992a) and a 250 kDa (subunit molecular mass 40 kDa) proteasome inhibitor (Murakami and Etlinger, 1986; Li et al., 1991; Driscoll et al., 1992). Activator proteins have also been purified (Yukawa et al., 1991; Goldberg and Rock, 1992; Chu-Ping et al., 1992b) but their physiological function and mechanism of action have not been established.

It is difficult to measure the activity of proteasomes *in vivo* and the significance of observed differences in proteolytic activity attributed to proteasomes during differentiation of murine erythroleukaemia cells (Tsukahara et al., 1990), during development (Ahn et al., 1991) and during maturation of red blood cells (DiCola et al., 1991) cannot be evaluated because assays of activities in crude extracts do not provide an accurate measure of levels of proteasome activity due to possible interference by other proteins.

In addition to modulation of proteasome activities and function by association with other proteins, the mammalian proteinase complex may also be activated by certain substrates. For example, it shows **positive co-operativity** with a substrate of the peptidylglutamyl-peptide hydrolase activity (Orlowski et al., 1991; Djaballah and Rivett, 1992) and high concentrations of this substrate can induce a conformational change (Djaballah et al., 1993). Proteolysis may also be regulated by a requirement for **modification of some protein substrates** (e.g. ubiquitination, oxidation or other modification) prior to their recognition by proteasomes (Rivett, 1985b, 1989a).

FUNCTIONS OF INDIVIDUAL COMPONENTS OF THE COMPLEX

Dissociation of mammalian proteasomes by denaturing agents causes rapid loss of the activities associated with the complex and it has not proved possible so far to demonstrate functions of the isolated subunits nor to reconstitute active molecules from the dissociated subunits. It is not yet clear exactly how many subunits are responsible for the proteolytic activities and, although it seems likely that individual subunits are responsible for each of the proteolytic activities, the possibility that active sites are each shared between two subunits cannot be ruled out. Two approaches have been taken to identify catalytic components of

the complex. In yeast, mutants defective in an individual proteasome gene (PRE1) show reduced chymotrypsin-like activity which suggests the possibility of a catalytic role for this subunit (Heinemeyer et al., 1991). The alternative method for identification of catalytic subunits involves active site labelling experiments which are also essential to learn something about the catalytic residues and mechanism. The problem with the latter approach has, until recently, been the identification of suitable selective inhibitors for the distinct catalytic centres. Leupeptin is a specific reversible inhibitor of the trypsin-like activity (Wilk and Orłowski, 1983) but subunits identified by leupeptin protection against modification by radiolabelled *N*-ethylmaleimide (Dick et al., 1992; Savory and Rivett, 1993) may not be the catalytic components. However, some selective affinity labels have now been identified (P. J. Savory, H. Angliker, E. Shaw and A. J. Rivett, unpublished work).

Although computer analysis has revealed no overall homology to other known proteins, **predicted tyrosine phosphorylation sites** have been identified in Taa, C3, C9, Dm29 and YC7a subunits (Tanaka et al., 1990a; Haass et al., 1990b; Zwickl et al., 1991), as have **putative cyclic AMP/cyclic GMP-dependent phosphorylation sites** in Dm28.1 and Taa (Haass et al., 1990a; Zwickl et al., 1991). These observations, as well as the fact that the archaeobacterial α subunit does not have a histidine residue and would therefore be unable to function independently as a classical serine protease, have led to the suggestion that that A-type subunits of the proteasome have a regulatory and targeting function, while the B-type subunits may be catalytic (Zwickl et al., 1992). However, this may be an oversimplification, especially for eukaryotic proteasomes.

SUBCELLULAR LOCALIZATION

Immunocytochemical studies have shown proteasomes to be present both in the nucleus and cytoplasm of a variety of cells and tissues (see Tanaka et al., 1990c for references; Beyette and Mykles, 1992). More detailed localization studies using electron microscopic immunogold labelling techniques with anti-proteasome antibodies have shown that a proportion of proteasomes are also close to or actually on the rough endoplasmic reticulum and in polysomes in rat hepatocytes and human L-132 cells (Rivett et al., 1992; Table 3). "Prosomes" have been suggested to be associated with intermediate filaments (Grossi de Sa et al., 1988; Briane et al., 1992) and a significant amount of the complex has been purified from erythrocyte membranes (Kinoshita et al., 1990).

The functional significance of the distribution of proteasomes and the relationship between particles in the different cellular compartments are not yet well understood (Rivett and Knecht, 1993). Proteasomes isolated from rat liver nuclei and cytoplasm show the same basic properties (Tanaka et al., 1989). Several proteasome components do contain **putative nuclear localization signals** and it has been suggested that tyrosine phosphorylation could affect exposure of nuclear localization signals and thereby regulate movement of proteasomes between the nucleus and cytoplasm (Tanaka et al., 1990c). The similarity between sequences in some A-type proteasome subunits and the nuclear targeting sequence of SV40 large T antigen has already been noted (Tanaka et al., 1990c; Zwickl et al., 1991; Figure 4). However, it has recently been suggested (Dingwall and Laskey, 1991) that many nuclear targeting sequences may be more complex than the SV40 large T antigen sequence, and that a bipartite motif comprising two basic amino acids, a spacer usually of ten amino acids, and a basic cluster in which three out

Table 3 Subcellular localization determined by immunogold electron microscopy

The data are from Rivett et al. (1992) obtained using polyclonal antibodies for rat liver proteasomes. Percentage labelling is calculated from the number of gold particles/ μm^2 . V_v is the volume density of the compartment. There was no significant labelling in mitochondria, lysosomes, peroxisomes or Golgi.

Cell compartment	Significant labelling (%)	
	Rat hepatocytes	Human L-132 cells
Nucleoli	3.2	3.3
Nuclear matrix	13.5 (V_v 8.4%)	47.3 (V_v 37.0%)
Mitochondria	—	—
Lysosomes	—	—
Endoplasmic reticulum	14.3	3.5
Peroxisomes	—	—
Golgi	—	—
Cytoplasmic matrix	69.0	45.9

of the next five amino acids are basic residues, is required. Of the mammalian proteasome subunits cloned so far, only the C9 component contains a putative bipartite motif (Figure 4), but this is incomplete in the closely related *Drosophila* Dm29 and yeast Y13 subunits.

The distribution of proteasomes between different cell compartments seems to vary with cell type (Haass et al., 1989) and changes in localization of proteasomes have been found to occur during oogenesis, embryogenesis and development in lower eukaryotic organisms (e.g. Grainger and Winkler, 1989; Klein et al., 1990; Kawahara and Yokosawa, 1992). There are also changes with the cell cycle (Knecht et al., 1991; Kawahara and Yokosawa, 1992; Amsterdam et al., 1992). In transformed cells and proliferating tissues, proteasomes have been found to occur predominantly in the nuclei (Grossi de Sa et al., 1988; Kumatori et al., 1990a; Kanayama et al., 1991).

PROTEASOMES AND CELL PROLIFERATION

Gene disruption experiments in yeast have demonstrated that several proteasomal proteins are each encoded by a single copy gene and that some of these genes (YC1, YC7 α or Y8, Y7, PRE1, PRS3 and PUP2; Fujiwara et al., 1990; Emori et al., 1991; Heinemeyer et al., 1991; Lee et al., 1992; Georgatsou et al., 1992) are essential for cell proliferation, whereas others are not (e.g. Y13; Emori et al., 1991). An increase in the level of expression of the gene for the one subunit of proteasomes which was investigated has been found in malignant human haematopoietic cell lines and growth-stimulated mononuclear cells, in leukaemic cells in bone marrow, and in cells during blastogenic transformation induced by phytohaemagglutinin and interleukin-2 (Kumatori et al., 1990a). Significantly increased levels of several proteasomal mRNAs have also been observed in malignant tumour cells and in transformed cells in culture (Kanayama et al., 1991; Balson et al., 1992; Shimbara et al., 1992) but there is no change in the level of proteasomal proteins detected by Western blotting. There appears to be an increase in the turnover of proteasomes under some conditions (Shimbara et al., 1992).

Although changes in proteasomal gene expression appear to be important during growth and differentiation of cells (Shimbara et al., 1992) the reason for this is presently unclear. Proteasomes may participate in the specific breakdown of certain cellular

SV40 large T	126- P K K K R K V -131
Ta α	50- S D K K V R S -56
RC3 (HC3, XC3)	48- T E K K Q K S -54
Dm28.1	242- E K K K Q K K -249
RC9	249- R E K K E K E -254
Dm29	252- A A K K E K Q -258
YC7 α	185- H F K K S K I -191
Nucleoplasmin	A V K R P A A T K K A G Q A K K K K L D K E D E
<i>Xenopus</i> N1	R K K R K T E E E S P L K D K D A K K S K Q E
RC9	L I K K H E E E E A K A E R E K K E K E Q R E K D

Figure 4 Putative nuclear localization signals in proteasomal subunits

Sequences related to the SV40 nuclear localization signal have been identified in several subunits (Tanaka et al., 1990c; Haass et al., 1990b) as shown. A sequence in the RC9 subunit is similar to the bipartite motif described by Dingwall and Laskey (1991) and illustrated by nucleoplasmin and the *Xenopus* N1 protein.

proteins, such as cyclins, for which rapid turnover is important in regulating the cell cycle. Short-lived proteins, including many key regulatory proteins, are primarily degraded by non-lysosomal pathways of intracellular protein degradation (Rivett, 1990; Rechsteiner, 1991; Goldberg and Rock, 1992; see below). Proteasomes are believed to play a major role in these pathways (Orlowski, 1990; Rivett, 1990) and decreased availability of functional proteasomes could allow increases in the levels of short-lived proteins. Alternatively, although such regulatory proteolytic properties of proteasomes may be important, the possibility that other functions of proteasomes predominate in cell proliferation cannot be ruled out. Their localization in the nucleus as well as in the cytoplasm and the increase in nuclear localization in rapidly proliferating cells argues for a more direct involvement in characteristic nuclear functions.

FUNCTIONS IN INTRACELLULAR PROTEIN DEGRADATION

The abundance and widespread distribution of proteasomes, as well as their ability to degrade protein substrates, suggest that they may play an **important role in non-lysosomal pathways of protein turnover** (Orlowski, 1990; Rivett and Knecht, 1993). There are both ubiquitin-dependent and ubiquitin-independent pathways. The **ubiquitin system**, a multicomponent, ATP-dependent pathway, which has been well-characterized in reticulocytes and yeast (see Hershko and Ciechanover, 1992; Jentsch, 1992, for reviews), is believed to be responsible for the removal of abnormal proteins and for the rapid degradation of normal short-lived proteins which are important for cell cycle progression such as cyclin and nuclear oncoproteins (Rechsteiner, 1991). Not all ubiquitinated proteins are rapidly degraded and the signal for ubiquitin-mediated proteolysis appears to be multiple ubiquitination. Other short-lived proteins and some long-lived proteins may be degraded by ubiquitin-independent non-lysosomal pathways, which are less clearly defined but may also be ATP-dependent (Rivett, 1990; Goldberg and Rock, 1992).

Although purified proteasomes are not activated by ATP (reviewed in Rivett, 1989a) and are apparently unable to degrade ubiquitin-protein conjugates (Hough et al., 1987), proteasomes have been implicated in both ATP- and ubiquitin-dependent

pathways of protein breakdown. Immunodepletion of proteasomes from crude cell extracts reduces both ATP-dependent and ubiquitin-dependent proteolysis (e.g. Matthews et al., 1989). Direct evidence for the involvement of proteasomes in the ubiquitin pathway is that ubiquitin-mediated proteolysis is decreased in the yeast PRE1 mutants which are defective in proteasomal chymotrypsin-like activity. Disruption of the PRE1 gene results in a decreased ability of cells to cope with stress conditions (Heinemeyer et al., 1991) and to degrade known substrates of the ubiquitin pathway (Richter-Ruoff et al., 1992; Seufert and Jentsch, 1992). The relative contribution of proteasomes to ubiquitin-dependent and ubiquitin-independent pathways may be determined by association with other proteins. They appear to form part of a larger (26 S) proteinase complex (see below) which is believed to be responsible for degradation of ubiquitin-protein conjugates (Hershko and Ciechanover, 1992) but which also appears to be involved in the ubiquitin-independent degradation of ornithine decarboxylase (Murakami et al., 1992).

In addition to their general role in intracellular protein turnover, proteasomes may have **specific proteolytic functions**. For example, a proteinase which is believed to contribute to cell-mediated cytotoxicity in interleukin-2-activated natural killer cells shows similar characteristics and immunochemical relatedness to proteasomes isolated from rat liver (K. Wasserman, R. P. Kitson, A. J. Rivett, S. T. Sweeney, M. K. Gabauer, R. B. Herberman, S. Watkins and R. H. Goldfarb, unpublished work) and proteasomes have also been implicated in antigen processing (see below). The balance of functions may vary in different cell types.

PROTEASOMES AND THE 26 S PROTEINASE

Based on results from a number of different laboratories it seems likely that the proteasome forms part of the 26 S proteinase (Hough et al., 1987; Eytan et al., 1989; Driscoll and Goldberg, 1990; Kanayama et al., 1992) but this view is not unanimous (Seelig et al., 1991) and the precise nature of the other components of the 26 S proteinase is not entirely clear. On SDS/PAGE gels a range of polypeptides of 35 kDa to 110 kDa, in addition to lower-molecular-mass bands corresponding to proteasome

subunits, has been reported for the 26 S proteinase isolated from reticulocytes (Hough et al., 1987), promyelocytic leukaemic HL60 cells (Orino et al., 1991) and *Xenopus* (Peters et al., 1991). The reticulocyte 26 S proteinase, which also has ATPase activity (Armon et al., 1990), is formed by the ATP-dependent association of proteasomes with two other components, CF1 and CF2 (Eytan et al., 1989; Driscoll and Goldberg, 1990). One of these has been suggested to be a 250 kDa inhibitor of the proteasome (Driscoll et al., 1992) while the other component may be an activator (Goldberg and Rock, 1992). However, in muscle, the 26 S proteinase has been reported to be composed of the proteasome and a 600 kDa ATP-dependent proteinase called multipain (Goldberg, 1992). There are some differences in the structural models which have been proposed from electron microscopy studies with negatively stained preparations (Peters et al., 1991; Ikai et al., 1991) and further work is required to clearly establish the components of the 26 S proteinase and its precise relationship to the proteasome.

PROTEASOMES AND ANTIGEN PROCESSING

Peptides which bind to newly synthesized major histocompatibility complex class I molecules in the endoplasmic reticulum are presumed to be generated in the cytoplasm prior to transport across the endoplasmic reticulum which is facilitated by peptide transporters (DeMars and Spies, 1992). A complex of low-molecular-mass proteins ("LMP"; Monaco and McDevitt, 1984), which was suggested to be involved in the generation of peptides from cellular proteins or viral proteins produced within infected cells for presentation in association with the class I major histocompatibility complex, has properties in common with proteasomes, suggesting that the two complexes might be related. The demonstration that immunoprecipitates of mouse macrophage or spleen cell extracts obtained with anti-LMP antibodies and anti-proteasome antibodies give very similar patterns on two-dimensional polyacrylamide gels (Brown et al., 1991; Ortiz-Navarette et al., 1991) supports this view.

A possible role of proteasomes in antigen processing was also suggested by the identification of two genes within the MHC class II region (LMP7/RING10 and LMP2/RING12; Glynne et al., 1991; Martinez and Monaco, 1991) which are up-regulated by γ -interferon (Kelly et al., 1991; Ortiz-Navarette et al., 1991; Yang et al., 1992) and which encode proteins containing deduced amino acid sequences similar to those of known proteasomal subunits (Lee et al., 1990; Lilley et al., 1990). Neither of the two proteins is essential for cell viability. Although the possibility that proteasomes contribute to antigen processing has received a lot of attention recently (see Goldberg and Rock, 1992, for a review) there is recent evidence that the two polymorphic MHC-encoded subunits are not essential for the processing of peptides bound by MHC class I molecules (Momburg et al., 1992; Arnold et al., 1992). However, such observations do not preclude some role for proteasomes in antigen processing.

CONCLUSIONS AND FUTURE DIRECTIONS

Proteasomes are high-molecular-mass cylindrical particles which contain at least five distinct types of catalytic components and are capable of degrading proteins to small peptides. They are found in all eukaryotic cells and tissues and are usually present in the nucleus as well as in the cytoplasm. They are composed of many different subunits which are encoded by members of the same gene family. Individual subunits can be grouped into A or

B types depending on whether they are most similar to the α or β subunit of the archaeobacterial proteasome. The latter enzyme provides a simple model for structural studies.

The diversity of eukaryotic proteasome localization and subunit composition, as well as the presence of RNA in only a small proportion of particles, suggests the possible existence of subpopulations of proteasomes. The precise subunit stoichiometry remains unclear as does the mechanism for the assembly or exchange of selected subunits to produce particles having different collections of polypeptide components. The fact that some but not all components of proteasomes are essential for cell viability presumably reflects essential functions of these subunits, which may either be structural or functional. Further knowledge of the function and distribution of individual components of the complex will help to elucidate some of these issues.

Since the proteinase does not fall into one of the recognized families of proteases, the identification of proteolytic components as well as the determination of catalytic residues and mechanism are of particular interest. The assembly of different proteolytic components into a single complex allows them to share regulatory or targeting mechanisms and provides an efficient means by which proteins recognized as substrates can be rapidly degraded to small peptides. The broad specificity of the proteinase would allow degradation of many different substrates by a single proteinase complex.

It is widely believed that proteasomes play a major part in both ubiquitin-dependent and ubiquitin-independent non-lysosomal pathways of protein breakdown. There is direct evidence for a role in ubiquitin-dependent proteolysis which may involve association of the proteasome with other components to form the 26 S proteinase which has recently also been implicated in ubiquitin-independent protein turnover (Murakami et al., 1992). Proposed mechanisms for the modulation of proteasome function and localization are speculative, and although it seems likely that the function of the proteasome can be modulated by association with other proteins such as endogenous inhibitors, activators, or other components of the 26 S proteinase, further studies are required to establish which of these factors are important *in vivo*. The functional significance of the proteasomal RNA remains to be established, as does the proposed role of proteasomes in the control of translation (Nothwang et al., 1992a).

A. J. R. is a Lister Institute-Jenner Research Fellow. Research in the author's laboratory was also supported by the Medical Research Council and the Wellcome Trust.

REFERENCES

- Ahn, J. Y., Hong, S. O., Kwak, K. B., Kang, S. S., Tanaka, K., Ichihara, A., Ha, D. B. and Chung, C. H. (1991) *J. Biol. Chem.* **266**, 15746–15749
- Aki, M., Tamura, T., Tokunaga, F., Iwanaga, S., Kawamura, Y., Shimbara, N., Kagawa, S., Tanaka, K. and Ichihara, A. (1992) *FEBS Lett.* **301**, 65–68
- Amsterdam, A., Pitzar, F. and Baumeister, W. (1992) *Proc. Natl. Acad. Sci. U.S.A.*, in the press
- Armon, T., Ganoth, D. and Hershko, A. (1990) *J. Biol. Chem.* **265**, 20723–20726
- Arnold, D., Driscoll, J., Androlewicz, M., Hughes, E., Cresswell, P. and Spies, T. (1992) *Nature (London)* **360**, 171–174
- Arribas, J. and Castaño, G. (1990) *J. Biol. Chem.* **265**, 13969–13973
- Arrigo, A.-P., Tanaka, K., Goldberg, A. L. and Welch, W. J. (1988) *Nature (London)* **331**, 192–194
- Balson, D. F., Skilton, H. E., Sweeney, S. T., Thomson, S. and Rivett A. J. (1992) *Biol. Chem. Hoppe-Seyler* **373**, 623–628
- Balzi, E., Chen, W., Capieaux, E., McCusker, J. H., Haber, J. E. and Goffeau, A. (1990) *Gene* **89**, 151
- Baumeister, W., Dahlmann, B., Hegerl, R., Kopp, F., Kuehn, L. and Pfeifer, G. (1988) *FEBS Lett.* **241**, 239–245
- Beyette, J. R. and Mykles, D. L. (1992) *Muscle Nerve* **15**, 1023–1035

- Briane, D., Olink-Coux, M., Vassy, J., Oudar, O., Huesca, M., Scherrer, K. and Fouchier, J. (1992) *Eur. J. Cell Biol.* **57**, 30–39
- Brown, M.G., Driscoll, J. and Monaco, J. J. (1991) *Nature (London)* **353**, 355–357
- Cardozo, C., Vinitsky, A., Hidalgo, M. C., Michaud, C. and Orlowski, M. (1992) *Biochemistry* **31**, 7373–7380
- Chu-Ping, M., Slaughter, C. A. and DeMartino, G. N. (1992a) *Biochim. Biophys. Acta* **1119**, 303–311
- Chu-Ping, M., Slaughter, C. A. and DeMartino, G. N. (1992b) *J. Biol. Chem.* **267**, 10515–10523
- Coux, O., Nothwang, H. G., Scherrer, K., Bergsma-Schutter, W., Arnberg, A. C., Timmins, P. A., Langowski, J. and Cohen-Addad, C. (1992) *FEBS Lett.* **300**, 49–55
- Dahlmann, B., Kopp, F., Kuehn, L., Nidel, B., Pfeifer, G., Hegerl, R. and Baumeister, W. (1989) *FEBS Lett.* **251**, 125–131
- Dahlmann, B., Kuehn, L., Ishiura, S., Tsukahara, T., Sugita, H., Tanaka, K., Rivett, J., Hough, R. F., Rechsteiner, M., Mykles, D. L., Fagan, J. M., Waxman, L., Ishii, S., Sasaki, M., Kloetzel, P. M., Harris, H., Ray, K., Behal, F. J., DeMartino, G. N. and McGuire, M. J. (1988) *Biochem. J.* **255**, 750–751
- DeMars, R. and Spies, T. (1992) *Trends Cell Biol.* **2**, 81–86
- DeMartino, G. N., Orth, K., McCullough, M. L., Lee, L. W., Munn, T. Z., Moomaw, C. R., Dawson, P. A. and Slaughter, C. A. (1991) *Biochim. Biophys. Acta* **1079**, 29–38
- Dick, L. R., Moomaw, C. R., DeMartino, G. N. and Slaughter, C. A. (1991) *Biochemistry* **30**, 2725–2734
- Dick, L. R., Moomaw, C. R., Pramanik, B. C., DeMartino, G. N. and Slaughter, C. A. (1992) *Biochemistry* **31**, 7347–7355
- DiCola D., Pratt, G. and Rechsteiner, M. (1991) *FEBS Lett.* **280**, 137–140
- Dingwall, C. and Laskey, R. A. (1991) *Trends Biochem. Sci.* **16**, 478–481
- Djabballah, H. and Rivett, A. J. (1992) *Biochemistry* **31**, 4133–4141
- Djabballah, H., Harness, J. A., Savory, P. J. and Rivett, A. J. (1992) *Eur. J. Biochem.* **209**, 629–634
- Djabballah, H., Rowe, A. J., Harding, S. E. and Rivett, A. J. (1993) *Biochem. J.*, in the press
- Driscoll, J. and Goldberg, A. L. (1990) *J. Biol. Chem.* **265**, 4789–4792
- Driscoll, J., Frydman, J., Goldberg, A. L. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4986–4990
- Emori, Y., Tsukahara, T., Kawasaki, H., Ishiura, S., Sugita, H. and Suzuki, K. (1991) *Mol. Cell Biol.* **11**, 344–353
- Eytan, E., Ganoth, D., Armon, T. and Hershko, A. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 7751–7755
- Falkenberg, P. E., Haass, C., Kloetzel, P. M., Nidel, B., Kopp, F., Kuehn, L. and Dahlmann, B. (1988) *Nature (London)* **331**, 190–192
- Frentzel, S., Gräf, U., Hämmerling, G. J. and Kloetzel, P.-M. (1992a) *FEBS Lett.* **302**, 121–125
- Frentzel, S., Troxell, M., Haass, C., Pesold-Hurt, B., Glätzer, K. H. and Kloetzel, P.-M. (1992b) *Eur. J. Biochem.* **205**, 1043–1051
- Fujii, G., Tashiro, K., Emori, Y., Saigo, K., Tanaka, K. and Shiokawa, K. (1991) *Biochem. Biophys. Research Commun.* **178**, 1233–1239
- Fujiwara, T., Tanaka, K., Kumatori, A., Shin, S., Yoshimura, T., Ichihara, A., Tokunaga, F., Aruga, R., Iwanaga, S., Kakizuka, A. and Nakanishi, S. (1989) *Biochemistry* **28**, 7332–7340
- Fujiwara, T., Tanaka, K., Orino, E., Yoshimura, T., Kumatori, A., Tamura, T., Chung C. H., Nakai, T., Yamaguchi, K., Shin, S., Kakizuka, A., Nakanishi, S. and Ichihara, A. (1990) *J. Biol. Chem.* **265**, 16604–16613
- Georgatou, E., Georgakopoulos, T. and Thireos, G. (1992) *FEBS Lett.* **299**, 39–43
- Glynne, R., Powis, S. H., Beck, S., Kelly, A., Kerr, L. A. and Trowsdale, J. (1991) *Nature (London)* **353**, 357–360
- Goldberg, A. L. (1992) *Eur. J. Biochem.* **203**, 9–23
- Goldberg, A. L. and Rock, K. L. (1992) *Nature (London)* **357**, 375–379
- Grainger, J. L. and Winkler, M. M. (1989) *J. Cell Biol.* **109**, 675–683
- Grossi de Sa, M. F., Martins de Sa, C., Harper, F., Coux, O., Akhayat, O., Pal, J. K., Florentin, Y. and Scherrer, K. (1988) *J. Cell Sci.* **89**, 151–165
- Grziwa, A., Baumeister, W., Dahlmann, B. and Kopp, F. (1991) *FEBS Lett.* **290**, 186–190
- Haass, C. and Kloetzel, P. M. (1989) *Exp. Cell Res.* **180**, 243–252
- Haass, C., Pesold-Hurt, B., Multhaup, G., Beyreuther, K. and Kloetzel, P. M. (1989) *EMBO J.* **8**, 2373–2379
- Haass, C., Pesold-Hurt, B., Multhaup, G., Beyreuther, K. and Kloetzel, P. M. (1990a) *Gene* **90**, 235–241
- Haass, C., Pesold-Hurt, B. and Kloetzel, P. M. (1990b) *Nucleic Acids Res.* **18**, 4018
- Haffter, P. and Fox, T. D. (1991) *Nucleic Acids Res.* **19**, 5075
- Harris, J. R. (1988) *Ind. J. Biochem.* **25**, 459–466
- Hegerl, R., Pfeifer, G., Pühler, G., Dahlmann, B. and Baumeister, W. (1991) *FEBS Lett.* **283**, 117–121
- Heinemeyer, W., Kleinschmidt, J. A., Saidowsky, J., Escher, C. and Wolf, D. H. (1991) *EMBO J.* **10**, 555–562
- Hendil, K. B. (1988) *Biochem. Int.* **17**, 471–477
- Hershko, A. and Ciechanover, A. (1992) *Annu. Rev. Biochem.* **61**, 761–807
- Hough, R., Pratt, G. and Rechsteiner, M. (1987) *J. Biol. Chem.* **262**, 8303–8313
- Ikai, A., Nishigai, M., Tanaka, K. and Ichihara, A. (1991) *FEBS Lett.* **292**, 21–24
- Jentsch, S. (1992) *Trends Cell Biol.* **2**, 98–103
- Kaltoft, M. B., Koch, C., Uerkvitz, W. and Hendil, K. B. (1992) *Hybridoma* **11**, 507–517
- Kanayama, H., Tanaka, K., Aki, M., Kagawa, S., Miyaji, H., Satoh, M., Okada, F., Sato, S., Shimbara, N. and Ichihara, A. (1991) *Cancer Res.* **51**, 6677–6685
- Kanayama, H., Tamura, T., Ugai, S., Kagawa, S., Tanahashi, N., Yoshimura, T., Tanaka, K. and Ichihara, A. (1992) *Eur. J. Biochem.* **206**, 567–578
- Kawahara, H. and Yokosawa, H. (1992) *Dev. Biol.* **151**, 27–33
- Kelly, A., Powis, S. H., Glynne, R., Radley, E., Beck, S. and Trowsdale, J. (1991) *Nature (London)* **353**, 667–668
- Klein, U., Gernold, M. and Kloetzel, P. M. (1990) *J. Cell Biol.* **111**, 2275–2282
- Kinoshita, M., Hamakubo, T., Fukui, I., Murachi, T. and Toyohara, H. (1990) *J. Biochem. (Tokyo)* **107**, 440–444
- Knecht, E., Palmer, A., Sweeney, S. T. and Rivett, A. J. (1991) *Biochem. Soc. Trans.* **19**, 293S
- Kopp, F., Steiner, R., Dahlmann, B., Kuehn, L. and Reinauer, H. (1986) *Biochim. Biophys. Acta* **872**, 253–260
- Kreutzer-Schmid, C. and Schmid, H. P. (1990) *FEBS Lett.* **267**, 142–146
- Kumatori, A., Tanaka, K., Inamura, N., Sone, S., Ogura, T., Matsumoto, T., Tachikawa, T., Shin, S. and Ichihara, A. (1990a) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 7071–7075
- Kumatori, A., Tanaka, K., Tamura, T., Fujiwara, T., Ichihara, A., Tokunaga, F., Onikura, A. and Iwanaga, S. (1990b) *FEBS Lett.* **264**, 279–282
- Lee, L. W., Moomaw, C. R., Orth, K., McGuire, M. J., DeMartino, G. N. and Slaughter, C. A. (1990) *Biochim. Biophys. Acta* **1037**, 178–185
- Lee, D. H., Tanaka, K., Tamura, T., Chung, C. H. and Ichihara, A. (1992) *Biochem. Biophys. Res. Commun.* **182**, 452–460
- Li, X., Gu, M. and Ellinger, J. D. (1991) *Biochemistry* **30**, 9709–9715
- Lilley, K. S., Davison, M. D. and Rivett, A. J. (1990) *FEBS Lett.* **262**, 327–329
- Martinez, C. K. and Monaco, J. J. (1991) *Nature (London)* **353**, 664–667
- Martins de Sa, C., Grossi de Sa, F., Akhayat, O., Broders, F., Scherrer, K., Horsch, A. and Schmid, H.-P. (1986) *J. Mol. Biol.* **187**, 479–493
- Mason, R. W. (1990) *Biochem. J.* **265**, 479–484
- Matthews, W., Tanaka, K., Driscoll, J., Ichihara, A. and Goldberg, A. L. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 2597–2601
- McDermott, J. R., Gibson, A. M., Oakley, A. E. and Biggins, J. A. (1991) *J. Neurochem.* **56**, 1509–1517
- McGuire, M. J., McCullough, M. L., Croall, D. E. and DeMartino, G. N. (1989) *Biochim. Biophys. Acta* **994**, 181–186
- Momburg, F., Ortiz-Navarette, V., Neefjes, J., Goulmy, E., van de Wal, Y., Spits, H., Powis, S. J., Butcher, G. W., Howard, J. C., Walden, P. and Hämmerling, G. J. (1992) *Nature (London)* **360**, 174–177
- Monaco, J. J. and McDevitt, H. O. (1984) *Nature (London)* **309**, 797–799
- Murakami, K. and Ellinger, J. D. (1986) *Proc. Natl. Acad. Sci. U.S.A.* **83**, 7588–7592
- Murakami, Y., Matsufuji, S., Kameji, T., Hayashi, S., Igarashi, K., Tamura, T., Tanaka, K. and Ichihara, A. (1992) *Nature (London)* **360**, 597–599
- Mykles, D. L. and Haire, M. F. (1991) *Arch. Biochem. Biophys.* **288**, 543–551
- Nothwang, H. G., Coux, O., Bey, F. and Scherrer, K. (1992a) *Biochem. J.* **207**, 621–630
- Nothwang, H. G., Coux, O., Keith, G. Silva-Pereira, I. and Scherrer, K. (1992b) *Nucleic Acids Res.* **20**, 1959–1965
- Orino, E., Tanaka, K., Tamura, T., Sone, S., Ogura, T. and Ichihara, A. (1991) *FEBS Lett.* **284**, 206–210
- Orlowski, M. (1990) *Biochemistry* **29**, 10289–10297
- Orlowski, M. and Michaud, C. (1989) *Biochemistry* **28**, 9270–9278
- Orlowski, M. and Wilk, S. (1988) *Biochem. J.* **255**, 751
- Orlowski, M., Cardozo, C., Hidalgo, M. C. and Michaud, C. (1991) *Biochemistry* **30**, 5999–6005
- Ortiz-Navarette, V., Seelig, A., Gernold, M., Frentzel, S., Kloetzel, P. M. and Hämmerling, G. J. (1991) *Nature (London)* **353**, 662–664
- Pereira, M. E., Nguyen, T., Wagner, B. J., Margolis, J. W., Yu, B. and Wilk, S. (1992) *J. Biol. Chem.* **267**, 7949–7955
- Peters, J. M., Harris, J. R. and Kleinschmidt, J. A. (1991) *Eur. J. Cell Biol.* **56**, 422–432
- Pühler, G., Weinkauff, S., Bachmann, L., Müller, S., Engel, A., Hegerl, R. and Wolfgang Baumeister, W. (1992) *EMBO J.* **11**, 1607–1616
- Rawlings, N. and Barrett, A. J. (1993) *Biochem. J.* **290**, 205–218
- Ray, K. and Harris, H. (1985) *Proc. Natl. Acad. Sci. U.S.A.* **82**, 7545–7549
- Rechsteiner, M. (1991) *Cell* **66**, 615–618
- Richter-Ruoff, B., Heinemeyer, W. and Wolf, D. H. (1992) *FEBS Lett.* **302**, 192–196
- Rivett, A. J. (1985a) *J. Biol. Chem.* **260**, 12600–12606
- Rivett, A. J. (1985b) *Arch. Biochem. Biophys.* **243**, 624–632
- Rivett, A. J. (1989a) *Arch. Biochem. Biophys.* **268**, 1–8
- Rivett, A. J. (1989b) *J. Biol. Chem.* **264**, 12215–12219
- Rivett, A. J. (1990) *Curr. Opin. Cell Biol.* **2**, 1143–1149

- Rivett, A. J. and Knecht, E. (1993) *Curr. Biol.* **3**, 127–129
- Rivett, A. J. and Sweeney, S. T. (1991) *Biochem. J.* **278**, 171–177
- Rivett, A. J., Palmer, A. and Knecht, E. (1992) *J. Histochem. Cytochem.* **40**, 1165–1172
- Savory, P. J. and Rivett, A. J. (1993) *Biochem. J.* **289**, 45–48
- Schliephacke, M., Kremp, A., Schmid, H. P., Kohler, K. and Kull, U. (1991) *Eur. J. Cell Biol.* **55**, 114–121
- Schmid, H. P., Akhayat, O., Martins de Sa, C., Puvion, F., Koehler, K. and Scherrer, K. (1984) *EMBO J.* **3**, 29–34
- Seelig, A., Kloetzel, P.-M., Kuehn, L. and Dahmann, B. (1991) *Biochem. J.* **280**, 225–232
- Seufert, S. and Jentsch, S. (1992) *EMBO J.* **11**, 3077–3080
- Shimbara, N., Orino, E., Sone, S., Ogura, T., Takashina, M., Shono, M., Tamura, T., Yasuda, H., Tanaka, K. and Ichihara, A. (1992) *J. Biol. Chem.* **267**, 18100–18109
- Skilton, H. S., Eperon, I. C. and Rivett, A. J. (1991) *FEBS Lett.* **279**, 351–355
- Sorimachi, H., Tsukahara, T., Kawasaki, H., Ishiura, S., Emori, Y., Sugita, H. and Suzuki, K. (1990) *Eur. J. Biochem.* **193**, 775–781
- Tamura, T., Tanaka, K., Kumatori, A., Yamada, F., Tsurumi, C., Fujiwara, T., Ichihara, A., Tokunaga, F., Aruga, R. and Iwanaga, S. (1990) *FEBS Lett.* **264**, 91–94
- Tamura, T., Lee, D. H., Osaka, F., Shin, S., Chung, C. H., Fujiwara, T., Tanaka, K. and Ichihara, A. (1991) *Biochim. Biophys. Acta* **1089**, 95–102
- Tanaka, K., Li, K., Ichihara, A., Waxman, L. and Goldberg, A. L. (1986) *J. Biol. Chem.* **261**, 15197–15203
- Tanaka, K., Yoshimura, T., Ichihara, A., Ikai, A., Nishigai, M., Morimoto, Y., Sato, M., Tanaka, N., Katsube, Y., Kameyama, K. and Takagi, T. (1988a) *J. Mol. Biol.* **203**, 985–996
- Tanaka, K., Yoshimura, T., Kumatori, A., Ichihara, A., Ikai, A., Nishigai, M., Kameyama, K. and Takagi, T. (1988b) *J. Biol. Chem.* **263**, 16209–16217
- Tanaka, K., Kumatori, A., Li, K. and Ichihara, A. (1989) *J. Cell. Physiol.* **139**, 34–41
- Tanaka, K., Fujiwara, T., Kumatori, A., Shin, S., Yoshimura, T., Ichihara, A., Tokunaga, F., Aruga, R., Iwanaga, S., Kakizuka, A. and Nakanishi, S. (1990a) *Biochemistry* **29**, 3777–3785
- Tanaka, K., Kanayana, H., Tamura, T., Lee, D. H., Kumatori, A., Fujiwara, T., Ichihara, A., Tokunaga, F., Aruga, R. and Iwanaga, S. (1990b) *Biochem. Biophys. Res. Commun.* **171**, 676–683
- Tanaka, K., Yoshimura, T., Tamura, T., Fujiwara, T., Kumatori, A. and Ichihara, A. (1990c) *FEBS Lett.* **271**, 41–46
- Tokunaga, F., Aruga, R., Iwanaga, S., Tanaka, K., Ichihara, A., Takao, T. and Shimonishi, Y. (1990) *FEBS Lett.* **262**, 373–375
- Tomek, W., Adam, G. and Schmid, H. P. (1988) *FEBS Lett.* **239**, 155–158
- Tsukahara, T., Ishihara, S., Kominami, E. and Sugita, H. (1990) *Exp. Cell Res.* **188**, 111–116
- Van Riel, M. C. H. M. and Martens, G. J. M. (1991) *FEBS Lett.* **291**, 37–40
- Weitman, D. and Etlinger, J. D. (1992) *J. Biol. Chem.* **267**, 6977–6982
- Wilk, S. and Orlowski, M. (1980) *J. Neurochem.* **35**, 1172–1182
- Wilk, S. and Orlowski, M. (1983) *J. Neurochem.* **40**, 842–849
- Yang, Y., Waters, J. B., Früh, K. and Peterson, P. A. (1992) *Proc. Natl. Acad. Sci. U.S.A.* **89**, 4928–4932
- Yu, B., Pereira, M. and Wilk, S. (1991) *J. Biol. Chem.* **266**, 17396–17400
- Yukawa, M., Sakon, M., Kambayashi, J., Shiba, E., Kawasaki, T., Ariyoshi, H. and Mori, T. (1991) *Biochem. Biophys. Res. Commun.* **178**, 256–262
- Zwickl, P., Pfeifer, G., Lottspeich, F., Kopp, F., Dahmann, B. and Baumeister, W. (1990) *J. Struct. Biol.* **103**, 197–203
- Zwickl, P., Lottspeich, F., Dahmann, B. and Baumeister, W. (1991) *FEBS Lett.* **278**, 217–221
- Zwickl, P., Grziwa, A., Pühler, G., Dahmann, B., Lottspeich, F. and Baumeister, W. (1992) *Biochemistry* **31**, 964–972