Chaperone-assisted assembly of the proteasome core particle

Ana C. Matias, Paula C. Ramos and R. Jürgen Dohmen

Abstract

The 26S proteasome is a non-lysosomal protease in the cytosol and nucleus of eukaryotic cells. Its main function is to mediate ubiquitin-dependent proteolysis. The 26S proteasome is a multimeric complex composed of the 20S proteasome CP (core particle) and the 19S RPs (regulatory particles). Although the atomic structure of the 26S proteasome has not yet been determined, high-resolution structures are available for its CP. Studies on the complicated assembly pathway of the proteasome have revealed that it involves an unprecedented number of dedicated chaperones. Assembly of the CP alone involves three conserved proteasome-assembly chaperones [PAC1–PAC2, PAC3–PAC4 and UMP1 (ubiquitin-mediated proteolysis 1)]. Whereas the two heterodimeric PACs have been implicated in the formation of rings of the seven distinct α subunits, UMP1 is important for the formation and dimerization of proteasome precursor complexes containing β subunits. Dimerization coincides with the incorporation of the last β subunit (β7). Additional modules important for the assembly of precursor complexes and their dimerization reside in the β subunits themselves, either as transient or as permanent extensions. Particularly important domains are the propeptide of β5 and the C-terminal extensions of β2 and β7. Upon maturation of the active sites by autocatalytic processing, UMP1 is degraded by the native proteasome.

Introduction

In eukaryotic cells, the main route for selective degradation of short-lived or abnormal proteins is the ATP-dependent UPS (ubiquitin–proteasome system) [1]. In this system, proteins are targeted for degradation by covalent attachment of polyubiquitin chains, which are recognized by the 26S proteasome, a 2.5 MDa protease complex [2–4]. This proteolytic complex is composed of the catalytic CP (core particle), the 20S proteasome, to the ends of which two 19S RPs (regulatory particles) are attached. The degradation of a substrate involves the release of the ubiquitin moieties, unfolding and translocation of the polypeptide into the 20S CP [5,6].

X-ray crystallography revealed that the overall structure of mature 20S proteasomes is highly conserved in species ranging from archaea, bacteria and Saccharomyces cerevisiae to mammals [6]. The 20S CP is composed of four stacked heptameric rings of either the α or the β type, in an α7β7α7β7α7 architecture. Whereas archaeal 20S proteasomes, in most cases, are made up of homo-oligomeric α and β rings, the eukaryotic 20S CP is composed of seven distinct α and seven distinct β subunits. In eukaryotic proteasomes, only three of the seven β subunits, namely β5, β2 and β1, mediate proteolytic activities, which are classified as, respectively, chymotrypsin-like (cleavage after bulky hydrophobic residues), trypsin-like (cleavage after basic residues) and caspase-like (cleavage after acidic residues) activities [6].

Biogenesis of the 20S CP in eukaryotes from its 14 distinct subunits occurs via an ordered set of steps, which is, by and large, conserved from yeast to humans. First a ring of α subunits is assembled that subsequently serves as a platform for the addition of β subunits leading to the formation of precursor complexes. Dimerization of such precursors is triggered by the incorporation of subunit β7. As discussed below, these processes are promoted by dedicated assembly chaperones and by specialized features of certain β subunits that can be regarded as intramolecular chaperones [7–9].

Assembly pathway of 20S proteasomes

Proteasomes from bacteria such as Rhodococcus assemble from heterodimers composed of α and β subunits [10]. Assembly of the 20S proteasome from archaea as well as from eukaryotes initiates with the formation of rings composed of seven α subunits. This was first observed upon expression of genes from archaea encoding such subunits in Escherichia coli or in vitro [11]. These experiments demonstrated that these subunits have the propensity to self-assemble into heptameric rings without the help of any chaperones. The
assembly and/or stability of rings composed of the seven distinct α subunits in eukaryotic cells, however, are promoted by dedicated chaperones (as discussed below). In eukaryotes, α rings then serve as platforms on to which subunits β1–β6 assemble stepwise in an ordered fashion together with an additional chaperone called UMP1 (ubiquitin-mediated proteolysis 1) to form proteasome precursor complexes [12]. Addition of β7, the last of the 14 CP subunits, coincides with the dimerization of such precursor complexes (Figure 1A) [13,14]. Dimerization triggers processing of N-terminal propeptides of β subunits and thereby activation of peptidase sites [15–19].

Dedicated proteasome-assembly chaperones

Two heterodimeric proteasome-assembly chaperones, termed PAC1–PAC2 and PAC3–PAC4 in mammals, interact with α subunits, promote the correct assembly of α rings and prevent their dimerization [20–24]. PAC3–PAC4 leaves the complex during subsequent assembly of β subunits, while PAC1–PAC2 remains bound until the assembly of the CP is completed [9].

Work in our laboratories has focused on another assembly chaperone, UMP1, which is found in proteasome precursor complexes containing a full set of α subunits and unprocessed β subunits. Experiments in which the expression of UMP1 or of individual β subunits was knocked down by RNAi (RNA interference) suggested that UMP1 and subunit β2 are the first polypeptides to associate with α rings complexed with PACs [9]. UMP1 appears to be essential for CP formation and viability in mammalian cells [12,22,25]. Deletion of UMP1 in the yeast S. cerevisiae, in contrast, is not lethal, but instead leads to severe growth defects and stress hypersensitivity [19]. In the absence of UMP1, precursor complexes and proteasomal complexes with incompletely processed β subunits accumulate, suggesting that UMP1 promotes formation of precursor complexes that are competent for efficient dimerization and maturation (Figure 1B) [19]. When two precursor complexes bearing UMP1 dimerize, UMP1 is enclosed in the nascent CP and degraded once the active sites have matured (Figure 1A) [13,14].

Role of β subunit propeptides in proteasome biogenesis

As is typical for proteases, the active β subunits are synthesized as precursor polypeptides containing N-terminal propeptides. Only upon cleavage of the propeptide is the catalytic Thr\(^1\) exposed and the β subunit becomes active. The presence of the propeptides prevent acetylation of Thr\(^1\) in the cytosol [27]. Propeptide processing occurs via intramolecular autolysis of the Gly-Thr\(^1\) bond, which takes place following dimerization of precursor complexes [17,28]. In eukaryotes, five of the seven distinct β subunits are synthesized as precursors, but only three habitat active sites. A combination of the conserved residues forming the catalytic centre (Thr\(^1\), Asp\(^{17}\) and Lys\(^{35}\)) plus a glycine residue preceding

---

**Figure 1 | Schematic model of the role of UMP1 and β subunit propeptides or extensions in proteasome maturation**

(A) In wild-type cells, UMP1 and unprocessed β subunit are detected in CP precursor complexes containing all α and β subunits except for β7. Dimerization of these precursors is triggered by incorporation of β7, the CTE of which contributes to a stabilization of the nascent CP. Upon dimerization, the entrapped UMP1 and β subunit propeptides or N-terminal extensions (NTEs) impose conformational changes on each other, thereby promoting autocatalytic processing of active β subunits and subsequent degradation of UMP1 and processing of inactive β subunits. (B) In a yeast mutant lacking UMP1, dimerization occurs assisted by incorporation of β7. The necessary conformational and/or positional shifts to reach autocatalytic processing, however, are not efficient, resulting in only partially mature CP. (C) Deletions either of the β5 propeptide or the β6 NTE (β5-Δpro, β6-ΔNTE), results in cell lethality, apparently because UMP1 remains in a position that blocks subsequent steps in proteasome maturation. (D) Removal of these mutations (β5-Δpro, β6-ΔNTE) is rescued by elimination of UMP1 (ump1Δ). In these double mutants, partially mature CPs are found.

---
The Try3 features are only present in the subunits β1, β2 and β5 [6]. The remaining subunits are inactive and are found in the proteasome structures in either unprocessed (β3 and β4) or processed (β6 and β7) form [29]. Processing of the latter subunits depends on the proteolytic action of neighboring active subunits [30,31].

Although propeptides of the β subunits of Thermoplasma or mycobacterial proteasomes could be deleted without severe impact on proteasome assembly, they proved to be important for efficient assembly of Rhodococcus proteasomes [28,32,33]. The propeptides of eukaryotic β subunits are highly divergent both in sequence and in length and display a rather low degree of conservation in contrast with a high conservation of the mature parts of these subunits. A particularly important role in proteasome biogenesis was found for the exceptionally long propeptide of the β5 subunit (75 residues) in S. cerevisiae. Deletion of this propeptide is lethal (Figure 1C) [17]. Interestingly, however, lethality of this propeptide deletion was suppressed by an additional deletion of UMP1 (Figure 1D) [19]. This finding suggested that the propeptide of β5 is required to induce a change of the conformation or position of UMP1 within the complex that is required to proceed with the assembly and maturation of the CP. A similar inhibitory role of UMP1 was observed upon truncation of an N-terminal extension of the β6 subunit, consistent with the notion that UMP1 blocks proteasome maturation, thereby conferring a checkpoint function, until the presence of the N-terminal extensions of certain β subunits as well as incorporation of the β7 subunit (see below) signals completion of the assembly [14]. The observations that, on one hand, the β5 propeptide is required to overcome an inhibition of maturation by UMP1 and that, on the other, UMP1 is required for efficient maturation of β5 and the other β subunits suggested that these polypeptides impose conformational changes on each other that promote CP formation and maturation (Figure 1A) [19]. Deletion of the β5 propeptide in human HEK (human embryonic kidney)-293T cells led to an accumulation of precursor complexes lacking the subunits β6 and β7 [12]. A simultaneous knockdown of UMP1 did not rescue this phenotype, but instead led to the accumulation of complexes with α subunits, but lacking all β subunits. These data indicate that human UMP1 serves an essential role early in precursor complex assembly, apparently in recruiting β2 as the first β subunit, while the propeptide of β5 is required for stable incorporation of β6 [12]. Together, these findings indicate that there are differences in the requirements for UMP1 or the β5 propeptide for distinct steps in the assembly pathway of proteasomes between yeast and human cells.

Role of C-terminal extensions of β subunits in the assembly of 20S proteasomes

An inspection of the crystal structure of the yeast 20S CP revealed two exceptional features of their β subunits, which are absent from their prokaryotic counterparts [29]. The subunits β2 and β7 bear C-terminal extensions which turned out to be important for proteasome assembly. The ∼30 residue extension of β2 wraps around the β3 subunit, its direct neighbour within the same ring of β subunits (Figure 2A). Deletion of this extension is lethal for yeast cells. The extension is not essential for the incorporation of β2 into yeast CP precursors. Such precursors containing the truncated β2 are, however, apparently not competent to efficiently form functional proteasomes [34]. In human cells, deletion of the corresponding C-terminal extension of β2 blocked precursor complex assembly after binding of β2 by preventing incorporation of the neighbouring β3 subunit into nascent precursor complexes. The same result was obtained upon deletion of the β2 propeptide [12].

These β2 extensions are therefore critically important for the incorporation of the second β subunit (β3) and thus contribute to the directionality and specificity of the steps in the assembly of β rings on α ring platforms.

The CTEs (C-terminal extensions) of the β7 subunits provide prominent connections between the two opposing halves of the proteasome (Figure 2B) [29,34]. Truncation of this extension in yeast resulted in a reduced efficiency of precursor complex dimerization, which is triggered by incorporation of the β7 subunit (Figure 1A) [13,14,34]. CPs and 26S proteasomes, once formed and matured, however, appeared to be stable and functional without the β7 extension, but lacked caspase-like activity [34]. C-terminal residues of this extension are important to stabilize a conformation of the β7 active site required for its activity [34]. Another important function of the β7 CTE appears to be the stabilization of the nascent 20S CP. Incorporation of β7 is the rate-limiting step in precursor complex dimerization as overexpression of this subunit in S. cerevisiae led to a strong reduction of the steady-state levels of these precursors [13]. Moreover, overexpression of β7 was sufficient to suppress the lethal effect of the deletion of the β5 propeptide. The CTE of β7 was required for this effect [14]. Deletion of the propeptide of β6 was also capable of suppressing lethality of the β3∆pro mutation similarly to what was shown previously for the deletion of UMP1 (see above) [14]. Together, these findings indicate that the N-terminal extensions of β5 and β6 may cooperate in chaperoning UMP1 into a conformation or position that is compatible with precursor complex dimerization and/or maturation (Figure 1). Increasing the concentration of the dimerization-driving subunit β7 with its contact-making CTE can suppress defects in these functions. Experiments in human cells showed that the β7 CTE is essential for the formation of CPs, indicating that its function in precursor dimerization is conserved from yeast to humans.

Experiments with prokaryotic proteasome subunits impaired in β subunit maturation indicated that they form loosely associated half-proteasomes [35,36]. Processing of β subunits, and maybe degradation of UMP1, may also contribute to the stabilization of nascent CPs in eukaryotic cells. This notion was supported by the finding that proteasomes isolated from a yeast mutant impaired in the maturation of β5 showed decreased stability during native PAGE [17]. Furthermore, it was observed that simultaneous

Ubiquitin–Proteasome System, Dynamics and Targeting 31
impairment of the Blm10 and 19S RP proteasome activators resulted in increased amounts of precursor complexes [13]. One interpretation of these results is that binding of these activators favours a conformation of nascent CPs that promotes maturation and thereby their stabilization.

Concluding remarks
Although, as discussed above, the principal steps in proteasome CP assembly are understood, many details of the process are still to be clarified and additional chaperones will probably be implicated in this complicated process.

Figure 2 | Surface structure representations of CTEs of S. cerevisiae β subunits
(A) The long β2 CTE wraps around the β3 subunit, its direct neighbour in the same ring. (B) β7 CTEs resemble clamps projecting from one half of the proteasome to the other. Each tail is located between the β1 and β2 subunits on the opposing ring of the mature CP. The Figure was prepared with data from PDB code 2F16 using PyMOL (DeLano Scientific, http://pymol.sourceforge.net).

Funding
Work in our laboratories is supported by grants from Fundação para a Ciência e Tecnologia (to P.C.R.) and by the Deutsche Forschungsgemeinschaft (to R.J.D.).

References

Received 29 October 2009
doi:10.1042/BST0380029