Chaperone-driven proteasome assembly

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Abstract

Assembly of the 34-subunit, 2.5 MDa 26S proteasome is a carefully choreographed intricate process. It starts with formation of a seven-membered α-ring that serves as a template for assembly of the complementary β-ring-forming 'half-proteasomes'. Dimerization results in a latent 20S core particle that can serve further as a platform for 19S regulatory particle attachment and formation of the biologically active 26S proteasome for ubiquitin-dependent proteolysis. Both general and dedicated proteasome assembly chaperones regulate the efficiency and outcome of critical steps in proteasome biogenesis, and in complex association.

Introduction

In cells, numerous regulatory pathways are controlled via the timely removal of critical proteins. These include proteins involved in the cell cycle, transcriptional regulation, DNA repair, development and differentiation, long-term memory, circadian rhythms, stress response, transcriptional silencing, cell-surface signalling, antigen presentation, and in combating cancer or viral infection [1–3]. Other proteins which must be properly discarded are damaged, abnormal or foreign (viral) proteins. In the nucleus, cytoplasm and even endoplasmic reticulum of eukaryotic cells, almost all proteins of the aforementioned types are degraded in an ATP-dependent manner by a single, highly conserved, multisubunit enzyme, the proteasome [4–6]. Most proteins that are destined for degradation by the proteasome are covalently labelled with multiple ubiquitin molecules by a cascade of ubiquitinating enzymes [6]. The active form of the proteasome is considered to be a 26S holoenzyme, which possesses an intrinsic ability to recognize and bind ubiquitinated targets.

The 26S proteasome holoenzyme is composed of a catalytic 20S CP (core particle) and a 19S RP (regulatory particle). In eukaryotes, the 20S proteasome is composed of seven homologous α and seven homologous β subunits (α1–α7 and β1–β7 respectively), all of which occupy distinct positions within the 20S particle. Mammals encode four additional catalytic β-subunits: three interferon-γ-inducible (β1i, β2i and β5i) and one thymus-specific (β5t), which are incorporated upon induction in place of their most closely related β-subunits, thus forming proteasome subtypes called immunoproteasomes and thymoproteasomes, each with altered catalytic activities [7,8]. The resulting $\alpha_7\beta_7\beta_7\alpha_7$ barrel-like structure is characterized by a two-fold horizontal symmetry, with each half containing an outer ring of seven α subunits and an inner ring of seven β subunits. The two β-rings contact each other to form the catalytic chamber of the 20S CP [9]. Five of the seven β subunits are synthesized as precursors with N-terminal propeptides [10]. After assembly into an inactive 20S form, these extensions are cleaved off, resulting in mature 20S CP. Maturation of three of the β subunits, β1, β2 and β5, exposes the N-terminal threonine residue that serves as the active-site nucleophile, providing the post-acidic, post-basic and post-hydrophobic proteolytic specificities respectively [11,12]. The resulting broad proteolytic specificity gave the 20S CP one of its names, the 'multicatalytic protease' or MCP. The two outer α-rings contact the β-rings on one side, and the base of the 19S activator on the other side. The 19S RP is composed of two subcomplexes, the base and the lid, that are linked by the Rpn10 subunit [13]. The 19S RP recognizes ubiquitinated proteins and mediates their unfolding and translocation into the 20S CP. It should therefore not come as a surprise that such an intricate assemblage is put together in a carefully executed manner, mediated by dedicated assembly factors at each step of proteasome assembly [14] (Figure 1).

PAC1–PAC4/Pba1–Pba4 early-assembly chaperones

Proteasome assembly is initiated with formation of a heteromeric seven-membered α-ring. Owing to the hetromeric nature of the α-ring in eukaryotes, subunits must be incorporated in an exact order to guarantee correct ring formation. This intricate process is compounded, as subunits α7 [15] and α5 [16] are known to spontaneously dimerize to form homomeric double-ring-like structures. Likewise, co-expression of α6 and α1 with α7 results in formation of misassemblies characterized by a high variation of subunit positioning [15]. These observations imply that not all α subunits intrinsically contain the necessary information for correct positioning within the α-ring, but instead probably require additional guidance. Responsibility for the proper assembly of the α-ring can now be attributed (at least in part) to a set of recently discovered Pba (proteasome biogenesis-associated) 1–4 chaperones. These proteins have been found to be widely conserved, including in humans, where the orthologues are referred to as PAC (proteasome-assembly chaperone) 1–4 [17,18].

Both PAC1–PAC2 and Pba1–Pba2 in the respective systems were shown to form dimers that interact with

Key words: chaperone, half-proteasome, proteasome assembly, proteolysis, ubiquitin.
Abbreviations used: CP, core particle; Hsc, heat-shock cognate; Hsp, heat-shock protein; PA, proteasome activator; PAC, proteasome-assembly chaperone; Pba, proteasome biogenesis-associated; POMP, proteasome maturation protein; RP, regulatory particle.
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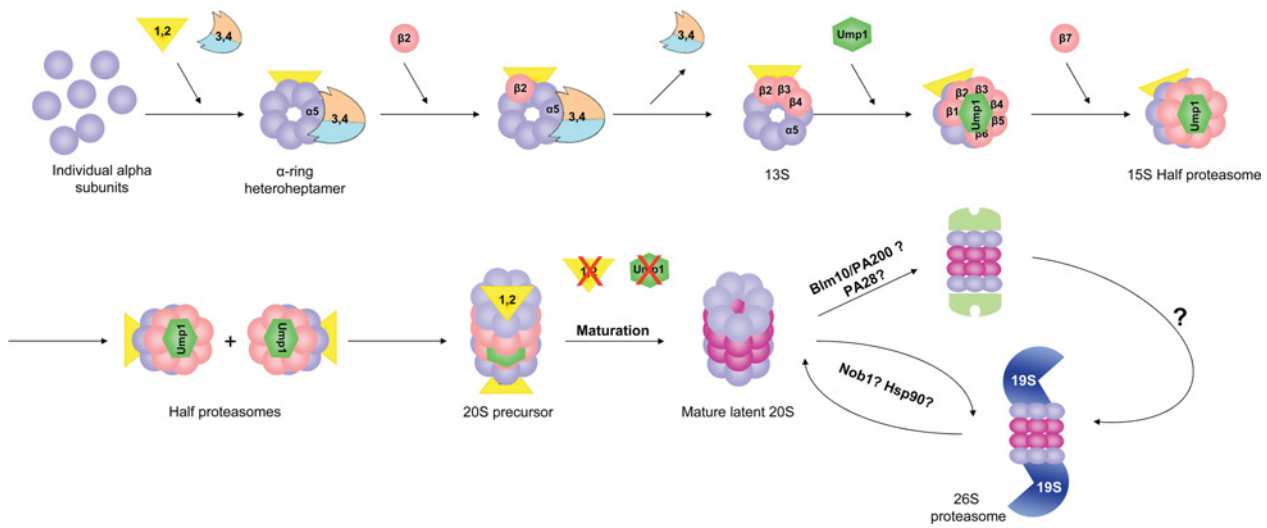
Figure 1 | Chaperone-facilitated proteasome assembly

Proteasome assembly in eukaryotes requires addition of chaperone molecules. Pba1–Pba4 (known as PAC1–PAC4 in mammals) bind to early-assembly intermediates of proteasome α-subunits and promote α-ring formation. The Pba3–Pba4 chaperone complex is required for efficient completion of the α-ring, and in particular for incorporation of α4. The Pba1–Pba2 heterodimer remains attached to the nascent α-ring and suppresses non-productive α-ring dimerization, thereby promoting attachment of the β-subunits to the proper surface of the α-ring. The resulting ring serves as a template for assembly of the β-ring: first β2, followed by β3 and β4 [17,20,22]. This intermediate is also referred to as the 13S precursor. At this stage, the Pba3–Pba4 complex dissociates due to steric hindrance with β3 [30a]. Another chaperone, Ump1, attaches to the still-incomplete β-ring, inhibiting dimerization until β7 is properly incorporated into half-proteasomes (15S). The N-terminal tail of β7 is a key player in the association of two half-proteasomes by overcoming Ump1 inhibition and allowing the formation of the 20S precursor [21]. Once all β-subunits are in place, the resulting half-proteasomes dimerize and the β-subunit propeptides are removed, leading to the mature 20S proteasome and degradation of Ump1 and Pba1–Pba2. Mature 20S proteasome, particularly the now-exposed α-ring surface, functions as a template for the initial assembly of the 19S RP. Therefore the 20S proteasome itself is also a 19S RP assembly factor [22]. Other factors such as Hsp90, Nob1, Ecm29 and Blm10, have been loosely implicated in RP or 26S assembly [21,52,56,65,72]. The sheer complexity of the 19S RP suggests that many more chaperones or assembly factors, yet to be identified, participate in proteasome biogenesis.



α-ring assembly intermediates containing as many as five subunits, but not α3 or α4 [19–21]. As we noted above, these are the α-subunits that have a propensity to form higher-molecular-mass combinations, suggesting that these early-assembly chaperones may protect from misassemblies. The necessity of PAC1–PAC2/Pba1–Pba2 to the assembly process was elucidated further by the observation that, in knockdown cells, there is an accumulation of off-pathway non-productive α-ring homoheptamers [19]. Thus it would appear that PAC1–PAC2/Pba1–Pba2 remain attached to the outer surface of the α-ring until completion of 20S assembly, probably until they are ether degraded by the 20S itself or replaced by 19S RP or another regulator [19]. To summarize, PAC1–PAC2/Pba1–Pba2 direct correct insertion of α-subunits into the nascent ring, prevent unwanted dimerization to additional α-ring copies, thus freeing one surface to serve as a template for the β-ring assembly.

Correct assembly of the α-ring and the initiation of β subunit incorporation is aided by a second set of proteasome-assembly chaperones. PAC3 (probably alongside PAC4) or Pba3–Pba4 dimers bind to early α-subunit assembly intermediates that contain a restricted subset of α subunits that includes α5 [17,18,22]. In the absence of these chaperones, an additional α4 subunit is inserted into the α-ring in place of the α3 subunit, and overall proteasome assembly will be less efficient [17]. Thus the major function of these chaperones may be to correctly seal the α-ring by precisely prioritizing the entry of the last α-subunits, α4 and α3, apparently in that order. The Pba3–Pab4 dimer then remains attached to the completed α-ring on the opposing surface to Pba1–Pab2 [22] until part way through completion of the β-ring (also called 13S complexes), when Pba3–Pba4 is released due to steric hindrance from β subunits [18], particularly β3, and recycled [20,22]. To summarize, Pba3–Pab4 act as a chaperone for 20S CP formation by properly sealing the α-ring and ensuring proper initiation of β-ring assembly by locating the β2 subunit at its proper position on the α-ring [17].

Ump1/POMP and proteasome maturation

Later steps of proteasome assembly and maturation require the support of additional accessory protein Ump1 or its mammalian orthologue POMP (proteasome maturation protein). No proteasome assembly intermediates that contain both Pba3–Pba4 and Ump1 have been detected to date, thus

apparently Ump1 binds to the proteasome precursor after Pba3–Pba4 detachment [17,22]. In the Δ*ump1* mutant, the formation of 20S structures from two half-proteasome precursors appears to be less efficient, and the maturation of the active-site subunits β1, β2 and β5 is drastically reduced [23]. It has been suggested that both Ump1 and the β5 propeptide mutually induce conformational or positional changes on each other upon dimerization of half-proteasome precursor complexes (also called 15S) [24]. When the β5 propeptide is absent, Ump1 remains in a position or conformation that is incompatible with subsequent maturation steps and thus results in a slowing down of proteasome assembly. This may explain how *ump1*-null mutations suppress the lethality of β5 propeptide deletion [23]. In addition to aiding the association of two 15S half-proteasomes, Ump1 also inhibits premature dimerization until the last subunit, β7, is inserted [21,23,25–28]. When the final subunit β7 is inserted, its propeptide tail helps to overcome Ump1 inhibition, allowing for the two 15S half-proteasomes to interact stably. This dimerization is coupled with the maturation of β1, β2 and β5, during which their propeptides are cleaved, revealing their functional threonine protease active sites [9,27,29,30]. In summary, Ump1 has multiple roles in proteasome maturation: first, it helps to keep the half-proteasome precursor complex in a conformation that is best suited for dimerization; secondly, it inhibits premature dimerization until the β-ring is properly assembled; and, subsequently, it is required for triggering the maturation of active sites within the 20S complex.

Recent studies in assembly of mammalian 20S indicate that, although the role of POMP is largely compatible with that of Ump1, it differs in a number of specifics. Work by Hirano et al. [30a] demonstrates that incorporation of POMP is coupled to that of β2, the first β subunit assembled on the α-ring, since loss of either β2 or POMP results in dissociation of the other. These observations may point to an important role of POMP in β-ring assembly on the α-ring template.

Another chaperone, Hsc (heat-shock cognate)73, was found to associate with proteasome-precursor complexes (15S) [31]. It has been suggested that Hsc73 might keep assembly intermediates in a partially unfolded state to allow subunit processing, correct folding and incorporation of late proteasome subunits [31], yet, to date, no evidence for this type of activity has been documented for any of the other known Hsp (heat-shock protein) 70 orthologues.

Mature 20S proteasomes are nevertheless maintained in a latent form due to N-terminal tails of α-subunits that block the entrance pore into the inner proteolytic chamber [32]. A number of PAs (proteasome activators) are able to attach to the α-ring surface and regulate gating of the central pore and activate proteolysis. The best-studied activator is the 19S RP (also known as PA700) which, in complex with the 20S, forms the 26S proteasome that performs the degradation of polyubiquitinated targets.

26S assembly

In contrast with 20S CP assembly, little is known so far about the pathway and mechanism of 19S RP assembly or about auxiliary factors that guide this process. How more than 20 divergent subunits assemble into a stable and functional 19S RP is still an enigma. One can only assume that, since the 19S RP is more heterogeneous and less symmetrical than the 20S CP to which it attaches, an even greater number of specific or general chaperones will be found to choreograph this process. Because the 20S proteasome alone, or in complex with non-ATPase activators such as PA28 or Blm10/PA200, does not use polyubiquitinated proteins as substrates, understanding the association of the 20S proteasome with the 19S RP is critical to our understanding of the ubiquitin–proteasome pathway. The 19S RP can be biochemically divided into two further substructures: the base and the lid [13]. The base itself is also composed of an inner central unit that extends the proteolytic channel at the surface of the base, and a peripheral unit that contains the RP ATPase subunits and additional ubiquitin-binding proteins [33,34].

At least *in vitro*, the 26S proteasome may dissociate into discrete 20S CP and 19S RP components, which in turn are able to reassociate in an energy-dependent manner [35–37]. Moreover, the central unit of the base, consisting of Rpn1 and Rpn2, can reattach to the α-surface of the 20S CP in the absence of any other 19S RP subunit, indicating that this unit may serve as a nucleating agent for the remainder of the base or 19S RP with the 20S CP [33]. It is unclear whether, *in vivo*, all proteasomes are synthesized *de novo*, or whether cycles of proteasome assembly and disassembly occur naturally within cells as well. Experimental evidence has been brought forth that some dynamic rearrangements do take place between pre-assembled 19S RP and 20S CP components [38–40]. Likewise, the ratio of free 20S CP to 26S proteasome holoenzyme is dynamic and can respond to cellular conditions, suggesting the possibility of dissociation and reassociation *in vivo* [41]. Together, such observations suggest that a continuous and dynamic assembly of 19S RP or of its subcomplexes with the 20S CP is a distinct possibility. One interesting insight is that the mature 20S proteasome, particularly the exposed α-ring surface, may function as a template for the initial assembly of the 19S RP base subcomplex. Therefore the 20S proteasome itself may double up as a 19S RP-assembly factor [22].

We note that association of pre-assembled 19S subcomplexes may be distinct from *de novo* synthesis of 19S RP from individual subunits; the two may even utilize a distinct set of assembly chaperones. Numerous proteins have been documented to interact with the 26S in high-throughput proteomic screens or with affinity-purified proteasome samples, and indeed many are general chaperones [42–50]. Most proteasome-interacting proteins probably reflect the dynamic nature of the proteasome complex, its wide repertoire of substrates and broad cellular localization, and may not influence proteasome assembly or biogenesis as such. But a few proteins do seem to play some role in assembly. For instance, a general chaperone known to aid in assembly of multicomponent complexes or in quaternary structure assemblies is Hsp90. Hsp90 was found to associate with the 26S proteasome through the 19S RP complex and inactivation of Hsp90 causes dissociation of the active 26S proteasome

into its constituents containing the 20S proteasome base and lid [51,52].

Two proteasome-specific proteins were proposed to facilitate 26S assembly: Blm10, a 200 kDa α-helix-rich repeat protein (also called PA200), and Nob1. Blm10/PA200 can be found attached to the 20S α-surface and apparently activates some proteolytic properties by gating the entrance pore [53–55]. A higher concentration of Blm10 attached to 20S CP was found in some proteasome mutants that led to inefficient 26S assembly and accumulation of incomplete intermediates, suggesting that Blm10 may play a role in proteasome-assembly pathways [21,25]. In one study, Blm10 was shown to join the 20S precursor during the transition into the mature 20S CP and interacts with *de novo*-synthesized mature 20S CP [56]. It was proposed that Blm10 acts as a co-ordinator of a late stage of nuclear 20S CP maturation [56]. Another non-ATPase ring that attaches to the 20S α-surface is PA28 [57–61], although no role for PA28 in 26S assembly has been documented. Nob1 is nuclear protein that is present only during the cell-growing phase [62]. This protein is proposed to function in the 26S proteasome assembly [62,63] and to be required for nuclear transfer of the 20S proteasome. Nob1 forms a complex with the 19S RP and is degraded just after the doubly capped 26S proteasome is completed [62].

There is some evidence that the lid and base are assembled independently. For instance, the lid appears to exist as a stable complex [13,64–66], although whether it is merely a proteasome-assembly intermediate or a standalone complex with an independent role has yet to be elucidated. In contrast, there is no documentation of an independent base or base–20S CP accumulating independently of the lid. Defects in the base seem to be a limiting factor for lid incorporation into the 19S RP. Thus some base-assembly mutants (for example in *RPN2* or *RPN10*) can lead to elevated levels of detached lid that accumulate in these mutant cells [13,25,65]. Other mutations in lid subunits interfere with lid assembly or with association of the lid and base subcomplexes (e.g. rpn11-1/mpr1-1, rpn9, rpn6, rpn5 and rpn9 [65,67–71]). Participation of exogenous chaperones aiding in lid assembly or association to the base has yet to be documented.

Summary

Under normal conditions, proteasome synthesis appears to be a rapid and efficient process. The overall flux through multiple steps proceeds smoothly, with few intermediates accumulating, suggesting the absence of a truly rate-limiting step. Interestingly, none of the dedicated proteasome assembly chaperones identified to date is strictly essential, therefore chaperone-deletion mutants can be a useful tool to study proteasome-assembly intermediates that are normally transient and do not accumulate. In particular, the cellular accumulation of these intermediates can be used to map the assembly pathway.

A multisubunit complex such as the proteasome requires accurate fitting of interaction surfaces between its building blocks. True to their definition as chaperones, the 20S CP assembly factors PAC1–PAC4/Pba1–Pba4, as well as Ump1/POMP, not only promote proper assembly, but also limit side reactions that would lead to dead-end intermediates or miscomplexes. They do so by binding to the very same surfaces of the exposed subunits and protect them from side reactions. In order to carry out these functions and fit properly on to their targets, the specific proteasome chaperones apparently share structural similarities with the proteasome subunits that they replace until their target is properly oriented within the assemblage. For example, the 20S CP-assembly chaperone Pba3–Pba4 shares clear structural features with the 20S β-subunits [17], explaining how the dimer can attach to the nascent α-ring only to be replaced by the higher-affinity correct β-subunit. We expect that some of the 19S RP-assembly factors that are awaiting discovery will be found to share some structural similarities with various classes of 19S RP subunits.

The large numbers of subunits that must associate through a series of discrete steps to form the proteasome holocomplex require multiple assembly factors. Apparently, some of these are general chaperones (such as members of the Hsc70 or Hsc90 families) that facilitate myriad protein–protein interactions, whereas others will be dedicated to specific proteasome subassemblies exploiting structural resemblances. It is extremely likely that other large protein complexes, if not most, employ dedicated chaperones for their assembly. The large portion of cellular proteins found in, or shuttling between, complexes raises the somewhat paradoxical situation that a significant portion of the proteome simply serves to keep the rest of these proteins in place.

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